EFFECT OF IMPROVED COMPLEMENTARY FOODS ON LEAN BODY MASS, ESSENTIAL FATTY ACIDS AND GROSS MOTOR DEVELOPMENT OF KENYAN INFANTS

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DECLARATION

This thesis is my original work and has not been submitted for an award of a degree in any other University.

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DEDICATION

This thesis is dedicated to my father, the late Dr. Walter Habil Odima Onyango

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DEFINITION OF OPERATIONAL TERMS

Anthropometric measurements: a set of noninvasive, quantitative techniques for determining an individual's growth and body fat composition by measuring, recording, and analyzing specific dimensions of the body, such as height and weight; mid upper arm circumference and head circumference. It is a readily accessible, inexpensive, objective method to ascertain the nutritional status of an individual or community.

Complementary foods: Any non-breastmilk foods or nutritive liquids that are given to young children during this period are defined as complementary foods. Given alongside breastmilk after the age of 6 months when breastmilk alone is no longer adequate to meet nutritional needs for growth and development.

Essential fatty acids (EFAs): these are fatty acids that humans and other animals must ingest because the body requires them for good health but cannot synthesize them.

Formulated supplementary foods: foods specifically formulated to provide nutrients which either are lacking or are present in insufficient quantities in the local diets. These foods include porridges, ready-to-use products such as pastes and compressed bars, and food based home fortificants. The WHO definition defined "supplementary foods" as formulated foods used to supplement the diet in order to rehabilitate malnourished persons or to prevent a deterioration of nutritional status of those most at risk.

Gross motor development: these are skills are tasks that utilize the gross or large muscles of the body like those in the arms, legs and core. Examples of motor milestones include the first time a baby sits without support, hands and knees crawling, standing with support, standing without support, walking with support and walking without support.

Lean Body Mass: also known as fat free mass (FFM). It is normally defined to be the body weight minus the body fat. This includes muscle as well as bones and other nonfat tissue.

Malnutrition: a state when the body does not have enough of the required nutrients (under-nutrition) or has excess of the required nutrients (over-nutrition).

LIST OF ACRONYMS AND ABBREVIATIONS

AA:	Arachidonic acid
ALA:	Alpha linolenic acid
ASAL:	Arid and Semi-Arid Areas
ASF:	Animal source foods
CHD:	Coronary heart disease
CHWs:	Community Health Workers
CI:	Confidence Interval
CLA:	Conjugated linoleic acid
CSB+:	Corn Soy Blend Plus
DANIDA:	Danish International Development Agency
DHA:	Docosahexaenoic acid
DRC:	Democratic Republic of Congo
DPA:	Docosapentaenoic acid
EFA:	Essential Fatty Acids
FAO:	Food and Agriculture Organization
FFM:	Fat free mass
FFMI:	Fat free mass index
FM:	Fat Mass
FMI:	Fat mass index
FTIR:	Fourier transformed infrared spectrometry
GoK:	Government of Kenya
GSIYCF:	Global Strategy for Infant and Young Children Feeding
HFA:	Height-for-age
HiNi:	High impact nutrition interventions

HIV/AIDS: Human Immuno-deficiency Virus/ Acquired Immuno-deficiency Syndrome

IAEA: International Atomic Energy Agency

IRMS: Isotope ratio mass spectrometry

ISRCTN: International Standard Randomized Controlled Trials

IYCF: Infant and Young Child Feeding

JKUAT: Jomo Kenyatta University of Agriculture and Technology

KDHS: Kenya Demographics and Health Survey

KEBS: Kenya Bureau of Standards

KEMRI: Kenya Medical Research Institute

KIRDI: Kenya Industrial Research and Development Institute

KNH-UONERC: Kenyatta National Hospital-University of Nairobi Ethics and Research Committee

LA: Linolenic acid

LCPUFA: Long chain polyunsaturated fatty acids

LDL: Low density lipoproteins

MAM: Moderate Acute Malnutrition

MCH: Maternal and Child health centers

MCTC: Mother-to-child (transmission of HIV)

MGRS: Multicenter Growth Reference Study

MOH: Ministry of Health (Kenya)

MUAC: Mid Upper Arm Circumference

MUFA: Monounsaturated fatty acids

NCD: Non communicable diseases

NCHS: National Center for Health Statistics

PAHO: Pan American Health Organization

PUFA:	Polyunsaturated fatty acids
RNI:	Recommended Nutrient Intake
SAM:	Severe Acute Malnutrition
SCN:	United Nations Standing Committee on Nutrition
SD:	Standard Deviation
SFA:	Saturated fatty acids
SOP:	Standard Operating Procedure
TBW:	Total Body Water
TC:	Total cholesterol
UNICEF:	United Nations Children's Fund
UNITID:	University of Nairobi Institute of Tropical and Infectious Diseases
UNSCN:	United Nations Standing Committee on Nutrition
UNSUN:	United Nations Scaling Up Nutrition
WFA:	Weight-for-age
WFC:	WinFood Classic
WFH:	Weight-for-height
WFL:	WinFood Lite
WHO:	World Health Organization
WHZ:	Weight-for-height z score
WLZ:	Weight-for-length z score

ABSTRACT

Background: Kenya, like many other resource poor countries suffers from high prevalence of undernutrition as depicted by high stunting (35%), wasting (7%) and underweight (16%) among children under 5 years of age. Malnutrition is associated with retarded growth, psychomotor development and limited work capacity and achievement in later life. Undernutrition in early infancy may be addressed via sound nutrition practices including optimum breastfeeding and complementary foods. Specifically, complementary foods with animal source foodstuffs and vitamin and mineral fortificants may positively influence growth and development among infants.

Objective: To assess the efficacy on lean mass accrual, essential fatty acids profile and gross motor development among Kenyan infants receiving a daily portion of either two versions of locally produced complementary foods based on maize and germinated amaranth grains with (Winfood Classic [WC]) or without (Winfood Lite [WL]) animal source foods namely edible termites and small fish compared to a standard food aid product Corn-Soy-Blend plus [CSB+]).

Study design and participants: This was a randomised double-blinded trial in which a total of 499 infants were individually randomized to receive one of three food products (WFC, WFL and CSB+) for nine months from 6-15 months of age.

Setting: A rural resource poor setting in Mumias District in Kenya characterized by high dependency on sugarcane production for cash with minimal food crop production.

Methodology: Primary outcome was increment in fat-free body mass (FFM). Secondary outcomes were whole blood Essential Fatty Acids (EFA) status and attainment of gross motor milestones. FFM was measured by deuterium-dose- to-the-infant technique in which each infant received 3g deuterium oxide upon collection of 2ml pre-dose saliva sample. Two further postdose saliva samples were collected at 2-hour and 3-hours. Deuterium oxide enrichment in saliva was determined by Fourier Transform Infrared Spectrophotometry and fat free mass derived from total body water. Whole blood EFA was measured by fast gas chromatography. Gross motor milestones were determined based on windows of achievement by World Health Organization (WHO) Multicentre Growth Reference Study (MGRS) standards.

Results: There were no significant differences in FFM increment in WFC and WFL compared to CSB+ (WFC +0.14 (95% CI -0.19 to 0.46) and WFL +0.19 (95% CI -0.13 to 0.51). No significant differences were observed among the three foods in essential fatty acid profile. However, the infants receiving WFC had significantly greater concentration of arachidonic acid than infants receiving CSB+ group (p=0.004). There were no significiant differences among the three groups in gross motor milestone attainment. Secondary analysis showed that WFC group attained standing without assistance (p= 0.02) and walking without assistance (p= 0.004) earlier than the WFL group.

Conclusions: No significant differences in impact on lean mass accrual, essential fatty aacids profile and gross motor miletone attainment were observed from feeding locally produced complementary foods with or without animal source foods (termites and small fish) compared to a standard plant-based product. Since the WFC and WFL have similar health outcomes to CSB+, they may be utilized as improved complementary foods in populations where they are culturally aceptable.

CHAPTER ONE

INTRODUCTION

1.1 Background

The importance of nutrition as a foundation for healthy development is underestimated. Poor nutrition leads to ill health, and ill health causes further deterioration in nutritional status. These effects are most dramatically observed in infants and young children, who bear the brunt of the onset of malnutrition and suffer the highest risk of disability and death associated with it (WHO, 2003).

The central role of nutrition to development is emphasized by the growing international awareness that the magnitude of malnutrition as a global health problem will prevent many countries from achieving the United Nations Millennium Development Goals (UN, 2010). The urgent need for effective nutritional interventions is clearly indicated by the current global situation where, on the one hand, 170 million children are underweight and under-nutrition is an important factor in more than half of all child deaths worldwide and, on the other hand, more than a billion adults are overweight. "The double burden of malnutrition", i.e., overlapping under- and over-nutrition, results in a very heavy burden on health systems in countries where treatment of diet-related non-communicable diseases will be increasingly needed at the same time as under-nutrition is still prevalent (UNSCN, 2004).

Children under the age of five years are most vulnerable to malnutrition. The World Health Organization (WHO) Infant and Young Child Feeding (IYCF) strategy 2003 recommends that all infants be exclusively breastfed for the first 6 months of age, then introduced to nutritionally complete complementary foods with continued breastfeeding until at least 2

years and beyond (WHO 2003). In addition, the WHO recommends provision of safe and appropriate complementary foods processed from indigenous nutrient rich foodstuffs (WHO 2008).

A large body of anthropometric data, accumulated over the past several decades, shows the existence of significant constraints to normal growth that have affected millions of children, especially in the developing countries (UNICEF, 2010). Anthropometric data also provide evidence of when children in different populations are most likely to be affected by these constraints. Regardless of how protracted or short the period of risk is, in virtually all populations in which growth is constrained the period of greatest vulnerability is the second semester of life (6 to 12 months of age) and well into the second year (until 18 months of age and often longer) (UNICEF, 2010). It begins when maternal milk is no longer adequate to supply all of the child's needs and continues until he or she is able to meet the nutritional requirements through consumption of the usual family diet and requires no special assistance with eating (UNICEF, 2010).

In developed countries and countries in economic transition, an epidemic of overweight and obesity is emerging (UNSUN, 2012). This is a major public health challenge affecting people of all ages and backgrounds. Overweight and obesity often start early in childhood. Globally, about 43 million children under the age of five years are overweight, according to the World Health Organization's (WHO) 2011 figures. As a consequence, rates of diabetes, cardiovascular disease and other diet-related non-communicable diseases (NCDs) are escalating worldwide. The costs of NCDs are increasing the burden on already overstretched health systems and government and family budgets.

1.2 Malnutrition in Kenya

Malnutrition is an important public health issue particularly for children under five years old who have a significantly higher risk of mortality and morbidity than well-nourished children (MOH/UNICEF, 2011). In Kenya, the infant and the under-five mortality rates are 77 and 115 per 1000 live births, respectively. The national figure for acute malnutrition of children under five years old is estimated at 6%, however there are huge variations in different regions of the country (MOH/UNICEF, 2009). In the Arid and Semi-Arid Areas (ASAL) where food insecurity and natural disasters have affected the population, rates of acute malnutrition are 15-20% of children under five, and sometimes substantially higher (MOH/UNICEF, 2011).

In Kenya, HIV and AIDS and malnutrition are intrinsically linked. Although the prevalence of HIV in the general population reduced from 13.5% in 1999 to 5.9% in 2006, the prevalence among pregnant women is approximately 7.8%, resulting in an estimated 90,000 children at risk of mother-to-child (MCTC) transmission of HIV; it is estimated that between 33,500 and 65,500 children in Kenya are becoming infected with HIV from their parents each year, reversing the gains in child survival in the country over the past two decades (MOH/UNICEF, 2011).

The Ministry of Health (MOH) in partnership with international and national non-governmental organizations (NGOs) has addressed the high rates of acute malnutrition in the ASAL areas. The cyclical nature of events in the ASAL calls for a systematic approach to build government health system capacity to address malnutrition in the long term. The limited capacity of the Government of Kenya (GoK) health staff to manage the growing burden of malnutrition is a challenge. The strengthening of communities for an integrated approach to malnutrition, especially to maintain sustainability and increase the access to services, is vital in mitigation of malnutrition in Kenya (MOH, 2009).

Kenya's current development blueprint, officially known as Kenya Vision 2030, aims to promote preventive health care (as opposed to curative intervention) and promote healthy lifestyles among individual citizens (GoK, 2007). Kenya's High Impact Nutrition Interventions (HiNi) include: promotion of exclusive breast feeding for the first six months of life; promotion of optimal complementary feeding for infants after the age of six months; Vitamin A supplementation (2 doses per year for children 6-59 months); zinc supplementation for diarrhea management; multiple-micronutrients for children under five years; de-worming for children (2 doses per year for children 12-59 months); iron-folic acid supplementation for pregnant mothers; prevention or treatment of Severe Acute Malnutrition (SAM) and Moderate Acute Malnutrition (MAM); promotion of improved hygiene practices including hand washing; salt iodization; and iron fortification of staple foods (MOH/UNICEF, 2011).

1.3 Complementary feeding

Complementary feeding refers to the introduction of solid foods to an infant's diet alongside breastfeeding from the age of 6 months when breast milk alone is not sufficient to meet all nutrient requirements for the infant. The age range for complementary feeding is generally 6-24 months. Breastfeeding is recommended until 24 months and beyond (WHO/UNICEF, 2002).

Infants are especially vulnerable to malnutrition and infection during the complementary feeding period. Nutritional needs for growth and development between 6-24 months of age are greater per kilogram of body weight than at any other time of life (WHO/UNICEF, 2002). Good nutrition is essential at this time to ensure healthy brain and body development. Insufficient nutrient intake and illness resulting from low quality food and poor feeding practices are main causes of malnutrition. Consequently, growth faltering occurs in the first

two years of life, especially in developing countries. If not addressed, malnutrition and infection can lead to death or long term irreversible consequences on future learning ability, economic productivity, immune response, and reproductive outcomes (WHO/UNICEF, 2002).

On the other hand, the period of complementary feeding is a crucial time of opportunity. Nutrition interventions during this stage can lead to great benefits. Feeding practices appropriate for the child's age, nutritionally adequate foods and continued breastfeeding can ensure optimal growth and development. Approximately 6 percent of deaths of children under five years old could be prevented through improvements in complementary foods and feeding practices (WHO/UNICEF, 2002).

Improving nutrition in this age group requires a combination of strategies. The WHO recommends the following actions to improve Infant and Young Child Feeding (IYCF) efforts:

• Protection, promotion, and support of breastfeeding. Breastfeeding remains an important source of energy, fat and essential fatty acids, protein, protective factors, and some essential vitamins and minerals for older infants and young children. Nutrients from breastmilk are optimally absorbed.

• Provision of timely and targeted counseling on feeding practices. Optimal complementary feeding depends not only on what is fed, but also on how, when, where, and by whom the child is fed. Assessments should be conducted to identify nutritional gaps in local diets and current good practices. They should be followed by trials of options for improving the diet and feeding practices, identification of priority audiences, and effective strategies for reaching these audiences. One key strategy is skilled, timely, and targeted counseling on recommended feeding practices.

• Enhancing access to nutritionally adequate complementary foods and/or fortified products to enrich home-prepared foods. Education alone to improve the use of available foods can be highly effective in some settings, but fortified foods and food assistance may be needed to prevent and treat stunting where food insecurity is a major constraint. Opportunities to address longstanding barriers to improved nutrition include "quick-to-prepare" fortified porridges, lower-priced lipid-based nutrient supplements such as fortified peanut-based spreads, and micronutrient powders that can be added to home-cooked complementary foods.

• Prevention and treatment of common early childhood illnesses. Reducing the frequency and duration of illness and promoting increased food intake after illness is an important strategy for improving growth.

1.4 Traditional complementary foods

Traditional complementary foods are often bulky, have a low energy density and contain insufficient amounts of micronutrients, in particular iron and zinc (Dewey, 2005). This is because complementary foods are largely cereal based and contain considerable amounts of phytate, which negatively affects micronutrient bioavailability, thereby inducing deficiencies in minerals. They also have a risk of contamination and often contain too much water with little solids (WHO, 1998; Owino *et al* 2007). Low infant feeding frequency further contributes to the undernutrition of children with studies carried out in Tanzania (UNICEF, 1998; Mamiro *et al*, 2004) showing that most children are fed only 2 or 3 times a day. Poor maternal nutrition, inappropriate breastfeeding and complementary feeding practices thus contribute to child malnutrition (Dewey, 2005; Owino *et al*, 2007). According to Faber *et al* (2005), exclusive breastfeeding was the predominant method of child feeding for the first 6 weeks of life in South Africa after which mixed feeding was predominantly practiced through to 6 months. In Latin America only 12% of infants were exclusively breastfed at 1 month of

age while in Kenya less than 32% of children are exclusively breastfed during the first 6 months of their life with the introduction of fluids other than breast milk before the age of 6 months doubling the prevalence of diarrhea (UNICEF, 2009).

To ensure higher nutrient density which is often a common problem in complementary foods, different processing technologies either singly or in combination have been proposed among other means. According to Owino *et al*, (2007), naturally occurring toxins and nutrient inhibitors occur in complementary foods which are mostly made from cereals, legumes and starchy roots and tubers with inherent anti-nutrients that adversely affect the availability of nutrients from foods. Processing must thus take into consideration such issues and contaminations from secondary metabolites of fungi like aflatoxins. The processing technologies used in complementary foods production include: heat processing; germinating (malting) and fermentation; soaking/steeping; use of enzymes; and extrusion cooking.

The current study had an aim of alleviating childhood malnutrition by improved utilisation of traditional foods, with the overall objective of contributing to the development of guidelines for utilisation of local foods for improving child nutrition. Maize and grain amaranth have been used as food items in the Kenyan diet along with a diverse selection of other indigenous animal foods such as small fish *(omena)* and edible insects.

The use of edible insects as human food goes back to the dawn of mankind. The Bible talks about certain insects as kosher foods in Leviticus 11, 1-32. Bodenheumer (1951) is credited with first presentation of a global review of edible insects as rich in essential food nutrients necessary for human and livestock feeding. Several authors have discussed potential of insects as minilivestock with the FAO conference held in Chiang Mai Thailand in 2008 unanimously recommending popularization of edible insects as an option for increasing sources of foods in the world (FAO, 2010). Consumption of edible insects is, thus, not a new

idea. It is how the insects are served that is new in food science. Scientists now have the challenge to find means and ways of popularizing the use of the abundant edible insects, thus part of the focus of work.

In some communities in Kenya, entomophagy-the art of eating insects- has been common and widespread depending on the availability of the insects and the ability to trap them when out of season. Other commonly consumed insects include the grasshoppers *tsenetsene* among the Luo and Luhya of Western part of Kenya, the fat bottomed female insect locally referred to as *Onyoso* among the Luo, crickets among the Western Kenya tribes of Luo and Luhya, and the lake flies (*sam or samo*) among the Luo of Western Kenya. It is for this reason that termites based on acceptability, relativly wide availability and high nutritional value was included in the intervention food formulation and aimed for use in the Western Kenya region.

1.4.1 Impact of complementary feeding on lean mass

Dewey and Adu-Afarwuah have suggested that growth might not be the most sensitive indicator of benefits of complementary feeding, because of several constraints which limit the extent to which a child's length responds to complementary feeding interventions (Dewey and Adu-Afarwuah, 2008). They suggest that it would be useful to include other outcome measurements such as behavior development, morbidity and micronutrient status when evaluating interventions. This thesis focuses on measurement of healthy growth. An exploration was performed, regarding which outcome measures would be best to determine healthy growth while at the same time applying to the contextual challenges seen in the WinFood trial in Cambodia and in Kenya.

This trial had increment in FFM as the primary outcome. The Winfood Cambodia trial was the first larger trial on complementary feeding which used such an outcome measure (Skau JKH, 2014). It was pre-assumed that the two component model would be a sensitive

measure, capable of detecting differences in FFM between the food groups. The FFM is composed of total body water, muscle tissue, bone mass and other minerals. Loss in lean mass is considered an important parameter of body wasting in starvation and a valuable indicator in disease progression and risk of death. A positive lean mass change is associated with overall health and wellbeing and functional outcomes such as timely attainment of gross motor milestones.

The main objective of the study was to assess the impact of improved complementary foods namely, 1) maize-amaranth-termite-fish complementary food naturally enriched with iron and zinc (Winfood Classic), 2) multi-micronutrient fortified maize-amaranth complementary food (Winfood Lite) and 3) multi-micronutrient fortified corn soy blend plus (CSB+) on lean body mass after 9 months of supplementation of infants from 6-15 months of age. Additionally, the study aimed to assess impact of the above mentioned improved complementary foods on essential fatty acids and gross motor development among infants

1.5 Justification

The Kenya Demographic and Health Survey (KDHS) for 2008-09 indicated that 7% of children under 5 years are wasted and 16% are underweight. While the prevalence rates for wasting and underweight have declined over the past three decades, the stunting rate has increased to an astounding 35%. Major micronutrient deficiencies include iodine deficiency disorder, iron deficiency anemia, and vitamin A and zinc deficiency, with high rates among young children. Approximately 25% of children are iodine deficient, although rates of goitre have reduced in the last decade by 10% due to the near universal household consumption of iodized salt. Nearly three quarters of u5s experience iron-deficiency anemia and another 85% are vitamin A deficient. Over half of pregnant and lactating women and, almost a fifth of men experience significant micronutrient deficiencies.

Apart from widespread undernutrition, Kenya is currently experiencing a rise in diet-related non-communicable diseases (NCDs) such as overweight and obesity, diabetes, cancers, kidney and liver complications attributable to consumption of foods low in fibre and high in fats and sugars. NCDs are responsible for additional 40% and 50% mortality and hospital burden in Kenya, respectively. Currently, diabetes prevalence is 4.2%; hypertension 12.7% while cancer affects 28000 people annually. Nearly a fifth of pre-school children overweight while 4% are obese. Highly endemic diseases such as malaria, HIV and tuberculosis further worsen the impact of malnutrition.

If uncontrolled through interventions including education for behavior change and therapies to treat the maladies, malnutrition is likely to lead to increased loss of productivity and lives in Kenya and globally.

1.6 Rationale

The period from conception to 2 years of age – the first thousand days of a child's life – represents a critical window of opportunity for avoiding health risks later in life (UNSUN, 2011). The assessment of growth during this crucial period of early vulnerability has traditionally been largely based on anthropometric measurements such as body weight and length, with less attention to the quality of growth and the relative partitioning of nutrients to fat-free mass or fat mass. Currently, the amount and distribution of body fat and the amount and composition of lean mass are understood to be very important for the long term health prospects of infants and children (IAEA, 2014).

Recent longitudinal studies (Moreno & Rodriguez, 2007; Wu & Chen, 2009; Vidailhet, 2010; Fall *et al*, 2011) have demonstrated associations between the incidence and outcome of several metabolic diseases such as obesity, hypertension and cardiovascular disorders to consumption of high calorie, high protein complementary feeds.

On the other hand, micronutrients have been linked to improved gross motor and psychomotor milestones, and better linear growth (Morgan *et al*, 2004; Kariger *et al*, 2005; Adu-Afarwuah *et al*, 2007). However, studies have been focused mainly on the impact of supplemental micronutrients, mainly iron, vitamin A and zinc, rather than micronutrients intrinsically present in the food or those from fortification premix of vitamins and minerals. Several studies in Kenya have been carried out on the effect of animal source foods such as milk and meat on growth, lean body mass and development of school going children (Neumann *et al*, 2003; Murphy *et al*, 2003; Grillenberger *et al*, 2003). There is scarce information on use of edible insects as ingredients for complementary feeding (Bauserman *et al*, 2013). The effect of animal source foods, and multi-micronutrient fortified complementary foods on adiposity and psychomotor development needs further investigation in randomised controlled trials.

1.7 Objectives

1.7.1 The overall objective

The overall objective of the study was to assess the effect of improved complementary diets on accrual of lean body mass (fat free mass), essential fatty acids, and gross motor development of infants 6 to 15 months in Kenya.

1.7.2 Specific objectives

The specific objectives of the study were:

1. To assess the effect of the complementary foods (Winfood CF and Winfood Lite) compared to CSB+ on accrual of lean body mass.

2. To evaluate the effect of the complementary foods on essential fatty acid profile of 6 to 15 months old infants compared to CSB+

3. To determine the effect of the complementary foods on gross motor development milestones of infants 6 to 15 months compared to CSB+

1.8 Hypotheses

Primary hypothesis

- Infants consuming the trial complementary foods blends (Winfood CF and Winfood Lite) have greater lean mass accrual, than those consuming CSB+.

Secondary hypotheses

- Infants consuming the trial complementary foods blends (Winfood CF and Winfood Lite) will have better essential fatty acid profile, that is higher omega 3 and omega 6 levels, compared to infants consuming CSB+

- Infants consuming the trial complementary foods blends (Winfood CF and Winfood Lite) will have higher gross motor development indicators compared to infants consuming CSB+

1.9 Limitations of the study

There was no ideal control group in this study since it would be unethical to deny infants foods that are perceived to have nutritional and health benefits. The trial foods were compared against CSB+. Data on breastfeeding was self-reported. Compliance was primarily self-reported and home visits done for a sub sample of participants.

1.10 Significance of the study

The present study is an intervention trial where the impact on lean mass accrual, essential fatty acids and gross motor milestones of the developed local complementary food was assessed. The study aimed to include more sensitive outcome measures on growth in combination with traditional anthropometric measures.

Better understanding of FFM and FM in the early age and how body composition impacts the onset of healthy growth in infants and young children in low income settings can contribute to shaping of nutrition interventions in the future. Linking these findings from this thesis, to the consequences in adult life and risk of overweight and obesity, and related non-communicable diseases, can assist policy-makers in the challenging task of preventing the emergence of a double-burden of malnutrition in Kenya, and other food insecure settings facing a future of nutrition transition with unknown health consequences.

1.11 Implementation vision, expected benefits and potential uses of the study results

The vision of the study is to generate a simple, versatile and easy-to use tool specifically adapted for the situation in Kenya, for the development of 'Winfoods'. The WinFood CF and WinFood Lite developed for this study are to be viewed as models for how complementary foods can be made from local foods. Future 'Winfoods' are envisaged to be a range of energy-dense, high-nutrient complementary foods, whose formulation can be adapted to population and/or program and/or production requirements, and which make optimal use of locally available foods. Winfood-products can be produced on a local level or by (small) enterprises. Potential uses can be in (national) nutrition programs aimed at food-insecure populations, for production by NGO's working in the mother-child health field and/or income-generating projects, programs targeting especially vulnerable population groups and also commercial production by larger local food companies. The composition of WinFoods can also be

adapted in recipes for homemade improved complementary foods. The study will assess the effects of different complementary foods on health and growth based on the most recent nutritional knowledge as to what constitutes optimal growth and beneficial health effects, thereby assessing the quality of the complementary foods in the most relevant way possible by using end-point functional outcomes, to ensure short- and long term benefits for Kenyan children. The study results will contribute to the effective implementation of the guidelines by providing the scientific documentation for the nutritional quality of a complementary food based on local indigenous Kenyan food stuffs.At a policy level, these results from the Winfood project can act as good evidence-based guidance for policy-makers to develop guidelines which can improve and strengthen the national policies on nutrition in Kenya in the future.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of malnutrition

Malnutrition is defined as ‘a state when the body does not have enough of the required nutrients (under-nutrition) or has excess of the required nutrients (over-nutrition) (UNICEF, 2010). The components of nutrition are macronutrients and micronutrients. Macronutrients consist of protein, carbohydrates and fats that make up the bulk of a diet and supply the body’s building blocks and energy (UNICEF, 2010).

In resource poor populations, carbohydrates are often a large part of the diet (80%) and the main source of energy. Fats, also an important component in the diet, in resource poor populations make up about 10% of the diet. Proteins are required to build new tissue and are derived from both plant and animal sources. Proteins from animal sources contain essential amino acids that cannot be produced by the body but must be eaten in the diet. Proteins from cereals and pulses alone do not provide sufficient essential amino acids in the right proportions to meet physiological requirements. In order to obtain the correct balance of essential amino acids from plant sources, cereals and pulses must be correctly combined when planning a meal (UNICEF, 2010).

There are two categories of under-nutrition: acute under-nutrition and chronic under-nutrition. Children can have a combination of both acute and chronic under-nutrition. Acute under-nutrition is categorized into Moderate Acute Malnutrition (MAM) and Severe Acute Malnutrition (SAM), determined by the patient’s degree of wasting. All cases of bi-lateral oedema are categorized as SAM (UNICEF, 2010).

Chronic under-nutrition is determined by a patient's degree of stunting, that is, when a child less than his or her height for a particular age. To treat a patient with chronic under-nutrition requires a long term focus that considers household food insecurity in the long run; home care practices in regards to feeding and hygiene practices; and issues related to public health (WHO, 2010).

Malnutrition (under-nutrition), clearly, is not a simple problem with a single, simple solution. Multiple and interrelated determinants are involved in why malnutrition develops, and a similarly intricate series of approaches, multifaceted and multisectoral, are needed to deal with it(UNICEF, 1998).

The conceptual framework on the causes of malnutrition was developed in 1990 as part of the UNICEF nutrition strategy. The framework shows that causes of malnutrition are multi-sectorial, embracing food, health and caring practices. They are also classified as immediate, underlying, and basic, whereby factors at one level influence other levels. The framework is used, at national, district and local levels, to help plan effective actions to improve nutrition. It serves as a guide in assessing and analysing the causes of the nutrition problem and helps in identifying the most appropriate mixture of actions (UNICEF, 1998). Figure 2.1 shows the conceptual framework of the causes of malnutrition.

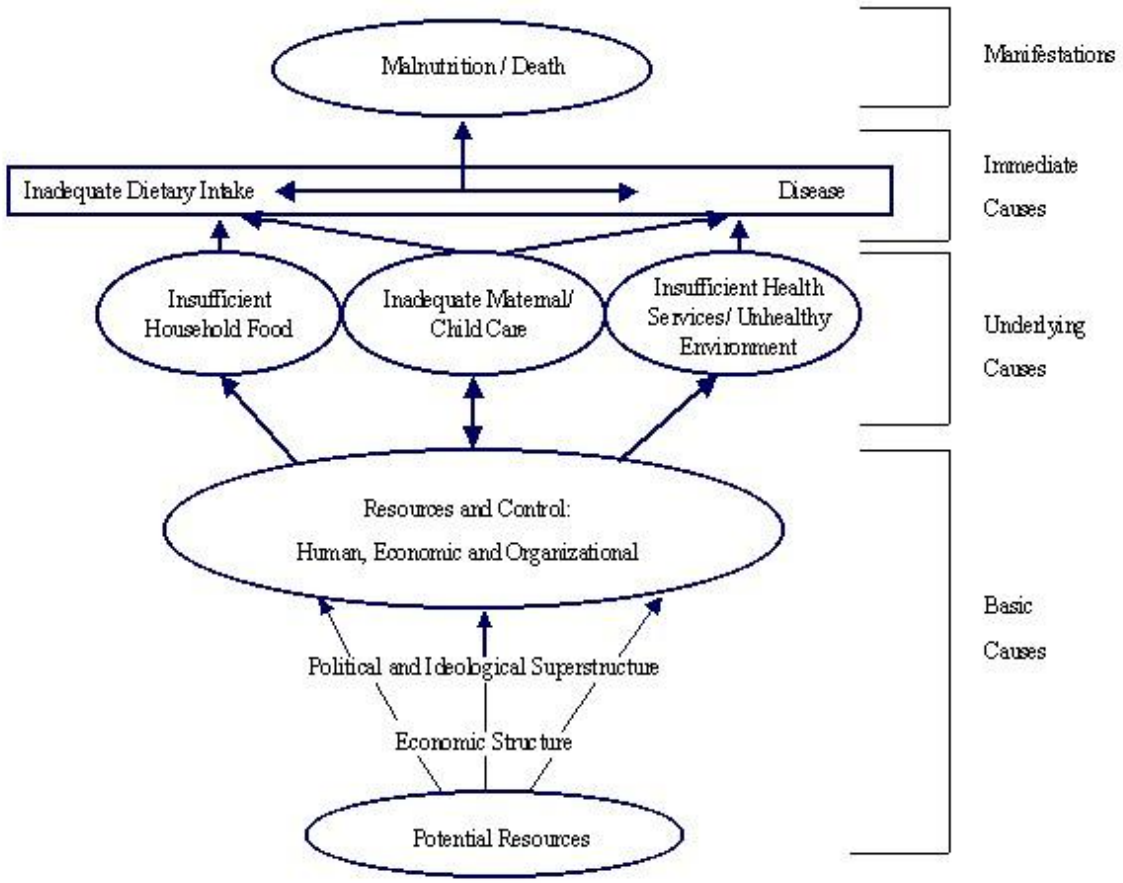


Figure 2.1: The UNICEF conceptual framework for the causes of malnutrition

Inadequate food intake and disease are immediate causes of under-nutrition and create a vicious cycle in which disease and malnutrition exacerbate each other. It is known as the Malnutrition Infection Complex (UNICEF, 1998). Thus, inadequate food intake and disease must both be addressed to support recovery from malnutrition.

There are three underlying causes of malnutrition: Inadequate household food security (limited access or availability of food); limited access to health services and/or inadequate environmental health conditions; and inadequate social and care environment in the household and local community, especially with regard to women and children.

The basic causes of under-nutrition in a community originate at the regional and national level, where strategies and policies that affect the allocation of resources (human, economic, political and cultural) influence what happens at community level. Geographical isolation and lack of access to markets due to poor infrastructure have a significant negative impact on food security.

2.2 Nutritional assessment

To determine the nutrition status of an individual, anthropometric measurements (such as their weight, height), age and presence of oedema is recorded. The measurements are compared to international reference standards. The nutrition standards are designed in respect to three indices: height-for-age (HFA), weight-for-height (WFH), and weight-for-age (WFA). To assess malnutrition by WFH, WFA and HFA, individual measurements are compared to an international reference standard. At present that standard is derived from surveys undertaken in the United States (WHO Child Growth Standard, 2006). The reference should not be considered 'ideal'; they are simply used as a standard to compare nutritional status in different regions and in populations over time. Anthropometric indices are usually expressed in two ways: as a percentage of the median value of the reference standard, or as z-scores derived from the reference standard. See Table 2.1 for anthropometric case definitions.

Table 2.1: Anthropometric criteria to identify severe, moderate and at risk categories of acute malnutrition children 0-5 years

Indicator	**Severe Acute Malnutrition (SAM)**	**Moderate Acute Malnutrition (MAM)**	**At Risk of Malnutrition**
Infants less than 6 months			
Weight-for-Age z-score (WAZ)	WAZ < -3 Z score	Static weight or losing weight at home	Static weight or losing weight at home
Oedema	Oedema present	Oedema absent	Oedema absent
Other signs	Too weak to suckle or feed	Poor feeding	Poor feeding
Children 6 months to 5 years			
Weight for Height z-score (WHZ)	WHZ < -3 z-score	Between -3 to < -2 z-score	Between -2 to < -1 z-score
Height-for-Age (HAZ)	HAZ <-3 z-score	Between -3 to <-2 z-score	Between -2 to <-1 z-score
Mid-Upper-Arm Circumference (MUAC)	< 11.5 cm	11.5cm to 12.4 cm	12.5cm to 13.4cm
Oedema	Oedema present	Oedema absent	Oedema absent

Mid-Upper-Arm Circumference (MUAC) is often the screening tool used to determine malnutrition for children in the community under five years old. A very low MUAC (< 11.5cm for children under five years) is considered a high mortality risk and is a criterion for admission with SAM (WHO, 2006).

Other nutritional assessments include, biochemical, clinical and dietary assessments. Biochemical assessments involve sampling of body fluids and secretions; such as blood, urine and saliva; and proceeding to analyze them in the laboratory for particular nutrients or biomarkers. Clinical assessments involve a trained medical practitioner examining the patient for outward manifestations of malnutrition, such as weight and muscle loss, obesity, presence of bilateral oedema, paleness of fingernails and inner eyelids. In addition developmental milestone attainment is assessed. Dietary assessments comprise establishing diet history to determine breastfeeding status, food adequacy, frequency and diversity as well as taking of food samples for laboratory analysis to determine nutrient composition.

Children who are malnourished are at high risk of mortality and morbidity (UNICEF, 2010). In Kenya, the Ministry of Health (MOH) programmes such as Integrated Management of Childhood Illnesses (IMCI) and Child Health (MCH) focus on children under five years old, and screening for malnutrition is part of the programme progress. When malnutrition screening is available in the community, Community Health Workers (CHWS) identify children who are malnourished with anthropometric measurements (such as MUAC) or evidence of oedema. Malnourished children are referred to the nearest health facility, nutrition unit, health post, or hospital out-patient department (MOH, 2009).

2.3 Complementary feeding

Complementary feeding is defined as the process starting when breast milk alone is no longer sufficient to meet the nutritional requirements of infants, and therefore other foods and liquids are needed, along with breast milk. On the other hand, appropriate diets for children who are not breastfed (such as those of HIV-positive mothers who choose not to breastfeed), are often referred to as "replacement feeding" (WHO, 2001).

Because of the rapid rate of growth and development during the first two years of life, nutrient needs per unit body weight of infants and young children are very high. Breast milk can make a substantial contribution to the total nutrient intake of children between 6 and 24 months of age, particularly for protein and many of the vitamins.

However, breast milk is relatively low in several minerals such as iron and zinc, even after accounting for bioavailability. At 9-11 months of age, for example, the proportion of the Recommended Nutrient Intake (RNI) that needs to be supplied by complementary foods is 97% for iron, 86% for zinc, 81% for phosphorus, 76% for magnesium, 73% for sodium and 72% for calcium (Dewey, 2000). Given the relatively small amounts of complementary foods that are consumed at 6-24 months, especially in resource poor settings, the nutrient density (amount of each nutrient per 100 kcal of food) of complementary foods needs to be very high (WHO, 2001).

The Global Strategy for Infant and Young Child Feeding (IYCF) was endorsed by WHO Member States and the UNICEF Executive Board in 2002. The Strategy is the framework through which WHO prioritizes research and development work in the area of IYCF, and provides technical support to countries to facilitate implementation. The strategy was founded on two decades of international public health consensus and action including the initiatives mentioned above.

The strategy places emphasis on the use of a comprehensive approach to breastfeeding promotion, and gives heightened attention to breastfeeding and complementary feeding in difficult circumstances such as HIV-prevalent areas. The strategy also emphasises the use of community based interventions to promote and support optimal IYCF.

In addition, it places emphasis on infants receiving nutritionally adequate and safe complementary foods while continuing to breastfeed for up to two years or more. This is particularly important during illness as infants are often willing to consume breast milk when they are reluctant to take water, so that dehydration may be prevented. Furthermore, besides nutrition, breastfeeding continues to provide protection to the child against many illnesses, and provides closeness, comfort, and contact that may help development.

The strategy is based on evidence that at around 6 months of age, an infant's nutritional requirements begin to exceed what can be met with breast milk alone. At this stage, age-appropriate, adequate and safe complementary foods are recommended to meet the infant's needs. Even with optimal breastfeeding practices, an infant's growth may falter if adequate and safe complementary foods are not received after 6 months of age.

Guidance on IYCF emphasizes the importance of ensuring that complementary foods are timely, adequate, safe and properly fed.

Timely: Foods are introduced when the need for energy and nutrients exceeds what can be provided through exclusive and frequent breastfeeding.

Adequate: Foods provide sufficient energy, protein, and micronutrients to meet a growing child's nutritional needs.

Safe: Foods are hygienically prepared and stored, and are fed with clean hands using clean utensils and not bottles and teats.

Properly fed: Foods are given consistent with a child's signals of appetite and satiety, and meal frequency and feeding method – actively encouraging the child to consume sufficient food using fingers, spoon or self-feeding and are suitable for age

Recent country surveys show that complementary feeding practices are far from ideal (UNICEF, 2011). While timely introduction of complementary foods (at 6 - 8 months) is a common practice in many countries, the quality of the available foods is often poor and knowledge of safe feeding practices can be limited. These outcomes strongly support the need for support for complementary feeding practices (see Figure 2.2).

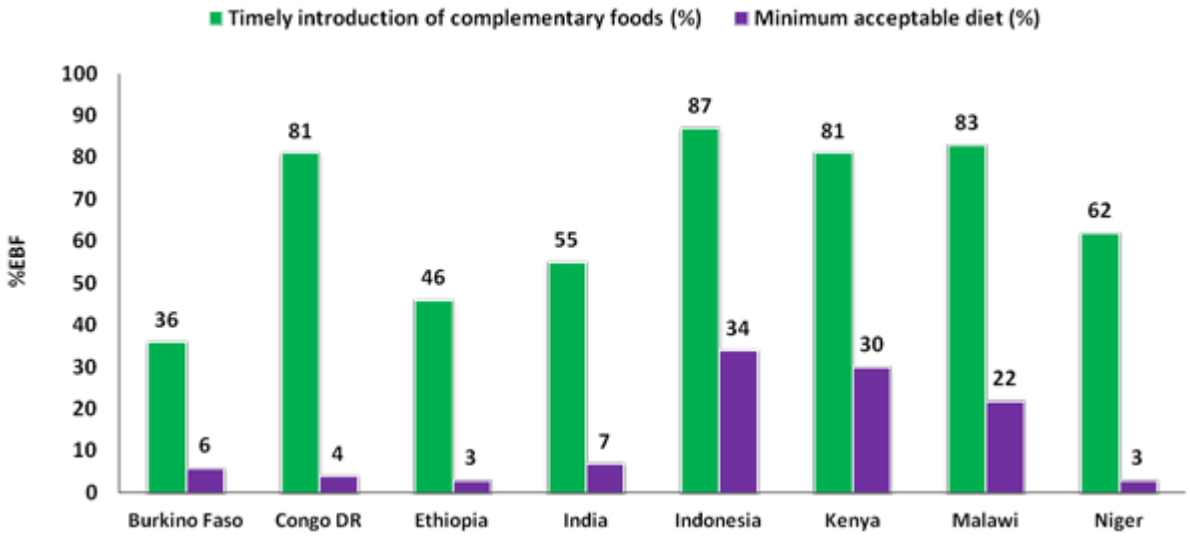


Figure 2.2: Status of complementary feeding in selected countries with data on 'timely introduction of complementary foods (6 - 8 months old), breastfed and non-breastfed children' and "minimum acceptable diet" (breastfed children 6 - 23 months). Data is from Demographic and Health Surveys, most recent survey for each country, from 2002 – 2008

Source: Adapted from UNICEF Childinfo (2011)

Although there is evidence to suggest that breastfeeding and complementary feeding promotion interventions can be effective, implementing these strategies in a variety of contexts may not be straightforward. As a result, WHO and UNICEF organised an informal

consultation in 2008 to develop guidance on complementary feeding programmes for decision makers and policy planners; entitled *Strengthening action to improve feeding of infants and young children 6 - 23 months of age in nutrition and child health programmes.*

The results of this review indicate that there is no single universal 'best' package of components in complementary feeding interventions because the needs of the target population vary greatly. They concluded that, the impact of such interventions is thus context specific, and depends on factors such as the initial prevalence of malnutrition, the degree of household food insecurity, the energy density of traditional complementary foods and the availability of micronutrient-rich local foods.

2.4 Long term effects of complementary feeding

There is growing evidence that complementary feeding has a 'programming' effect on health status of an individual later on in life (Farris 1982; Michaelsen, 2005; Gunther, 2007). Farris *et al* (1982) reported that risk of coronary heart disease (CHD) begins to emerge from childhood (Farris, 1982). Serum lipid levels especially serum cholesterol is a major risk factor for atherosclerotic heart disease. Although total cholesterol (TC) and low-density lipoprotein (LDL) are influenced by adult diet and adiposity, levels of these factors 'track' with increasing strength from early childhood (Farris *et al*, 1982). Docosahexaenoic acid (DHA), an Omega-3 EFA, is crucial from the moment of conception, throughout growth in the womb, in infancy and beyond. It cannot be manufactured by the body therefore must be continously supplied in the diet. A lack of DHA leads to developmental immaturity that can affect one throughout life (Stevens *et al*, 1996; Stordy, 1995).

Nutrition in the neonatal and early infancy may have major, long-term 'programming' effect on physiology and metabolism of cholesterol. Hence the influences of infants' eating patterns, on subsequent cholesterol levels, are of growing interest. It has been seen that

infants on different feeding regimens, have different lipid profiles (Kallio *et al*, 1992). Majority of studies have compared lipid levels in exclusively breastfed babies with formula-fed infants (Friedman and Goldberg, 1975; Wagner and Stock-hausen, 1988; Wong *et al*, 1993). These studies reported similar findings that breastfed infants had significantly higher total cholesterol and low density lipoprotein-cholesterol than formula-fed infants. The differences observed between the absolute values of total cholesterol, high density lipoprotein -cholesterol, low density lipoprotein -cholesterol, very low density lipoprotein -cholesterol and triglycerides in the studies could be owing to many factors, for example methodology, type of sample (venous/capillary), time interval between last feeding, preparation of feed (dilution) and genetic composition (Agostoni *et al*, 2000). These studies were carried out in developed countries; however, infants in resource poor settings are predominantly breastfed with early introduction of complementary foods (Dewey 2000; Harit *et al*, 2007).

Micronutrients have been linked to improved psychomotor milestones. Morgan *et al*, (2004) reported a positive and significant relation between meat intake from 4-12 and 4-16 months to psychomotor developmental indices. Adu-Afarwuah *et al* (2007) carried out randomized comparison of 3 types of micronutrient supplements for home fortification of complementary foods in Ghana, and its effects on growth and motor development. They found all three supplements had positive effects on motor milestone acquisition by 12 months compared with no intervention, but only fat-based Nutributter affected growth. Manno *et al* (2011) assessed the effects on mild morbidity of a richly micronutrient-fortified complementary food given to Zambian infants aged 6-18 months. They found that the richly micronutrient-fortified food was associated with more episodes of lower respiratory infections/pneumonia diagnosed at scheduled visits, compared with the basal diet.

2.5 Review of similar interventions and outcomes

Complementary feeding interventions are usually targeted at the age range of 6–24 months, which is the time of peak incidence of growth faltering, micronutrient deficiencies and infectious illnesses in developing countries. After 2 years of age, it is much more difficult to reverse the effects of malnutrition on stunting, and some of the functional deficits may be permanent (Dewey and Adu-Afarwuah, 2008).

The interventions generally include one or more components related to the Guiding Principles for Complementary Feeding of the Breastfed Child (PAHO/WHO, 2003). The 10 guiding principles cover: (1) duration of exclusive breastfeeding and age of introduction of complementary foods; (2) maintenance of breastfeeding; (3) responsive feeding; (4) safe preparation and storage of complementary foods; (5) amount of complementary food needed; (6) food consistency; (7) meal frequency and energy density; (8) nutrient content of complementary foods; (9) use of vitamin-mineral supplements or fortified products for infant and mother; and (10) feeding during and after illness.

In Dewey and Adu-Afarwuah (2008) review, interventions were grouped into five categories depending on the main strategy used:

(1) education about complementary feeding as the main treatment (2) complementary food or a food product offering extra energy (with or without added micronutrients) provided as the only treatment (3) provision of food combined with some other strategy, usually education for mothers (4) fortification of complementary foods (centrally processed fortified foods or home-fortification products) with micronutrients (with no difference in energy provided to intervention vs. control groups), and (5) increased energy density and/or nutrient bioavailability of complementary foods through the use of simple technologies.

Supplementation with micronutrient fortified or unfortified cereal/legume blends was associated with an increase of 0.52 in the WAZ of Ghanaian infants ($P < 0.001$) from 6 to 12 months of age (Lartey *et al*,1999), and an increase of 3.2 kg in the weight of Nigerian children (Obatolu, 2003) from 4 to 18months of age. Consumption of a fortified spread from 6 to 12 months increased WAZ (by 0.34 Z) among infants in Ghana (Adu-Afarwuah *et al*, 2007), when compared with a non-intervention group. In Malawi, 6–17-month-old underweight (WAZ < -2) children who consumed at least 50 g of a similar spread daily for 3 months gained 230 g more in weight compared with those receiving 0–5 g day-1. In contrast, provision of a complementary food did not affect the weight of children in South Africa (Oelofse *et al*, 2003), Zambia (Owino *et al*, 2007) or Indonesia (Beckett *et al*, 2000). Linear growth was increased in five of the seven studies. In the two studies in Ghana, 6 months of intervention was associated with an increase in LAZ of 0.26–0.69 (Lartey *et al*, 1999; Adu-Afarwuah *et al*, 2007), compared with non-intervention children. In Zambia, children consuming a fortified cereal/legume blend for 3 months grew 0.9 cm more than their counterparts not included in the intervention, and in Malawi, malnourished children consuming fortified fat-based spreads for 3 months gained 0.8 cm more than unsupplemented children. The greatest effect of food supplementation on linear growth was observed in Nigeria (Obatolu 2003), where the height of intervention children receiving large quantities of a cereal/ legume/fish oil blend from 4 to 18 months of age was reportedly 6 cm greater than that of the control group. However, no significant effect on linear growth was found in the studies in South Africa (Oelofse *et al*, 2003) and Indonesia (Beckett *et al*, 2000).

The effect of fortification of complementary foods on growth was tested in six efficacy trials in which there was no difference in the amount of energy provided to intervention and control groups. Three of these studies involved home fortification using micronutrient supplements (Sprinkles™ or crushable tablets), from 6 through 12 (Smuts *et al*, 2005; Adu-Afarwuah *et*

al, 2007) or 18 months (Giovannini *et al*, 2006) of age. The other studies used cereal/legume mixes (Lartey *et al*, 1999; Faber *et al*, 2005) or a milk formulation (Dhingra *et al*, 2004) to which the micronutrients were added during processing. In the latter studies, the control groups received unfortified products (Lartey *et al*, 1999; Dhingra *et al*, 2004; Faber *et al*, 2005).

In the milk-fortification study in India (Dhingra *et al*, 2004), intervention children gained significantly more weight (by 0.21 kg, 95% CI 0.12, 0.31 kg) and had greater WAZ (by 0.24 Z,95% CI 0.11, 0.36 Z) and WLZ (mean difference 0.16 Z,95% CI 0.03, 0.30 Z) at the end of 12 months of supplementation, compared with the control group. In the other five efficacy trials (Lartey *et al*, 1999; Faber *et al*, 2005; Smuts *et al*, 2005; Giovannini *et al*, 2006; Adu-Afarwuah *et al*, 2007), fortification of complementary foods had no significant effect on weight. Similar results were reported for linear growth: only in India (Dhingra *et al*, 2004) was there a significant impact of fortification. In that study, intervention children had greater mean length gain and LAZ at the end of study, compared with the control group.

Of five efficacy trials in five different countries that used strategies to increase energy density as the only intervention, only two had an impact on growth. In Bangladesh (Hossain *et al*, 2005), severely malnourished children (weight-for-age <60% of NCHS median) received a cereal/legume/oil blend containing amylase-rich flour from germinated wheat 6 days week-1 for 1.5 months. In India (John & Gopaldas, 1993), the food given was wheat gruel with industrial amylase that was consumed ad lib once daily for 6 months. Similar types of cereal/legume blends containing industrial amylase were provided to infants in Congo (Moursi *et al*, 2003) for 4.5 months and in Zambia (Owino *et al*, 2007) for 3months, but in Tanzania (Mamiro *et al*, 2004) the increased energy density of the mainly cereal/legume blend was achieved through traditional methods of soaking, germination and roasting. In each

of these studies, the control group received the same type of food but without the added amylase or processing.

In the study by Adu-Afarwuah *et al* (2007) in Ghana, the proportion of children who were able to stand or walk independently by 12 months of age was compared between the four groups (two of which are relevant to this intervention strategy: the group receiving the fortified spread and the nonintervention group). The groups did not differ in the ability to stand independently, but 49% of infants receiving the fortified spread were able to walk independently compared with only 25% in the nonintervention group ($p = 0.01$). In South Africa, where infants received fortified or unfortified cereal from 6 to 12 months of age, the level of mental and motor development was observed by research assistants using an instrument based on the Denver Development Screening Test (Oelofse *et al,* 2003). At 12 months, test scores (calculated by expressing the number of positive observations as a percentage of the maximum possible observations) did not differ significantly in the intervention and control groups. In the Indonesia study (Beckett *et al,* 2000) in which poorly nourished children 12 or 18 months of age received high- or low-energy milk products with micronutrients, the relatively short 18-month-olds who received the high energy diet over 1 year had higher mental test performance (based on the 1969 version of the Bayley Scales of Infant Development), compared with their counterparts who received the low-energy diet (Pollitt *et al,* 2002).

There is limited information on edible insects as complementary foods. In a study by Skau *et al* (2014) in Cambodia, edible spiders were used as an ingredient in an improved complementary food for 419 infants aged 6-15 months. The food sustained growth indicators similar to the standard foods Corn Soy Blend Plus and Corn Soy Blend ++. Bauserman *et al* (2013) carried out a study in The Democratic Republic of Congo (DRC) in which caterpillar

cereal was tested as a potential complementary feeding product for children and young. Nutritional content and acceptability were assessed in a group of 20 mothers and their 8-10 month old infants. A 30-g portion of the caterpillar cereal contained 6.9 g of protein, 6.3 g of fat, and 12.0 g of carbohydrate, and yielded 132 kcal. A 30-g portion also contained 3.8 mg of iron and 3.8 mg of zinc. The caterpillar cereal had positive growth indicators and was acceptable to mothers and infants in the DRC. In both studies in Cambodia and DRC, no serious adverse effects were reported.

2.6 Entomophagy in Kenya

Entomophagy - the practice of eating insects - goes back thousands of years and has been documented in nearly every part of the world (FAO, 2008). According to the Food and Agriculture Organization (FAO), consumption of insects has declined in many societies and is sometimes ridiculed as old-fashioned and unhealthy. Yet, it would be prudent to carefully consider the value of customary knowledge before discarding it too readily (FAO, 2008). Scientific analysis confirms, for example, the exceptional nutritional benefits of many insects, and studies point to the potential to produce insects for food with far fewer negative environmental impacts than for many mainstream foods consumed today (FAO, 2008).

The status of insects in Kenyan diets range from being a preferred and valued food to foods collected for food security in periods of scarcity (Kinyuru *et al*, 2012). Edible insects, consumed in the western region of Kenya may constitute the cheapest sources of macronutrients and micronutrients, especially providing minerals, dietary fiber, protein and polyunsaturated fatty acids that are essential for optimal human growth and development (Orech *et al*, 2007; Ayieko, 2007; Kinyuru *et al*, 2010a; Kinyuru *et al*, 2010b).

According to Owuor *et al* (unpublished), termites, (known as *Ng'wen* in Luo, *Kumbekumbe* in Swahili and *Tsiswa* in Luhya dialects in Kenya), appear to be of greater significance in

diets than most ants (Ayieko *et al*, 2010) in Kenya. However, different species exist and with their seasonality inhibits regular collection throughout the year (Nyeko and Olubayo, 2005; Kinyuru *et al*, 2009). The most popular are the sexual winged forms of the larger species within lowlands. These emerge from holes at the termitaria or moulds after the first rains and during the short rains, late during the last quarter of the year. The abundance of the insects may vary widely from year to year due to over-exploitation of the environment (Ayieko *et al*, 2009).

They are prepared by sun drying, shallow fried in own fat, or simply eaten raw directly from the emergence hole (Christensen *et al*, 2006; Ayieko *et al*, 2010). They are usually consumed as part of a meal or as a complete meal with tapioca, bread, roast corn, or simply eaten as snack food. Some mothers would even grind the dried termites into flour and use it as a sprinkle in baby porridge (Bergeron *et al*, 1988). In some parts of East Africa, termite mounds are considered so important that they are owned by individuals and sometimes form part of inheritance when one dies (Banjo *et al*, 2006).

In addition several fruits are consumed and entomophagy is an active practice (Ayieko *et al*, 2008; 2010). The Luhya principally consume (*tsiswa*) elate termites *Macrotermes* spp. and (*amatete*) locusts *Schistocerca gregaria* and a host of lesser used species like (*risami*) lakeflies *Chaoboridae* spp. and *Chironomidae* spp., (*amafunyi*) scarab beetles Scarabaeidae, (*amaana*) bee larvae *Apis mellifera* and (*tsisiche*) *Ruspolia differens*.

Kinyuru *et al* (2013) carried out proximate, iron, zinc, calcium and fatty acid composition of four species of edible winged termites from Western Kenya. Sun-dried insect samples (*Macrotermes subhylanus, Pseudacanthotermes militaris, Macrotermes bellicosus, Pseudacanthotermes spiniger*) were purchased from local harvesters and distributors in Maseno market in Kisumu West District, Luanda market in Emuhaya District and Kakamega

market in Kakamega District. Sun-dried termites were analysed by species which included four different species (Kinyuru *et al*, 2013).

The termites were analyzed by dry weight basis in order to ascertain their potential in food-based strategies to improve the nutritional health. The termite species contained 44.8 – 47.3 g/100g fat; 33.5-39.3 g/100g protein; 0.7- 8.7g/100g available carbohydrate; 53.3 - 115.9 mg/100g iron and; 7.1-12.8 g/100g zinc. The level of unsaturated fatty acids in edible termites was 50.5 – 66.2%, while n-6:n-3 ratio ranged between 5:1 to 57:1 signifying potential nutritional and public health significance.The termite species were found to contain 44.8 – 47.3 g/100g fat; 33.5-39.3 g/100g protein; 0.7- 8.7g/100g available carbohydrate; iron 53.3 - 115.9 mg/100g and zinc 7.1-12.8 g/100g. The level of unsaturated fatty acids in edible termites was found to be 50.5 – 66.2% while n-6:n-3 ratio ranged between 5:1 to 57:1 signifying potential nutritional and public health significance. The termites may be exploited in provision of high quality diets especially in the developing countries which have been plagued by iron and zinc deficiencies as well as poor supply of dietary polyunsaturated fatty acid sources. (Kinyuru *et al*, 2013).

Aside from their nutritional and environmental benefits, experts see considerable opportunity for edible insects to provide income and jobs for rural people who capture, rear, process, transport and market insects as food. These prospects can be enhanced through promotion and adoption of modern food technology standards to ensure that the insects are safe and attractive for human consumption (FAO, 2008).

The complementary foods in the current study have been prepared using locally available food products,that contain that has the appropriate macro- and micronutrient contents for infant complementary feeding. These cereals are acceptable to both mothers and infants in a rural area of Kenya. Because the ingredients are locally available and the production of this

cereal is simple, this cereal is likely to be a sustainable alternative fortified and animal-source food for complementary feeding.

2.7 Anthropometry – assessment of body composition

Body composition measurements are ideal to study the long-term consequences of early growth and nutrition (Wells, 2012). Firstly, body composition measurements quantify the mass of lean and fat tissue which are important contributors to human capital in terms of their influence on physical work capacity, reproductive function and robusticity (Victoria, 2008). Secondly, body composition measurements are ideal to estimate the risk of chronic disease in later life (Wells, 2012) There is a growing recognition of the need to measure body composition in children (Wells, 2007). Partially due to a rise in the prevalence of childhood obesity in high, middle and low-income countries which increases the demand for determining body fatness (UNSUN, 2011) and partially because measurement of body composition is important for the optimum of clinical care, where measurement of body composition can assist the treatment of growth disorder and other chronic diseases (Wells, 1999; UNSUN, 2013).

2.8 Body composition assessment by stable isotope techniques in nutrition

The most common approach in body composition assessment is to divide body mass into two compartments, fat mass and fat-free mass. The three commonly recognised primary body composition assessment techniques are densitometry, elemental analysis and the measurement of total body water. Densitometry involves the estimation of body density which has conventionally been made by underwater weighing. More recently, air displacement plethysmography has provided a simpler alternative. Both densitometry approaches are laboratory-based and therefore not suitable for use in field settings. Elemental analysis techniques, including total body in vivo neutron activation analysis and total body

potassium analysis, are also limited in terms of wider application. Dual-energy x-ray absorptiometry is a widely used body composition method although not commonly used in field studies. The third primary body composition measurement technique is the assessment of total body water. The technique is based on the assumption that the water content of fat free mass is relatively constant (approximately 73.2% in adults) and that a negligible amount of water is associated with fat in adipose tissue (IAEA, 2009).

Total body water assessment using stable isotope labels is the criterion method of body composition analysis and ideally suited for nutrition applications in field settings (IAEA, 2009). Less exacting techniques, including anthropometry and bioelectrical impedance have been used in large nutrition interventions and population studies with validation against total body water in a representative sample.

Deuterium oxide is a stable isotope of water. Deuterium is naturally present in nature and thus it is not toxic at all. Approximately 0.02% of human body water is deuterium. Several studies of body composition of infants in resource poor settings have been carried out using deuterium. (Arsenault *et al*, 2008; Wells, 2005).

Deuterium oxide ($^{2}H_2O$) and ^{18}O-labelled water ($H_2{}^{18}O$) are both used for body composition assessment, however due to substantial differences in cost, the technique of choice is deuterium oxide dilution. The labelling of water molecules with deuterium enables the measurement of the dynamic character of body water. After consuming a dose of $^{2}H_2O$, the deuterium-labelled water is distributed throughout the body water pool and commonly reaches a steady state concentration in approximately 3-5 hours. The body water pool size, or deuterium dilution space, can be measured based on the concentration of deuterium oxide in body water and the exact dose of deuterium-labelled water consumed. Comparisons are made between pre-dose and post-dose samples of urine (IRMS

only) or saliva (IRMS or FTIR). Table 2.2 shows the standardized doses of deuterium oxide for the estimation of body water in children.

Table 2.2: Standardized doses of $^{2}H_2O$ for the estimation of Total Body Water in children (Source IAEA, 2009)

Body weight (kg)	**Weight of $^{2}H_2O$ required (g)**	**Volume of 1 in 5 dilution (mL)** (deuterium oxide added to ordinary water)
<10	3	15
10-20	6	30
20-30	10	50
30-50	20	Not applicable
>50	30	Not applicable

The IAEA is currently supporting projects on body composition assessment and total energy expenditure to address a wide range of priority areas in nutrition, including childhood obesity, acute severe malnutrition and HIV/AIDS in Latin America, Asia and Africa. Kenya has been a Member State of the IAEA since 1965.

2.9 Importance of body composition assessment

The period from conception to 2 years of age – the first thousand days of a child's life – represents a critical window of opportunity for avoiding health risks later in life (UNSUN, 2011). The assessment of growth during this crucial period of early vulnerability has traditionally been largely based on anthropometric measurements such as body weight and length, with less attention to quality to the quality of growth and the relative partitioning of nutrients to fat-free mass or fat mass. Currently, the amount and distribution of body fat and

the amount and composition of lean mass are understood to be very important for the long term health prospects of infants and children (IAEA, 2014).

Recent longitudinal studies (Moreno & Rodriguez, 2007; Wu & Chen, 2009; Vidailhet, 2010; Fall *et al*, 2011) have demonstrated associations between the incidence and outcome of several metabolic diseases such as obesity, hypertension and cardiovascular disorders to consumption of high calorie, high protein complementary feeds.

Many populations are experiencing profound shifts in nutrition, such that a proportion of individuals are exposed to both undernutrition and overnutrition as they develop, an extreme version of a scenario known as the 'dual burden' (Doak *et al*, 2005). For example, in India, where the prevalence of intrauterine growth retardation is the highest worldwide (Lahariya *et al*, 2010), many children and adults in urban Indian populations are however overweight (Shetty, 2002). Wells argues that research into the causes and consequences of reduced fetal growth is hindered by lack of understanding of the underlying body composition (Wells, 2012).

In populations prone to malnutrition, lean mass is positively associated with health. Greater muscle mass improves physical work capacity, and has been hypothesized to protect against diabetes in developing country populations (Schooling *et al*, 2011). According to Wells, greater adiposity is beneficial up to a point, improving female reproductive function and benefiting immune function in both sexes (Wells, 2009). According to James and colleagues, data from low BMI and underweight adults have greater morbidity and mortality than those of 'normal' weight (James *et al*, 1988).

In early life, poor weight gain is strongly associated with morbidity and mortality (Kramer *et al*, 1987). Low birth weight is the biggest risk factor for early mortality, while in Brazil, catch-up growth during infancy decreased this risk (Victoria *et al*, 2001). Historically,

therefore, there has been much interest in promoting early growth, including maternal supplementation during pregnancy to increase birth weight, and optimizing infant nutrition to increase the rate of infant growth. A recent authoritative review of data from developing countries showed that height at 2 years of age was positively related to a range of outcomes in later life, including adult size, metabolic health and educational attainment (Victoria *et al*, 2008).

Recent studies on infant body composition have focused on the consequences of poor early growth. In epidemiological studies, low birth weight emerged as a strong predictor of the metabolic syndrome, stroke, hypertension, type 2 diabetes and cardiovascular disease (Barker, 1998; Barker *et al*, 2005; Forsen *et al*, 1999). These findings have been broadly replicated in developing country settings (Victoria *et al*, 2008; Stein *et al*, 1996), although some differences have also emerged.

Several studies have been carried out to compare the body compositions of different races (Wells, 2009). In the Indian population, for example, in both early life (Yajnik *et al*, 2003) and later life (Deurenberg *et al*, 2003), the association between body composition and body mass index differs from that in Europeans.

Wells suggests that the first component of growth is 'metabolic capacity', which refers to a variety of aspects of organ structure and function which emerge during the period of hyperplasic growth, dominated by cell division. Individual traits contributing to metabolic capacity include muscle mass, beta cell mass, and nephron number, which collectively confer homeostatic function (Wells, 2009; Wells, 2011). These traits, strongly contingent on fetal and early infant growth, are assumed to be indexed by birth weight in the epidemiological investigations of chronic disease risk, although high birth weight 'macrosomic' babies may also have high adiposity and perturbations of insulin metabolism (Poston, 2011), less

favourable for long-term health. Although some authors have suggested that low birth weight babies are systematically different from normal weight babies, and hence might be characterized by metabolic "defects", the traits specified above appear broadly to scale linearly with birth weight, although with different scaling relationships. The thrifty phenotype may therefore best be represented as a 'continuum of thrift', with infants simply differing across a wide range of investment in metabolic capacity (Wells, 2011).

Wells further suggests that the second component of growth is 'metabolic load', which encompasses a variety of traits which challenge homeostasis, and which emerge during the hypertrophic growth period, of increases in cell size. Metabolic load is exacerbated by large tissue masses, a rich diet and sedentary behaviour, each of which challenges the ongoing regulation of cellular metabolism. Metabolic load is strongly contingent on postnatal weight gain and lifestyle, especially from childhood onwards, and reflects the exposure of the infant to external environmental factors impacting on behaviour and diet (Wells, 2009, Wells, 2011).

Nevertheless, recent studies highlight differences between populations. For example, the contribution of birth weight to disease risk appears less replicable across markers of cardiovascular risk in populations such as India than in European populations (Stein *et al*, 1996; Kumaran, 2000). Furthermore, infant growth does not have consistent associations with later body composition in industrialized versus developing country populations (Wells *et al*, 2007), suggesting that the developmental etiology of metabolic load may also differ.

Recent findings of randomized controlled trials of infant diets show that faster infant growth has been associated with greater blood pressure, insulin levels, blood lipids and adiposity in childhood or adolescence (Singhal *et al*, 2001; Singhal *et al*, 2003; Singhal *et al*, 2004a; Singhal *et al*, 2004b; Singhal *et al*, 2010).

Body composition measurements are ideal for researching the long-term consequences of early growth and nutrition, because on the one hand they are ideal for quantifying the masses of lean tissue and fat that contribute to human capital (physical work capacity, reproductive function and robusticity) (Victoria *et al*, 2009), whilst on the other, they are ideal for testing the capacity-load model of chronic disease risk. Broadly, lean mass may be taken as an index of metabolic capacity, whereas fat mass may be taken as an index of metabolic load. Thus, for populations such as India, currently undergoing the nutrition transition in urban environments whilst still demonstrating high levels of malnutrition in traditional rural or slum settings, sequential body composition measurements may help understand the life-course emergence of health and disease (Wells, 2012).

2.10 Essential fatty acids

The concept that specific components of fat may be necessary for the proper growth and development of animals and possibly humans was introduced in the 1930s (Burr and Burr, 1929). Yet EFAs were considered of marginal importance until the 1960s when signs of clinical deficiency became apparent in infants fed skim-milk-based formula and in those given lipid-free parenteral nutrition. The essentiality of n-6 and n-3 FAs for humans is best explained by the inability of animal tissue to introduce double bonds in positions prior to carbon 9 (Uauy *et al*, 1999).

According to the Food and Agriculture Organization (FAO) and World Health Organization (WHO) joint expert consultation on fats and fatty acids in human nutrition in Geneva in November 2008 (FAO/WHO, 2008); there are inherent limitations with the convention of grouping fatty acids based only on the number of double bonds, i.e. saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) insofar

as describing the effects of fatty acids on human health and in developing dietary recommendations.

The large body of epidemiological evidence about total fats, fatty acids, and human health apply these groupings and show that the major groups of fatty acids are associated with different health effects. However, the expert consultation recognised that individual fatty acids within each broad classification of fatty acids may have unique biological properties and health effects. This has relevance in making global recommendations because intakes of the individual fatty acids that make up the broad groupings will differ across regions of the world depending on the predominant food sources of total fats and oils.

The expert consultation also recognized that in spite of these limitations, the scientific community in general and an increasing proportion of the general population continues to use the groupings based on chemical structure and thus, there would be disadvantages in abandoning them. Moreover, few countries have food composition databases that enable dietary assessment of individual fatty acid intake.

Therefore for the sake of clarity and in recognition that often generalized terms are used to refer to specific fatty acids, the expert consultation thought it appropriate to provide details as to the use in this document. In particular:

• The expert consultation recognized that grouping of fatty acids into these three broad groups (SFA, MUFA and PUFA) is based on chemical classifications, but it is clear that individual fatty acids within these groups have distinct biological properties. However, most of the epidemiological evidence reviewed by the experts uses broad groupings, which make it difficult to distinguish and disentangle the effects of individual fatty acids.

• SFA refers to the major SFA in the diet, namely C14, C16, C18, except in the case of milk and coconut oil where SFA range from C4 to C18.

• MUFA refers to the major monounsaturated fatty acid in Western diets, which is oleic acid (C18:1n-9). It should be recognised that in some populations, a major monounsaturated fatty acid is erucic acid (C22:1n-9), as for example, found in culinary oils derived from some Brassica spp. such as rapeseed and mustard seed.

• PUFA refers to the major PUFA in the diet, which includes mainly linoleic acid (C18:2n-6), a lower proportion of alpha-linolenic acid (C18:3n-3), and depending on seafood intake a variable but relatively low proportion of long chain PUFA such as arachidonic acid (AA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA). For the purposes of food labelling, the terms EFA, PUFA, long chain PUFA, n-6 and n-3 lack precision and should not be used without fully specifying the actual fatty acids and their amounts. Many different fatty acids with quite different properties fall under these umbrella terms.

• TFA refers to the major trans fatty acids in the diet which are typically isomers of 18:1 trans derived from partially hydrogenated vegetable oils.

• Some fatty acids (e.g. trans monoenes, conjugated linoleic acid [CLA], etc.) are members of more than one chemical classification but by convention are interpreted as in only one category (trans monoenes in MUFA, CLA in PUFA, etc.).

• There are many fatty acids that are usually minor components of most foods but are major components of some specialty foods and/or of supplements. FAO/WHO recommendations must be carefully interpreted with respect to unusual fatty acids ["usual" = straight chain, all-

cis, methylene-interrupted (homoallylic); "unusual" = trans, branched chain, non-methylene interrupted double bond structure] (FAO/WHO, 2008).

Dietary essential fatty acids (EFA) have long been considered part of the lipid supply necessary for energy, growth, cellular metabolism, and muscle activity. The fact that some EFAs serve as indispensable dietary precursors for eicosanoid formation has provided greater significance to the study of their role in health and disease. During the 1980s and 1990s, attention was placed on the effect of n-3 and n-6 EFAs in normal fetal and infant development (Sprecher, 1981; Bazan, 1989; Willis, 1984; Uauy and Hoffman, 1991; Simopoulos, 1991; Innis, 1991; Uauy et al, 1989). Extensive research has been carried out in industrialized countries documenting sources and benefits of EFAs. There is however limited data on the same in low-income countries.

Michaelsen and colleagues conducted a review of the available data on dietary intake of fatty acids in low-income countries, with special focus on pregnancy and the first years of life. The review includes data on the fatty acid content of relevant foods, the amount of long-chain polyunsaturated fatty acids (LCPUFA) in breast milk, fatty acid intake during the complementary feeding period, and data on fatty acid availability in selected low-income and middle-income countries based on Food and Agriculture Organization (FAO) food balance data (Michaelsen *et al*, 2011).

The report emphasized the importance of breastfeeding during the complementary feeding period; breast milk being a key source of PUFA because complementary foods in low-income countries are typically cereal-based with low fat content, and therefore contribute very little PUFA.

In all the 13 countries included in the food balance calculations, there was a strong positive association between the economic status of the country (GDP) and the supply of total fat and

n-3 fatty acids. Most of the fat and PUFA intake comes from vegetable oils and cereals among families living on a primarily plant-based diet. Fat content of cereals is low and mainly located in the outer layers of the kernels, so whole grains are a better source of fatty acids than refined cereals. A very important source of PUFA in low-income countries is vegetable oils, but they differ considerably in PUFA content and especially in the LA/ALA ratio. Soybean oil and canola/ rapeseed oil have the highest ALA content (Michaelsen *et al*, 2011).

Furthermore Michaelsen *et al* (2011) identified fish and seafood as the most important sources of n-3 LCPUFA, with marine fish generally a better source than freshwater fish. 'For poor rural populations in particular, farming of small fish in rice fields and lakes could provide an important extra supply of n-3 fatty acids. Other potentially sustainable and cheap sources of LCPUFA are indigenous foods (e.g. amphibia, worms and insects) which are underutilized in many populations' (Michaelsen *et al*, 2011).

The official Government of Kenya national food composition tables were written in 1993 by Sehmi. The book contains foodstuffs commonly consumed in various parts of Kenya and their nutritional contents in terms of carbohydrates, proteins, fats and vitamins. The fats are presented as Total amount of fat g %; SFA g%; MUFA g%; PUFA g%; LIN g%; and CHOL mg% (Sehmi, 1993). The food composition tables are incomplete and should be updated to include more foods as well as analyses of specific EFAs.

2.10.1 Mechanism of Essential Fatty Acids

Fats have a variety of biological functions in the body but are primarily involved in: Provision of energy and fueling of cells, membrane structure and function; and communication within and between cells. (Innis, 1991).

Essential fats, in particular, play a critical role in neurobehavioral development, immune function, growth, mental health, and long-term metabolic health. Essential fatty acids are those that humans are unable to synthesize and must therefore obtain through their diet. They include alpha-linolenic acid (ALA), the building block for the longer-chain omega-3 fatty acids, and linoleic acid (LA), the building block for the longer-chain omega-6 fatty acids.

Fatty acids are especially important for brain function. More than 50 percent of the adult brain is made up of fatty acids (dry weight). Long-chain polyunsaturated fatty acids (LCPUFA) such as docosahexaenoic acid (DHA) and arachidonic acid (AA) are among the most important fatty acids in the brain. LCPUFA are incorporated into specialized cell connections (called synaptic membranes) through which transmission of messages between cells occurs. DHA has positive effects on nerve cell growth and differentiation and decreases nerve cell death. In addition, omega-3 fatty acids provide the building blocks for chemical messengers that relay nerve signals through the brain and thus could affect mental health (Innis, 1991).

In low-income countries, the availability of fat and omega-3 fatty acids in the food supply is generally low, often below the minimum recommended intake for vulnerable groups. In populations living on a predominantly plant-based diet, vegetable oils and cereals are important sources of fatty acids. Some vegetable oils such as soy and canola oil have high contents of omega-3 fatty acids while other oils from corn, peanut, safflower, and sunflower have very low omega-3 fatty acid levels. Among young children in low-income countries, fat intake is generally adequate while they are still breastfed but declines sharply after weaning (Michaelsen, 2011).

LCPUFA transfer from the mother to her fetus/newborn during pregnancy and lactation is mainly dependent upon maternal status. Transfer is highly variable because there are large

differences worldwide in dietary intake of LCPUFA, particularly DHA. This is reflected in the large differences in DHA content of breastmilk between and even within countries. For children under 2 years of age, the key sources of LCPUFA, especially omega-3 fatty acids, are breastmilk and fish (Michaelsen, 2011).

The relative amounts of omega-3 and omega-6 fatty acids in the diet may also influence fatty acid status because the conversion of ALA to long-chain omega-3 fatty acids is affected by omega-6 fatty acid intake. Both the total fatty acid content of the diet and the balance of LA to ALA are determinants of LCPUFA status. There are large differences in breastmilk DHA content within and across countries, probably related to differences in maternal dietary fatty acid intakes (Innis, 2007; Michaelsen, 2011)

2.11 Child motor development milestones

Several evaluations indexes have been developed to assess infants with suspected or known risk of developmental delay, typically in gross and fine motor development. Most criteria are based on scores from standardized tests that document delays in development, calculated from either age-equivalent scores or standard scores. Of standardized tests, the Motor Scale of the Bayley Scales of Infant Pediatric Physical Therapy Concurrent Validity of the BSID II and the PDMS-2 Development II (Bayley, 1993) and the Peabody Developmental Motor Scales-2 (Folio, 2000) are two of the most commonly used discriminative measures in early intervention.

The WHO Multicentre Growth Reference Study (MGRS) in 2006 had as its primary objective the construction of curves and related tools to assess growth and development in children from birth to 5 years of age. Six milestones were selected for study: sitting without support, hands-and-knees crawling, standing with assistance, walking with assistance, standing alone and walking alone. These milestones were considered to be universal,

fundamental to the acquisition of self sufficient erect locomotion, and simple to test and evaluate (WHO, 2006).

The MGRS curves were designed to replace the previously recommended reference curves for child growth (i.e. the NCHS/WHO growth reference), which are now known to suffer from a number of deficiencies. The group recommended that ‘‘windows of achievement’’ be used rather than percentile curves. These windows should be bounded by the 1st and 99th percentiles of the pooled distribution of all sites and should be interpreted as normal variation in ages of achievement among healthy children. The concept of a ‘‘window’’ offers a simple tool that can be easily used to assess children since it requires no calculations.

There are two independent sources of information about the achievement of motor milestones in the MGRS. The first, by the caretaker, provides the actual date when the milestone was first observed and/or tested. The second, by the fieldworker, provides a date when the performance was first demonstrated on a scheduled visit (WHO, 2006).

Children begin to explore their environments through the attainment of specific motor skills, and engage in new experiences that promote learning and development of other component processes (Bertenthal *et al*, 1990). As early as 1909, Tracey documented a natural progression of motor milestone development (Tracey, 1909). Tracking motor milestone acquisition has informed researchers about the motor development proficiency of young children (Burnside, 1927). Robson argued that the time that children spend in any given milestone or take to achieve some of the more advanced milestones (e.g., walking without support) is variable and often depends on whether they achieved a preliminary milestone (Robson, 1984). Later on it was discovered that advancement to locomotion and beyond is dependent upon the young child’s musculoskeletal system, body size, mass, and proportions (Pollitt, 1994; Adolph, 1998; Thelen, 1983; McGraw, 1943).

In developing countries, growth faltering is prevalent (deOnis, 2000). This is because children in developing countries are susceptible to infection and malnutrition (Rivera and Montorell, 1988; Habitch *et al*, 1995). Globally, an estimated 43% of children less than 4 years of age have been found to be anemic (Ezzati *et al*, 2002). Children less than 2 years of age are particularly vulnerable due to their increased demand for nutrients as they transition from breastfeeding exclusively to consuming complementary foods (WHO/UNICEF, 1998). Limited variety of complementary foods has been associated with low nutrient density adequacy of the foods, which may cause malnutrition and developmental delays (Dewey *et al*, 2005).

Animal protein intake was positively associated with earlier walking acquisition among Guatemalan infants (Kuklina *et al*, 2005). Anemia and iron deficiency were independently associated with walking in a cohort of Zanzibari infants (Kariger *et al*, 2004). Delays in motor milestone acquisition, including walking, were found among cohorts of stunted, underweight Indonesian (Pollitt *et al*, 1994), Zanzibari (Kariger *et al*, 2004), and Guatemalan infants (Kuklina *et al*, 2005), and among stunted and wasted Pakistani infants (Cheung *et al*, 2001).

On the other hand, Vestergaard *et al* (1999) reported that achievement of two motor skills (crawling and pincer grip) was linked to the duration of breastfeeding in a large sample of Danish infants, even after adjustment for potentially confounding variables. Although motor development in infancy is not correlated with later cognitive development in well-nourished populations, Pollitt and Gorman (1990) reported that motor test scores (although not mental scores) of Guatemalan infants at 15 months were significantly associated with several indices of cognitive performance in adolescence and speculated that this may also be the case in other nutritionally at risk populations.

Dewey *et al*, (2001) studied the effects of exclusive breastfeeding for four versus six months on maternal nutritional status and infant motor development in two randomized trials in Honduras. They reported that infants in the Exclusive Breastfeeding (EBF) group crawled sooner in both studies; and were more likely to be walking by 12 months than infants in the Supplementary Feeding (SF) group (Dewey *et al*, 2001).

Siegel and group (2005) studied the effect of nutritional factors on the acquisition of walking in a cross-sectional cohort of 485 4- to 17-months Nepali children adjusting for age, sex, caste, and socio-economic status (SES). Multivariate logistic models that controlled for age, sex, caste, and SES revealed that children with higher length-for-age and weight-for-length Z-scores, no anemia, and meat consumption walked at an earlier age than children with lower scores, anemia, and no meat consumption. They concluded that growth, anemia, and diet are independently associated with delays in the onset of bipedal locomotion among young Nepali children (Siegel *et al*, 2005)

Adu-Afarwuah *et al* (2007) conducted a randomized comparison of 3 types of micronutrient supplements for home fortification of complementary foods in Ghana in order to determine effects on growth and motor development. They compared the home fortification of complementary foods with Sprinkles (SP) powder, crushable Nutritabs (NT) tablets, or energy-dense (108 kcal/d), fat-based Nutributter (NB) and randomly assigned 313 Ghanaian infants. All 3 supplements had positive effects on motor milestone acquisition by 12 months compared with no intervention, but only NB affected growth (Adu-Afarwuah *et al*, 2007).

Dewey and Adu-Afarwuah (2008) suggested a conceptual framework for the association between different factors affecting growth through the complementary feeding period: “quality of complementary food”, “quantity of complementary food”, “breast milk” and “morbidity” and for how these are likely to interact. Complementary feeding interventions

should ideally address all four components (Dewey and adu-Afarwuah, 2008). In a modified model (see Figure 2.3), "outcome measurement" is added as a crosscutting component because our choice of outcome measures is determinant for our understanding of the magnitude of a potential effect on growth by interventions addressing one or more of the underlying factors. Reliable, precise and accurate measuring techniques to measure growth are required to advance our understanding of how nutrition interventions can support healthy growth in infants and children during the critical complementary feeding period.

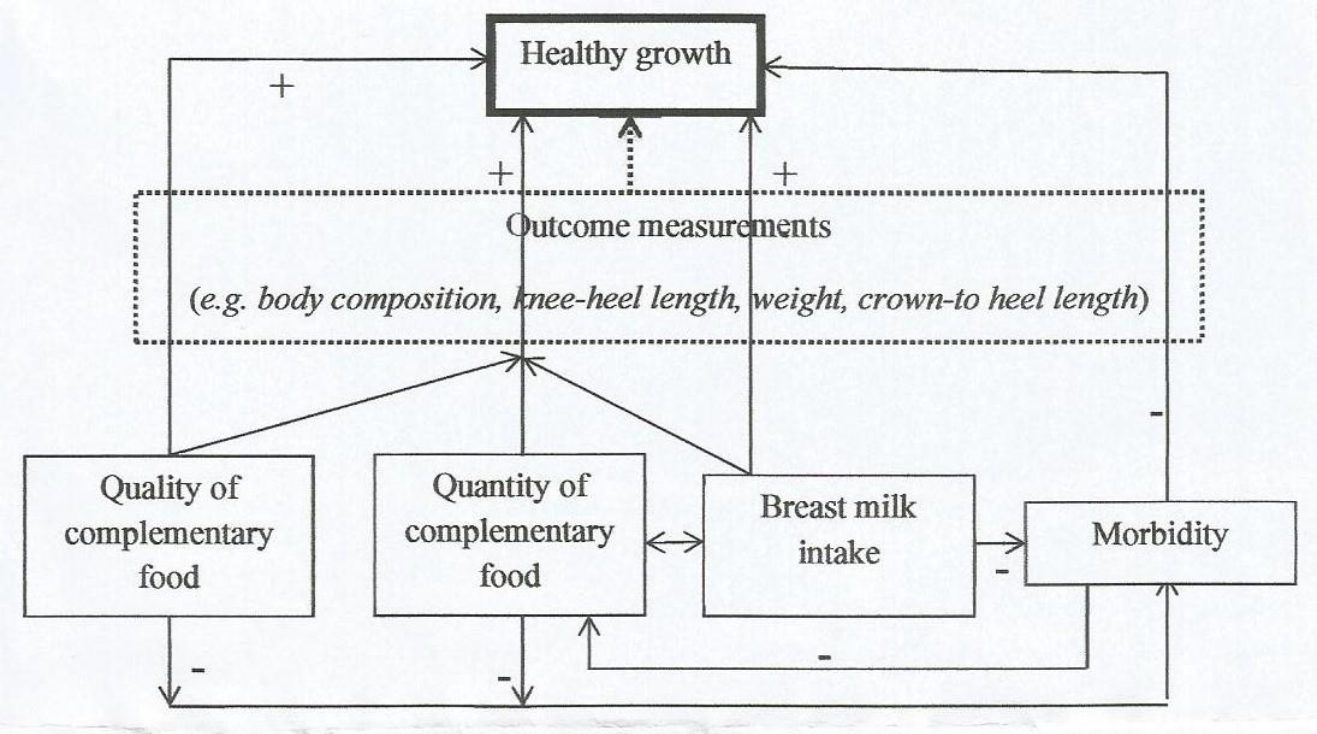


Figure 2.3: Conceptual model of proximal factors affecting healthy growth during the period oof complementary feeding. Modified from Dewey and Adu-Afarwuah, 2008

2.12 Summary of literature review

Complementary feeding is a complex set of behaviours, comprising timing of introduction, food choices and dietary diversity, preparation methods, quantity, feeding frequency, responsiveness to infant cues, and safe preparation and storage of foods. The home environment should ideally provide a clean, safe and stimulating environment to adequately nurture the mother and child (Dewey and Adu-Afarwuah, 2008).

In Dewey and Adu-Afarwuah (2008) grouped interventions of complementary feeding into five categories depending on the main strategy used: (1) education about complementary feeding as the main treatment (2) complementary food or a food product offering extra energy (with or without added micronutrients) provided as the only treatment (3) provision of food combined with some other strategy, usually education for mothers (4) fortification of complementary foods (centrally processed fortified foods or home-fortification products) with micronutrients (with no difference in energy provided to intervention vs. control groups), and (5) increased energy density and/or nutrient bioavailability of complementary foods through the use of simple technologies.

In addition, Dewey and Adu-Afarwuah (2008) evaluated the effectiveness and efficacy of the interventions according to growth outcomes, morbidity outcomes, child development, micronutrient intake, iron status, zinc status and vitamin A status. Table 2.3 shows a summary of studies relevant to the scope of the current study.

Table 2.3: Summary of literature review

Author,Year Skau, 2014	**Country:** Cambodia
	Title: Preventing under-nutrition in Cambodia: Assessing the effect of improved local complementary food on growth
	Participants: 419 Cambodian infants 6-15 months
	Key results: Gains in FFM did not differ between groups. The two fortified food products WF-L and CSB++, both containing ASF (fish or milk), sustained linear growth marginally better than the fortified but plant-based CSB+ and better than the non-fortified WF containing 14% ASF.
Author,Year Adu-Afurwuah et al, 2007	**Country:** Ghana
	Title: Randomized comparison of 3 types of micronutrient supplements for home fortification of complementary foods in Ghana: effects on growth and motor development
	Participants: 313 Gambian infants 6-12 months
	Key results: All 3 supplements had positive effects on motor milestone acquisition by 12 months compared with no intervention, but only Nutributter affected growth.
Author,Year Kinyuru et al, 2012	**Country:** Kenya
	Title: Nutrient composition of four species of winged termites consumed in Western Kenya
	Subject: 4 species of edible termites
	Key results: The termites may be exploited in provision of high quality diets especially in the developing countries which have been plagued by iron and zinc deficiencies as well as poor supply of dietary polyunsaturated fatty acid sources.
Author,Year Ettyang et al, 2004	**Country:** Kenya
	Title: Assessment of body composition in lactating mothers in a rural African community using deuterium oxide
	Participants: 10 lactating women
	Key results: The variability (5.8 – 17.5%) observed in the SF technique may result in lower prediction of % BF. This may be an important factor for community-based nutritional interventions that aim at improving the body composition of vulnerable groups such as pregnant and lactating

	women or subjects with severe under-nutrition.
Author,Year Grillenberger et al, 2003	**Country:** Kenya
	Title: Food Supplements Have a Positive Impact on Weight Gain and the Addition of Animal Source Foods Increases Lean Body Mass of Kenyan Schoolchildren
	Participants: 544 Kenyan school children
	Key results: Food supplements (meat and milk)had a positive impact on weight gain in the study children and the addition of meat increased their lean body mass
Author,Year Wells et al, 2009	**Country:** Gambia
	Title: Body composition by 2H dilution in Gambian infants: comparison with UK infants and evaluation of simple prediction methods
	Participants: 30 infants 3-18 months of age
	Key results: Gambian infants characterized by growth faltering had lean mass deficits that increased with age. However, adiposity increased with age, and showed indications of a more central distribution than in the reference infants.
Author,Year Andersen et al, 2013	**Country:** Ethiopia
	Title: Body composition from birth to 6 months of age in Ethiopian infants: reference data obtained by air-displacement plethysmography
	Participants: 378 infants aged 0-6 months
	Key results: Z scores and centile reference charts for an apparently healthy urban Ethiopian infant population. The study represents a first step toward providing reference data on FM and FFM for an urban African context, for future clinical care and research.
Author,Year Stanfield et al, 2012	**Country:** United Kingdom
	Title: Differences in body composition between infants of South Asian and European ancestry: the London Mother and Baby Study
	Participants: 30 South Asian and 30 White European infants aged 6–12 weeks
	Key results: The characteristic differences in body composition observed between adult South Asians and White Europeans are apparent in early infancy. Of particular note is that this is the first study to demonstrate that South Asians compared with White Europeans have reduced FFM in

	infancy. The early manifestation of this phenotype suggests that it is either genetic and/or determined through exposure to maternal physiology, rather than a consequence of behaviours or diet in childhood or at older ages.
Author, Year Fomon et al, 1985	**Country:** USA
	Title: Body composition of reference children from birth to age 10 years
	Participants: Children from birth to 10 years
	Key results: With increasing age during childhood a 'maturation' occurs in the chemical composition of fat free body mass: percentage of water decreases and percentages of protein and osseous mineral increases.
Author, Year Bauserman et al, 2013	**Country:** Congo
	Title: Caterpillar cereal as a potential complementary feeding product for infants and young children: nutritional content and acceptability
	Participants: 20 mothers and their 8–10-month-old Infants
	Key results: Cereal made from locally available caterpillars has appropriate macro- and micronutrient contents for complementary feeding, and is acceptable to mothers and infants in the DRC.

2.13 Gaps in Knowledge

The gaps in knowledge identified in literature that the current study sort to address are:

1. The lack of nutritious complementary foods in resource poor settings (Dewey, 2005). Cereal based gruels predominantly used in resource constrained settings do not meet nutrition requirements for rapid growth and development (WHO, 1998; Owino et al, 2007).

2. Traditional assessments eg anthropometric measurements are less sensitive to quality of weight gain; partitioning of FFM/FM. Body composition (UNSUN, 2010).

3. Inadequate documentation of functional outcomes of nutrition such as gross motor milestones (Dewey and Adu-Afarwuah, 2008)

4. Inadequate documentation of EFA food composition and blood profiles in resource poor settings (Michaelsen et al, 2011)

5. Dearth of evidence in randomized control trials of outcomes for nutrients intrinsic in food, especially in resource poor settings. (FAO, 2011)

CHAPTER THREE

METHODOLOGY

3.1 Study setting

3.1.1 Study area and population

The study was conducted in Mumias District, Kakamega County of the Western Province of Kenya between January 2012 and February 2013. The Mumias town, the capital of Mumias District, has an urban population of 116,358 (KDHS, 2009). The town is linked by road to Kakamega (in east), Busia (west), Bungoma (north), Butere (south). The village of Buchinga is located between Mumias and Kakamega. The population growth rate is 2.4 % (KDHS, 2009).

Most of the inhabitants of Mumias district are from the Luhya ethnic group. The population is mainly centered around the urban centre that houses the Mumias Sugar Company. Most of the inhabitants of the project area have small plantations of sugar cane with the average size of plot holdings of 4 acres. The sugar cane is harvested after 18-24 months; in the meantime therefore, farmers are left without a viable source of income till the next harvesting season. This type of farming has left many families with very small pieces of land that are used for food crops resulting into increased malnutrition in the area especially among children - hence increased family expenditure on health.

Figure 3.1 shows the location of Mumias district in the world map.

Figure 3.1: Position of Mumias district on the world map. Source: Maphill <www.maphill.com>

According to the Kenya Demographic and Health Survey, 40.4% of children in Kakamega County have low height for their age, indicating malnutrition, much of it being protein and mineral deficiency. Overall, in Kenya, 34.8% of children have stunted growth; 76% of children under five years old are Vitamin A deficient, 73% are anemic (low iron) and 55% of pregnant women are anemic (KDHS, 2010).

Konyole and group (unpublished) carried out a crossectional study of 600 children aged 6 to 24 months in Mumias in November 2011. The unpublished results show wasting (defined as WLZ <-2 standard deviations (SD) of the WHO Child Growth Standards median) at 11.2%. Stunting (defined as LAZ <-2 SD of the WHO Child Growth Standards median) was at 26.4% and underweight (defined as WAZ <-2 SD of the WHO Child Growth Standards median) was 14.9% (Konyole *et al* unpublished). According to the WHO classification for degree of population malnutrition, the prevalence of wasting in Mumias was "serious';

stunting was ‘medium’ and underweight was ‘medium’ (See Table 3.1). No data existed on the body composition, whole blood essential fatty acids or gross motor milestones status of the population by the time the current study was carried out.

Table 3.1: WHO Definition of malnutrition

- Underweight: weight for age < -2 standard deviations (SD) of the WHO Child Growth Standards median
- Stunting: height for age < -2 SD of the WHO Child Growth Standards median
- Wasting: weight for height < -2 SD of the WHO Child Growth Standards median
- Overweight: weight for height > +2 SD of the WHO Child Growth Standards median

WHO classification scheme for degree of population malnutrition

Indicator	Prevalence cut-off values for public health significance
Underweight	< 10%: Low prevalence 10-19%: Medium prevalence 20-29%: High prevalence = 30%: Very high prevalence
Stunting	< 20%: Low prevalence 20-29%: Medium prevalence 30-39%: High prevalence = 40%: Very high prevalence
Wasting	< 5%: Acceptable 5-9%: Poor 10-14%: Serious = 15%: Critical

Reference: WHO, 1995.

3.2 Recruitment of study participants

Mothers and infants pairs were recruited in the study as they came to the health facilities (Makunga, Lusheya and Khaunga child welfare clinics) for diphtheria, pertussis (whooping

cough) and tetanus (DPT; also DTP and DTwP) or oral polio vaccination at 5 months of age.

The inclusion criteria

Mothers accepting and ready to:

1) Prepare and feed their children with the complementary food blends and the CSB+
2) Stay in the study area for the next 12 months, and if consenting to participate
3) Attend clinic for growth monitoring as specified by policy (up to 5 years)
4) Allow blood sampling from the infant for whole blood EFA
5) The infant must have been free of any evidence of chronic disease and weighed at least 2500g at birth.
6) The mothers were not forced to take part in the study but consented to attend clinic for growth monitoring and blood sampling.
7) Infants 6 months old with a MUAC > 11.5 cm, no bilateral pitting oedema, no anaemia (Hb> 110g/L) or clinical signs of vitamin A deficiency (xerosis or Bitot spots) and no sign of chronic disease were enrolled in the study. This was regardless of breastfeeding status or HIV status.

The exclusion criteria

Children with WHZ< -3, a MUAC<115mm, and/or bilateral pitting oedema, or with anaemia (Hb< 110g/L) or clinical signs of vitamin A deficiency (xerosis or Bitot spots) were excluded and referred for treatment at the partnering health facilities. Likewise, infants with chronic illness requiring medication, genetic disorders interfering with normal growth as

well as severely acutely malnourished children were excluded from the study and referred for clinical and therapeutic nutrition care.

3. 3 Sample size calculation

In the current study, fat free mass was used as the key indicator.

The sample size calculation was done using the equation by Fisher in Gibson and Ferguson (2008)

Arsenault *et al* (2008) carrying out similar study in Peru which was used to compare means and standard deviations, used tha same equation:

$n= [(u + v)^2 \times (s_1^2 + s_2^2)] / (m_1 – m_2)$

Where:

n = sample size; u= 0.64 corresponds to β for the test of 95%; v = 1.94, corresponds to an α

of 5% two tailed test; s_1 = the standard deviation of the nutrient of interest in group A; s_2 = the standard deviation in group B, and m_1 and m_2 are the corresponding means.

In order to detect at least 0.4 kg difference in lean mass 0.7 SD at 80% power and 5% level of significance, and allowing for 15% loss to follow up, at least 150 infants per group were required.

3.4 Sampling procedure

Mother and infant pairs were recruited in the study as they came to the health facilities (Child welfare clinics). The sites were chosen to reflect the resource poor settings targeted in the study and in the region where the food ingredients of the blend(s) are already accepted. The

study thus had an on-going recruitment, which took three months (See Annex 3). Figure 3.2 shows the sampling procedure of the study.

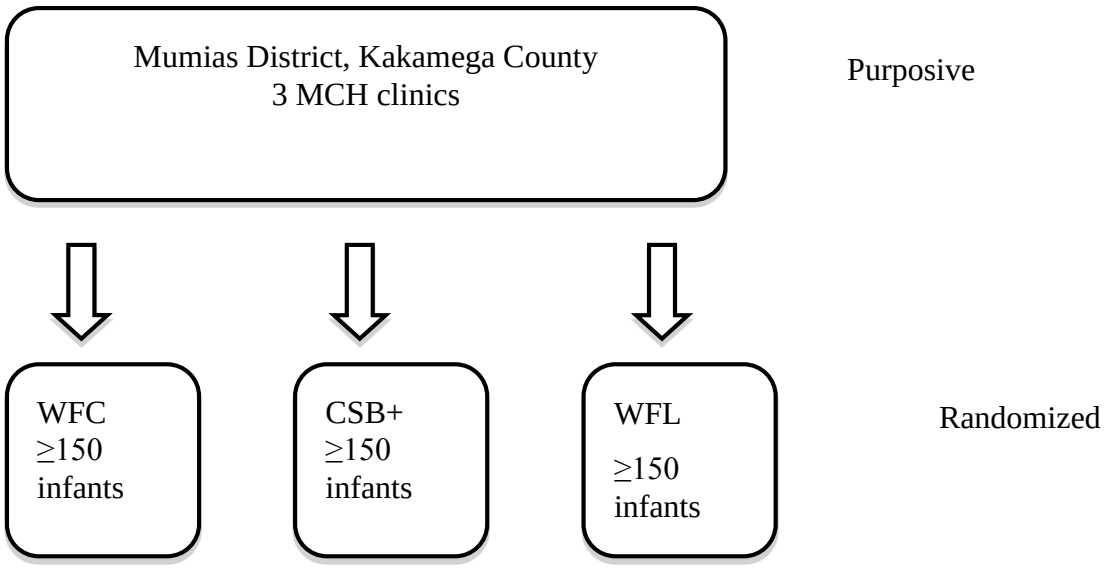


Figure 3.2: The sampling procedure

3.5 Study design

The study was a community-based double blind randomized trial in which infants were individually randomised at 6 months of age to receive one of the study complementary foods, namely, 1) maize-amaranth-termite-fish complementary food naturally enriched with iron and zinc (Winfood Classic), or 2) multi-micronutrient fortified maize-amaranth complementary food (Winfood Lite) or 3) multi-micronutrient fortified corn soy blend plus (CSB+) for 9 months. Table 3.2 shows the composition of the complementary foods (CSB+, WFC and WFL) in terms of ingredients and nutrient composition developed by Kinyuru and colleagues (Kinyuru *et al*, 2012).

Table 3.2a: Composition of the ingredients of the blended food products, g per 100g dry weight

	WFC	WFL	CSB+
Germinated amaranth	71.0	82.52	-
Whole maize	10.40	10.22	74.24
Whole soybean	-	-	19.00
Dagaa fish	3.0	-	-
Termite	10.0	-	-
Soybean oil	0.60	0.59	-
Sugar	5.0	4.91	5.0
Premix 1 (IS 723)*	-	0.20	0.20
Premix 2 (IS 730)**	-	1.56	1.56

* Mineral/vitamin premix – FBF-V-10

** Mono calcium phosphate and potassium chloride

Table 3.2b: Nutrient composition of food products, per 100g dry weight

	WFC	WFL	CSB+
Energy (kcal)	423.6	407.2	391.7
Protein (g)	19.1	14.6	15.1
Fat (g)	12.3	9.0	15.8
Iron (mg)	12.2	12.5	7.7
Zinc (mg)	6.3	5.5	5.1
Calcium (mg)	48.2	139.4	141.9

Table 3.2c: Fatty acid composition of food products, per 100g dry weight

	WFC	WFL	CSB+
Arachidonic	-	-	No data
Capylic	0.48	-	No data
Lauric	0.27	-	No data
Myristic	1.77	-	No data
Palmitic	28.90	27.40	No data
Palmitoleic	8.09	-	No data
Stearic	5.88	4.65	No data
Oleic	46.23	31.55	No data
Linoleic	6.45	32.78	No data
Linolenic	1.98	3.63	No data

The complementary foods were locally produced by processing to a pre-cooked semi-instant porridge (Kinyuru *et al*, 2012). WFC is composed of maize-amaranth-termite-fish complementary food naturally enriched with iron and zinc. The grain amaranth is germinated to enhance bioavailability. WFL is composed of maize-amaranth but does not contain the

animal source foods. It is enhanced by germinating the grain amaranth to enhance bioavailability and fortification with micronutrients (premix). CSB+ is a nutritional food prepared from heat treated maize and soya beans, and fortified by imported vitamins and minerals.

The ingredient foods' (insects, fishes, amaranth and maize) selection and prioritization was based on their availability, acceptability across communities, being traditional/ indigenous, having low anti-nutrients, high Fe/ protein and other nutrients criteria prescribed by WHO (2003). Ease of preparation for local adaptability was also considered before processing into final desired product. Vigorous referencing on analyzed Fe and Zn was done using the National Food Composition Tables and the planning of satisfactory diets in Kenya by Sehmi (1993). The base food (staple) and final product (complementary food) to be developed (flour for gruel/porridge) also influenced the choice of ingredient species.

3.5.1 Ingredient foods selection

Maize (a staple) and grain amaranth (for its high nutritional value, availability and acceptability) were identified. Small pelagic fish locally called *omena* was chosen because of its high (Ca 35mg/ 100g) dry weight, protein (44g/ 100g) dry weight and Fe (17 mg/ 100g) dry weight, ready availability, wide consumption by Kenyans, and because it is already sold in supermarkets and blended into flours meant for infant porridge preparation. A preliminary preparation had shown that small fish at 10% tasted too "fishy" while a composition at 3% was acceptable and undetectable. This informed the decision to fix the proportion of small fish in the formulations at 3% maximum. Termites emerged as widely consumed among target communities with distinct availability seasons associated with long and short rains. They are available in local markets when in season and harvesting is easily done when in and out of season. From the previous studies, the termites (*Macrotermes subhylanus*) locally

called *Agoro* were found to contain 83.38mg/100g dry weight Fe and 10.10mg/100g dry weight Zn while the protein content was 37.50mg/100g dry weight and significantly high levels of the unsaturated fatty acids (Kinyuru *et al*, 2013).

3.5.2 Formulation and optimization of the Winfoods

Using the foods chosen as the ingredients for the design of a complementary food, an excel worksheet (see sample in the annex 16) was used to optimize the complementary foods by varying the amounts of the various foods to meet the target requirements according to Lutter and Dewey (2003), Dewey (2003) and the Institute of Medicine (2003). Two formulations named Winfood CF and Winfood Lite were prepared based on different compositions and process technologies as initially explained. The WinFood CF and WinFood Lite were tested for acceptability in the target group of 20 mother infant pairs, using a 7 point Hedonic scale before the trial started.

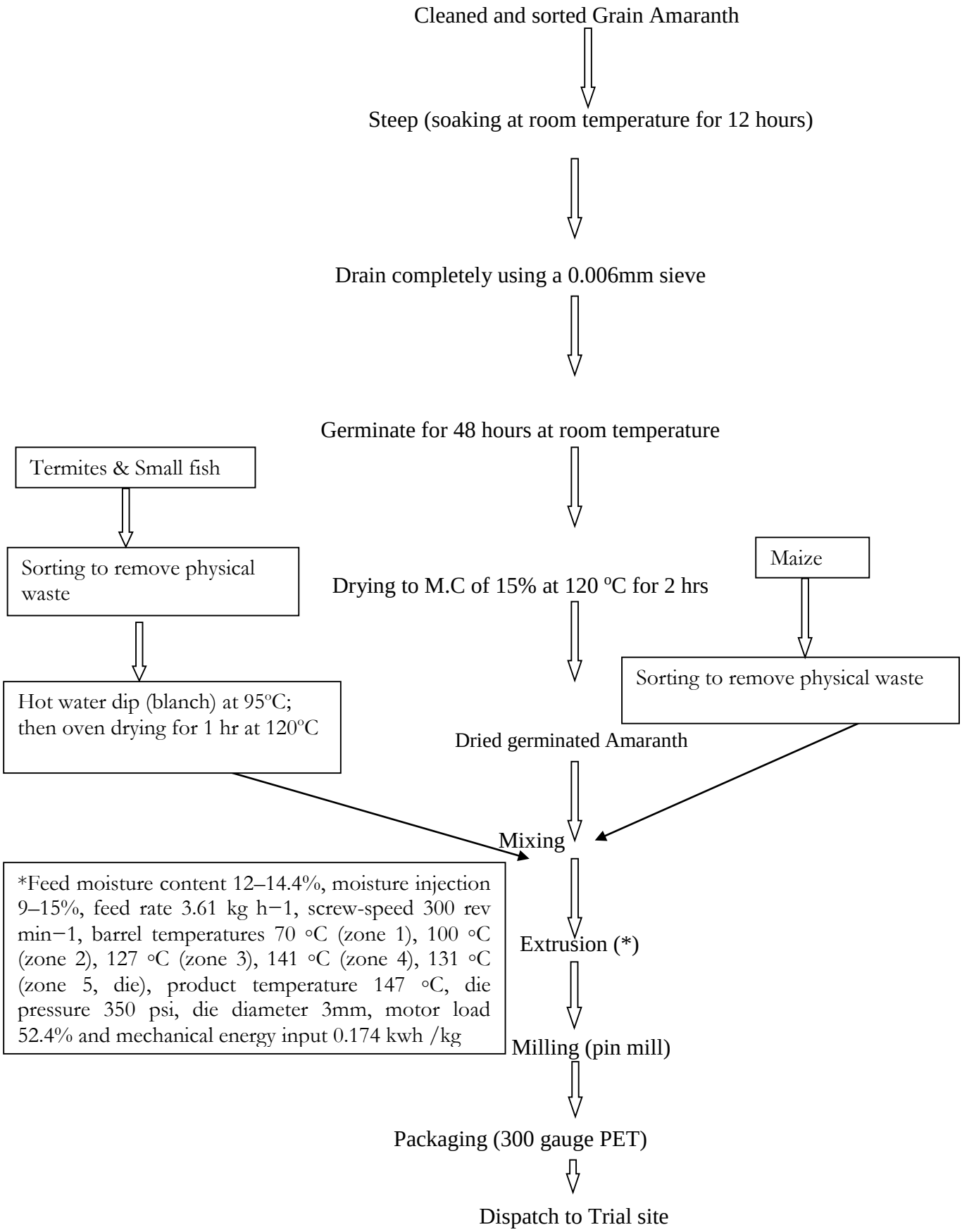
3.5.3 Composition of CSB+

Corn-Soya Blend Plus or CSB+ is a formulated supplementary food distributed mainly during Blanket Feeding or in Supplementary Feeding Programme (dry or wet rations) interventions by UNICEF. CSB+ evolved from the old UNIMIX. It contains maize (64 per cent), whole soya beans (24 per cent), sugar (10 per cent), vegetable oil, and vitamin & mineral premix. There is no milk powder in CSB+. It provides 380 kcal/ 100g dry product (14 per cent protein and 6 per cent fat). CSB+ is supplied in a 10 kg or 25kg airtight bag, and has a 12 months shelf-life.

From the theoretical formulations and the proximate analysis of the produced Winfood samples, they compare well with the CSB+ as "gold standard" currently recommended by the WFP. The development process of the Winfood products has been published separately by

Kinyuru *et al* (2012). Konyole *et al* (2012) carried out an acceptability test among 58 infants for all three foods in the target population to ensure that the foods were acceptable to the populace and safe for use in the randomized trial. A total of 57 children consumed each of the three foods on separate days with one-day washout between foods. Each food was considered acceptable if the child consumed at least 75% of the serving. Most mothers preferred WFL flour and porridge (63.2% and 70.2%, respectively) compared to WFC (24.4% and 10.5%) and CSB+ (12.3% and 19.3%). Children consuming at least 75% of served porridge were 43%, 19.6% and 21% for WFL, WFC and CSB+, respectively. No adverse effects were observed for all the foods throughout the study period and follow up lasting 4 weeks (Konyole *et al*, 2012).

Figure 3.3: Process flow diagram for production of the WinFoods (Kinyuru *et al*, 2012)



The ingredients were obtained from local producers, certified export quality. The foods were produced by KIRDI under a Kenya Bureau of Standards certified production facility conditions and under the responsibility of a graduate Food Scientist and Technologist. The WinFood-CF were conducted in batches during the trial period ensuring maximum storage time of the processed food of 2 months. The processed food was stored at -20°C at KIRDI and quality control checks carried out at the JKUAT laboratories before the transportation to the intervention sites in Western Kenya. The foods were stored for a maximum of 2 weeks at room temperature.

The foods were assessed for microbiological contamination at the Department of Food Science and Post Harvest Technology, Jomo Kenyatta University of Agriculture and Technology (JKUAT) and the Kenya Bureau of Standards (KEBS) food laboratories. Annex 19 shows the preliminary analysis from Kenya Bureau of Standards. Each batch was tested, and foods stored for more than 2 weeks at room temperature were tested.

3.6 Product labelling and blinding

All three products were packed in plastic containers with identical WinFood logo. Product identification was blinded for investigators and enumerators through the entire data collection until the preliminary analyses were completed. The product identification by codes was marked in small print on the backside of each sachet, allowing staff responsible for the food distribution to ensure correct distribution of the products to each participant. Similarly, mothers were blinded from the identity of the complementary foods.

3.7 Randomization

The study was a community-based double blind randomized trial in which infants were individually randomised at 6 months of age to receive one of the study complementary foods, namely, 1) maize-amaranth-termite-fish complementary food naturally enriched with iron and zinc (Winfood Classic), or 2) multi-micronutrient fortified maize-amaranth complementary food (Winfood Lite) or 3) multi-micronutrient fortified corn soy blend plus (CSB+) for 9 months.

Random allocation sequences were computer-generated and stratified by sex with varying block size of 6.

Figure 3.4 shows the randomized study design.

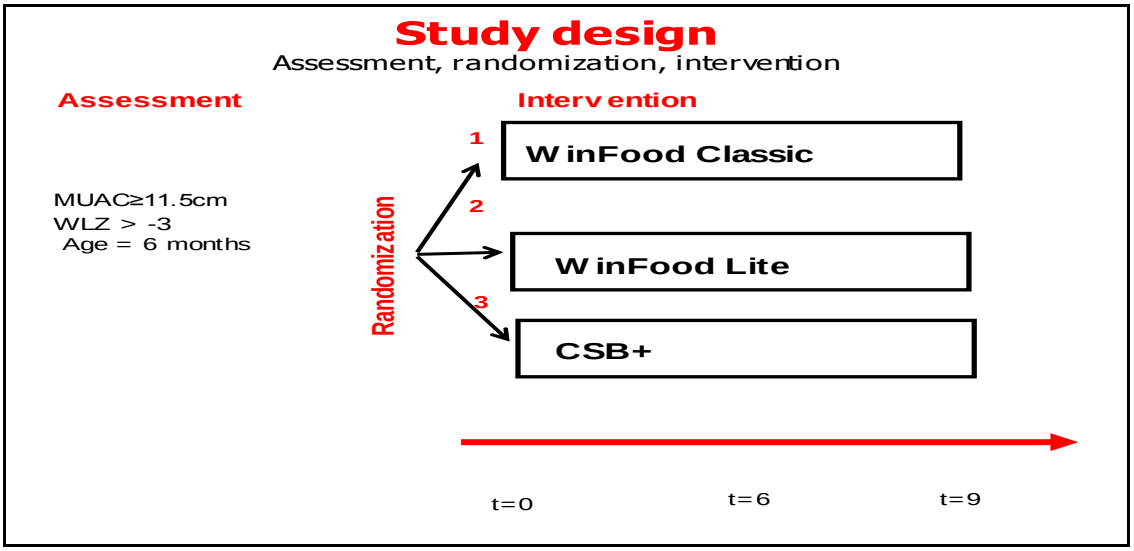


Figure 3.4: Randomized controlled study design

3.8 Description of the intervention

All foods (WinFood CF, Winfood Lite and CSB+) were packed in identical opaque plastic containers which were assigned secret barcodes. They were packed in monthly rations which were adjusted to the age of the child, as described below:

- Children in the age-group 6-8 months received 50 g/day
- Children in the age-group 9-12 months received 75 g/day
- Children in the age group of 13-15 months received 125 g/ day

These rations were adjusted with WHO recommendations for complementary feeding of breastfed infants supplying 200 kilocalories per day for the 6-8 months, 300 kilocalories per day for the 9-12 months and 550 kilocalories per day for 13 to 15 months (WHO, 2002).

Instructions and labels informed caregivers that the food is for babies, to restrict intra-household sharing. The daily rations packets were delivered in monthly rations. The distribution was coordinated alongside nutritional status assessment of the child.

3.9 Study outcomes

Primary outcome

- Lean body mass of children 6 to 15 months for the three complementary foods (WinFood Classic, WinFood Lite and CSB+)

Secondary Outcomes

- Whole blood essential fatty acid profile
- Windows of achievement of gross motor skills of children 6 to 15 months for the three complementary foods

3.10 Data collection

3.10.1 Questionnaire data

General information was obtained at baseline by trained enumerators. Data on the date of birth, sex of the child, demographic and socio-economic data for the family was collected. The socio-economic questions are based on the questions from the Kenyan Demographic and Health Survey 2008-09 (See Annex 4 and 5).

Compliance: The mother was asked how much of the WinfoodCF, Winfood Lite or CSB+ were consumed by the child. The degree of sharing of the assigned diets with other household members was assessed by interviewing the mother. The mothers were asked to keep all the distributed packets after these are empty so they can be collected and counted on a monthly basis (Annex 6).

Procedure: The mother was asked to answer the questions in the questionnaires. The questions were asked orally to the mother by trained Winfood staff in an interview, with additional explanations and standard examples provided if necessary.

3.10.2 Anthropometric assessments

Anthropometry: Weight was measured to the nearest 0.01 kg, using Seca-UNICEF scales (UniScale). Length was measured to the nearest 0.1 cm using calibrated length board. The head circumference was measured by using a fibreglass tape. Mid-upper-arm-circumference was measured to the nearest 0.1 cm, using a UNICEF MUAC tapes. For all anthropometric measurements and derived variables, the increment from baseline were computed (Annex 8).

Procedure (See Annex 9)

- Weight: The mother would step on the Uniscale, without child and her weight would be calibrated as zero. The mother would step down from the Uniscale and step back up with the child in her arms. The scale will then read the weight

- Length: The child would lie down on a length board and two trained Winfood staff would measure the child

- Head circumference and MUAC: The child would be sitting with the mother, when the trained Winfood staff would measure the head circumference with a tape and the circumference of the left arm

Justification: Anthropometry measurement is the traditional outcome indicator in nutritional studies, which makes it possible to compare the results to other nutritional studies carried out in other countries. All the above measurements were taken thrice by the same person to minimize variability. In order to determine any inter-observer variation in measurements each responsible Winfood staff took daily measurements for 3 days of weight, height, head circumferences, MUAC of the same subject volunteered to be measured with permission from the guardian until the three measurements agree within 0.5 units.

3.11 Blood sampling and analyses

A venous (3 mL) blood sample was collected at the 9 months endpoint visit by phlebotomists at the health facilities. The whole blood sample was collected using a field-friendly closed vacutainer system and kept in a cooler box with ice packs. On the same day of collection, samples were transported to Lusheya health center where they were stored in a chest freezer at -20^{0}C until processed. Samples were transferred in dry ice package to the UNITID laboratories in Nairobi where they were stored at -80^{0}C. They were afterward

shipped in dry ice to University of Waterloo in Canada where analysis was done by fast gas chromatography (Stark and Salem Jr., 2005).

3.12 Deuterium oxide dilution procedure for lean mass determination

The children were given a fixed standardized dose of deuterium labelled water which had been accurately weighed with analytical balance at KEMRI, following the guidelines from the International Atomic Energy Agency (IAEA) in Vienna, Austria. A predose 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 deuterium oxide solution (3g deuterium and 12 ml of mineral water) was given to the child orally via a syringe barrel. Infants fasted at least 15 minutes before the samples were taken. Post-dose saliva samples were taken at 2 hours and 3 hours. All samples were collected into a tightly capped cryogenic tube and kept in a cooler box with ice packs. On the same day of collection, samples were transported to Lusheya health center where they were stored in a chest freezer at -20^0C pending transfer for analysis. Samples were transferred in dry ice package to the Kenya Medical Research Institute (KEMRI) laboratories in Nairobi, where analysis was done. Enrichment of deuterium in saliva samples was determined using Fourier Transform Infrared (FTIR) spectrophotometer (Shimadzu, Vienna, Austria). Using the mean of deuterium enrichment based on the two post-dose samples, the dilution space and total body water (TBW) were calculated accordingly (IAEA, 2009). Fat free mass was calculated as: TBW/0.79 for both sexes. Raw values for body water were converted to fat-free body mass using age- and sex-specific values for the hydration of fat-free tissue (Schoeller, 1996).

Table 3.3 shows the standardized dose to give children from IAEA. Figure 3.5 shows the procedure of the Deuterium method.

Table 3.3: Dose preparation for infants (FTIR)

Standardized doses of ^2H_2O for the estimation of TBW in children (from unpublished document by C. Slater, 2009 - IAEA)

Body weight (kg)	Weight of ^2H_2O required (g)
<10	3
10-20	6
20-30	10
30-50	20
>50	30

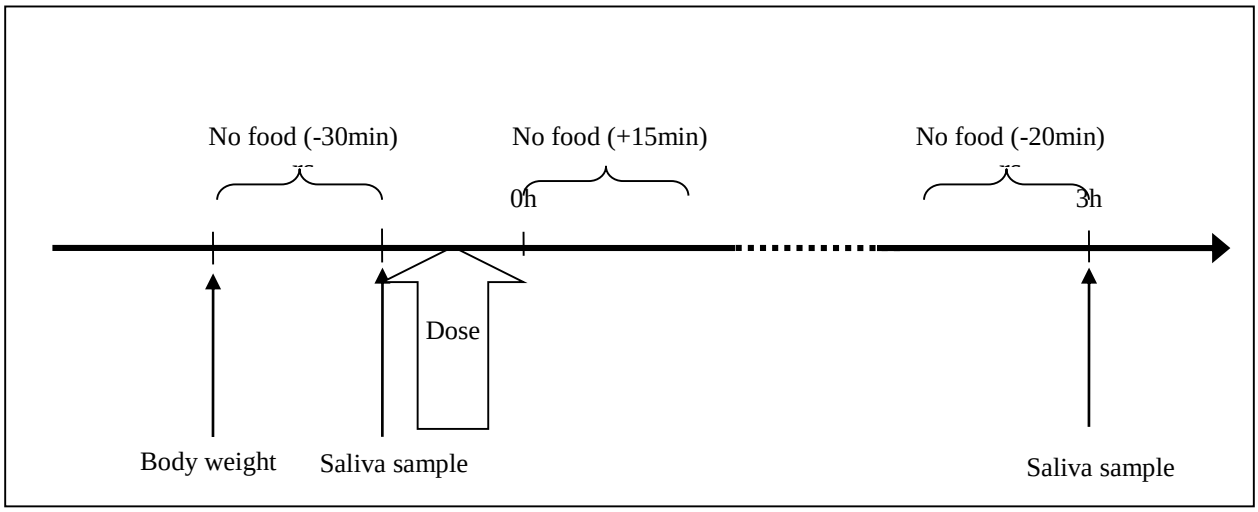


Figure 3.5: The procedure for the Deuterium method

3.12.1 Assumptions of the deuterium dilution technique

There are certain assumptions associated with the deuterium dilution technique for estimating TBW (Schoeller, 2005):

Assumption 1: The deuterium oxide is distributed only in body water.

This assumption is not true. Deuterium in body water enters other pools within the body, which is known as non-aqueous exchange:

—Deuterium exchanges with exchangeable hydrogen atoms in body protein. Exchangeable hydrogen atoms are those on amino ($–NH_2$), hydroxyl (–OH) and carboxyl (–COOH) groups of amino acids.

—Deuterium is also sequestered into fat and protein as these are synthesized. Therefore, the volume of distribution (V), sometimes known as the dilution space, of deuterium is slightly greater than TBW. The ^2H space (V_D) is 1.041 times that of TBW.

This is accounted for by dividing the calculated dilution space (V_D) by 1.041 to achieve TBW (kg).

Therefore,

TBW (kg) = Dose ^2H_2O (mg)/enrichment ^2H in saliva (mg/kg)

should read:

V_D (kg) = Dose ^2H_2O (mg)/enrichment ^2H in saliva (mg/kg)

and

TBW (kg) = V_D (kg)/1.041

Assumption 2: The deuterium oxide is equally distributed in all body water compartments.

This assumption is true for water in the body, but not for water leaving the body as water vapour, which is subject to isotopic fractionation. There is no fractionation in urine, faecal

water or sweat. Sweat is excreted from the sweat glands as liquid water, and evaporation occurs after it leaves the body water; thus, water vapour in breath and transdermal evaporation is subject to fractionation. Transdermal evaporation is insensible water loss from the skin through routes other than the sweat glands. The effect of increased insensible water losses, which contain less deuterium than body water, is to concentrate the deuterium oxide left behind, which leads to an underestimation of TBW and therefore an overestimation of body fat. It is important to avoid physical activity during the equilibration period to avoid increasing the rate of breathing and transdermal evaporation.

Assumption 3: The rate of equilibration of deuterium oxide is rapid.

This is true in healthy participants, but water turnover is slower in the elderly, in pregnant women, and in patients with expanded extracellular water volume (such as malnourished children with oedema). Therefore, a longer equilibration time should be allowed for these subjects:

—Equilibration is the process whereby the D_2O is evenly mixed throughout the body water. After equilibration, all body water compartments will contain the same concentration of deuterium.

—Equilibration between the enriched dose and body water is not instantaneous. Equilibration of body water with saliva is rapid, but equilibration with urine, especially in elderly subjects with residual urine post voiding, can take a few hours. The question is, “How long is the delay before equilibration is complete?”

—In healthy participants, equilibration is usually achieved after 2–5 h and saliva samples can be collected at 3 and 4 h. In general, children have faster water turnover than adults, and elderly adults have slower water turnover than younger adults.

Assumption 4: Neither deuterium oxide nor body water is lost during the equilibration time. This assumption is probably not true, but precautions should be taken to minimize losses. Body water is not a simple closed system; it is a dynamic system with a variety of inputs (drink, food and metabolic water) and outputs (urine, faeces, sweat, breath, etc.). In temperate climates, approximately 8% of body water is turned over in adults each day. Water turnover is 50–100% greater in tropical climates due to increased insensible water losses in the lungs and from the skin.

When TBW is measured using the equilibration technique described here, which lasts for 3–4 hours, participants can be asked to empty their bladder before the dose is taken, not to consume food or liquids, and to avoid physical activity during the equilibration period. As a result, the loss of deuterium in urine and sweat is minimized and can be ignored. If it is not possible to fast during the equilibration period, a note should be kept of the volume of fluids consumed; this volume should be subtracted from the calculated TBW.

3.13 Child gross motor development

Six milestones were selected for study: sitting without support, hands-and-knees crawling, standing with assistance, walking with assistance, standing alone and walking alone. These milestones were considered to be universal, fundamental to the acquisition of self sufficient erect locomotion, and simple to test and evaluate (WHO, 2006). There were two independent sources of information about the achievement of motor milestones in the study. The first, the caregiver, provided the actual date when the milestone was first observed and/or tested. The second, the fieldworker, provided a date when the performance was first demonstrated on a scheduled visit.

Annex 1 and 3 present a flow chart over the data collection process and a timeline table for the whole research. Table 3.4 shows a summary of the baseline and follow-up examinations in the intervention study.

Table 3.4: Summary of baseline and follow-up assessments in intervention study

Time (months)	0	1	2	3	4	5	6	7	8	9
Screening/recruitment [1]	●									
Randomisation	●									
Complementary feeding	●	●	●	●	●	●	●	●	●	●
Routine visits/supervision	●	●	●	●	●	●	●	●	●	●
Examinations	●	●	●	●	●	●	●	●	●	●
Questionnaire[2]	●	●	●	●	●	●	●	●	●	●
Clinical examinations[3]	●	●	●	●	●	●	●	●	●	●
Anthropometry[4]	●	●	●	●	●	●	●	●	●	●
Blood sampling	●									●
Body composition	●									●
Motor development	●	●	●	●	●	●	●	●	●	●

[1]Screening for eligibility, e.g. age 6 month and weight-for-height z-score> -3
[2] Demographic and socio-economic data at baseline. Dietary assessment, compliance and morbidity data at baseline at each follow-up
[3] Measuring temperature, blood pressure and looking for symptoms of malnutrition or other diseases
[4] Weight, Height, Left arm circumference and Head circumference

3.14 Statistical analysis

The initial analysis was done before breaking the code. Quantitative data was entered using Epi Info while frequencies, means and median values were calculated using SPSS software. Differences in continuous variables between intervention and control groups were tested by two way analysis of variance (ANOVA) and between groups by multiple comparisons using STATA 12.

Baseline characteristics of the food groups were summarized using descriptive statistics. ANOVA was used to estimate the mean change per group with corresponding 95% Confidence Interval (CI). Selected pairwise comparisons were considered: CSB+ was used as

reference group. CIs and p-values were adjusted for multiplicity. The statistical analysis was based on intention to treat. A significance level of 5% was used.

Nutritional status was defined by length-for-age (LAZ), weight-for-length z-scores (WLZ) and weight-for-age z-scores (WAZ). LAZ and WLZ were changed from continuous variables into categorical variables divided by z-score lower than -2; z-score from -2 to -1; z-score from -1 to 0 and z-score above 0. The FFM index (FFMI) and the FM index (FMI) were calculated by FFM (kg)/height^2 (m^2) and FM (kg)/ height^2 (m^2), respectively. Indices were preferred instead of absolute numbers, because the Indices can describe the FFM and FM normalized for height and expressed in the same unit as body mass index (BMI).

3.15 Case definitions for indicators

1. Lean mass (fat free mass)

TABLE 1
Body composition of reference children

Age	Length	Wt	Fat		FFBM	Components of FFBM (% of body wt)						
						Protein	Water			Minerals		Carbo-hydrate
							TBW	Extra-cellular water	Cellular water	Osseous	Non osseous	
	cm	g	g	%	g							
A. Boys												
Birth	51.6	3545	486	13.7	3059	12.9	69.6	42.5	27.0	2.6	0.6	0.5
1 mo	54.8	4452	671	15.1	3781	12.9	68.4	41.1	27.3	2.6	0.6	0.5
2 mo	58.2	5509	1095	19.9	4414	12.3	64.3	38.0	26.3	2.4	0.6	0.5
3 mo	61.5	6435	1495	23.2	4940	12.0	61.4	35.7	25.8	2.3	0.6	0.5
4 mo	63.9	7060	1743	24.7	5317	11.9	60.1	34.5	25.7	2.3	0.5	0.4
5 mo	65.9	7575	1913	25.3	5662	11.9	59.6	33.8	25.8	2.3	0.5	0.4
6 mo	67.6	8030	2037	25.4	5993	12.0	59.4	33.4	26.0	2.3	0.5	0.4
9 mo	72.3	9180	2199	24.0	6981	12.4	60.3	33.0	27.2	2.3	0.6	0.5
12 mo	76.1	10150	2287	22.5	7863	12.9	61.2	32.9	28.3	2.3	0.6	0.5
18 mo	82.4	11470	2382	20.8	9088	13.5	62.2	32.3	29.9	2.5	0.6	0.5
24 mo	87.2	12590	2456	19.5	10134	14.0	62.9	31.9	31.0	2.6	0.6	0.5
3 yr	95.3	14675	2576	17.5	12099	14.7	63.9	31.1	32.8	2.8	0.6	0.5
4 yr	102.9	16690	2656	15.9	14034	15.3	64.8	30.5	34.2	2.9	0.6	0.5
5 yr	109.9	18670	2720	14.6	15950	15.8	65.4	30.0	35.4	3.1	0.6	0.5
6 yr	116.1	20690	2795	13.5	17895	16.2	66.0	29.6	36.4	3.2	0.6	0.5
7 yr	121.7	22850	2931	12.8	19919	16.5	66.2	29.1	37.1	3.3	0.6	0.5
8 yr	127.0	25300	3293	13.0	22007	16.6	65.8	28.3	37.5	3.4	0.6	0.5
9 yr	132.2	28130	3724	13.2	24406	16.8	65.4	27.6	37.8	3.5	0.6	0.5
10 yr	137.5	31440	4318	13.7	27122	16.8	64.8	26.7	38.0	3.5	0.6	0.5
B. Girls												
Birth	50.5	3325	495	14.9	2830	12.8	68.6	42.0	26.7	2.6	0.6	0.5
1 mo	53.4	4131	668	16.2	3463	12.7	67.5	40.5	26.9	2.5	0.6	0.5
2 mo	56.7	4989	1053	21.1	3936	12.2	63.2	37.1	26.1	2.4	0.6	0.5
3 mo	59.6	5743	1366	23.8	4377	12.0	60.9	35.1	25.8	2.3	0.6	0.5
4 mo	61.9	6300	1585	25.2	4715	11.9	59.6	33.8	25.8	2.3	0.5	0.4
5 mo	63.9	6800	1769	26.0	5031	11.9	58.8	33.0	25.9	2.2	0.5	0.4
6 mo	65.8	7250	1915	26.4	5335	12.0	58.4	32.4	26.0	2.2	0.5	0.4
9 mo	70.4	8270	2066	25.0	6204	12.5	59.3	32.0	27.3	2.3	0.5	0.4
12 mo	74.3	9180	2175	23.7	7005	12.9	60.1	31.8	28.3	2.3	0.5	0.5
18 mo	80.2	10780	2346	21.8	8434	13.5	61.3	31.5	29.8	2.4	0.6	0.5
24 mo	85.5	11910	2433	20.4	9477	13.9	62.2	31.5	30.8	2.4	0.6	0.5
3 yr	94.1	14100	2606	18.5	11494	14.4	63.5	31.3	32.2	2.5	0.6	0.5
4 yr	101.6	15960	2757	17.3	13203	14.8	64.3	31.2	33.1	2.5	0.6	0.5
5 yr	108.4	17660	2949	16.7	14711	15.0	64.6	31.0	33.6	2.5	0.6	0.5
6 yr	114.6	19520	3208	16.4	16312	15.2	64.7	30.8	34.0	2.6	0.6	0.5
7 yr	120.6	21840	3662	16.8	18178	15.2	64.4	30.3	34.1	2.5	0.6	0.5
8 yr	126.4	24840	4319	17.4	20521	15.2	63.8	29.6	34.2	2.5	0.6	0.5
9 yr	132.2	28460	5207	18.3	23253	15.1	63.0	28.9	34.1	2.5	0.6	0.5
10 yr	138.3	32550	6318	19.4	26232	15.0	62.0	28.1	33.9	2.5	0.6	0.5

Source: Fomon *et al*, 1982

2. Essential fatty acids

Table 3.5 Recommended dietary intakes for total fat and essential fatty acids for infants and young children (6–24 months) (FAO/WHO 2008)

Fat and fatty acids	**Reccommended dietary intake**
Fat in food supply	Gradual reduction to 35%E, depending on physical activity
Polyunsaturated fatty acid (PUFA)	<15%E (U-AMDR)*
n-6 PUFA(18:2 undifferentiated)	3.0–4.5%E (AI)†
n-3 PUFA (18:3 undifferentiated)	0.4–0.6%E (AI) ††

†recommended intake for LA only; ††recommended intake for ALA only; *AMDR, acceptable macronutrient distribution range; *U-AMDR, upper value for AMDR; AI, adequate intake (range); LCPUFA, long-chain polyunsaturated fatty acids.

3. Developmental milestones

Table 3.6 Gross motor developmental milestones WHO,2006

Milestone	**Normal limits**
Sitting without support	5-9 months
Standing with assistance	8-11 months
Hands and knees crawling	9-13 months
Standing alone	10-16 months
Walking with assistance	10-14 months
Walking alone	12-18 months

4. Anthropometry

Table 3.7: Anthropometric criteria to identify severe, moderate and at risk categories of acute malnutrition children 0-5 years

Indicator	**Severe Acute Malnutrition (SAM)**	**Moderate Acute Malnutrition (MAM)**	**At Risk of Malnutrition**
Infants less than 6 months			
Weight-for-Age z-score (WAZ)	WAZ < -3 Z score	Static weight or losing weight at home	Static weight or losing weight at home
Oedema	Oedema present	Oedema absent	Oedema absent
Other signs	Too weak to suckle or feed	Poor feeding	Poor feeding
Children 6 months to 5 years			
Weight for Height z-score (WHZ)	WHZ < -3 z-score	Between -3 to < -2 z-score	Between -2 to < -1 z-score
Height-for-Age (HAZ)	HAZ <-3 z-score	Between -3 to <-2 z-score	Between -2 to <-1 z-score
Mid-Upper-Arm Circumference (MUAC)	< 11.5 cm	11.5cm to 12.4 cm	12.5cm to 13.4cm
Oedema	Oedema present	Oedema absent	Oedema absent

3.16 Training of staff

The research team consisted of 23 staff members made up of a food technologist, a nutritionist, nurses, clinical officers, phlebotomists and field assistants. Before implementation of the study all staff was trained by the research team and experts from Kenya Medical Research Institute (KEMRI) for two weeks on how to take anthropometric, biochemical, clinical, dietary and gross motor milestone measurements as well as how to fill questionnaires. Moreover, regular supervision was done to ensure adherence to the study protocol.

3.17 Ethical considerations

The intervention group received WinFood CF or Winfood Lite which are nutritiously justified by a composition of grain amaranth, maize and animal-source-foods, and that has been tested to be acceptable to mothers in taste and appearance and composition. The control group was given the CSB+.

The ingredients were obtained from local producers, certified export quality. The foods were produced by Kenya Industrial Research and Development Institute (KIRDI) under a Kenya Bureau of Standards (KEBS) certified production facility conditions and under the responsibility of a graduate Food Scientist and Technologist. Annex 19 shows the preliminary analysis from Kenya Bureau of Standards (KEBS).

The trial was registered with the International Standard Randomized Controlled Trials; number ISRCTN 30012997. Furthermore, approval by the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (KNH-UONERC) number P334/08/2011 (See Annex 19), Kenya Ministry of Health (MOH) officials and community permissions were obtained before commencing the study. Written informed consent was

given by all caregivers before recruitment into the study (Annex 15).

The deuterium oxide which is naturally occurring and non-radioactive isotope of water and there are no health concerns about its use in any age group (IAEA, 2009). The biological samples were used for the terms of the study, and after the analyses were carried out, the samples were destroyed. All samples and data obtained in the study was kept anonymous. Only qualified health workers were recruited as filed staff. They were trained and assessed prior to and during the study to ensure best practices and quality control.

CHAPTER FOUR

RESULTS

4.1 Baseline characteristics of the study participants

Out of the 499 randomized, 428 (86%) were followed to completion. Of the 71 (14%) children lost to follow up, 63 (89%) were relocated and 8 (11%) died due to illness. Figure 4.1 shows the flow chart of the intervention inception to completion. Anthropometry and milestone assessments were carried out for all 499 children. Body composition (FFM and FM) analysis and whole blood EFA analysis was done for n=431 and n=251 infants respectively.

Figure 4.1 Trial profile

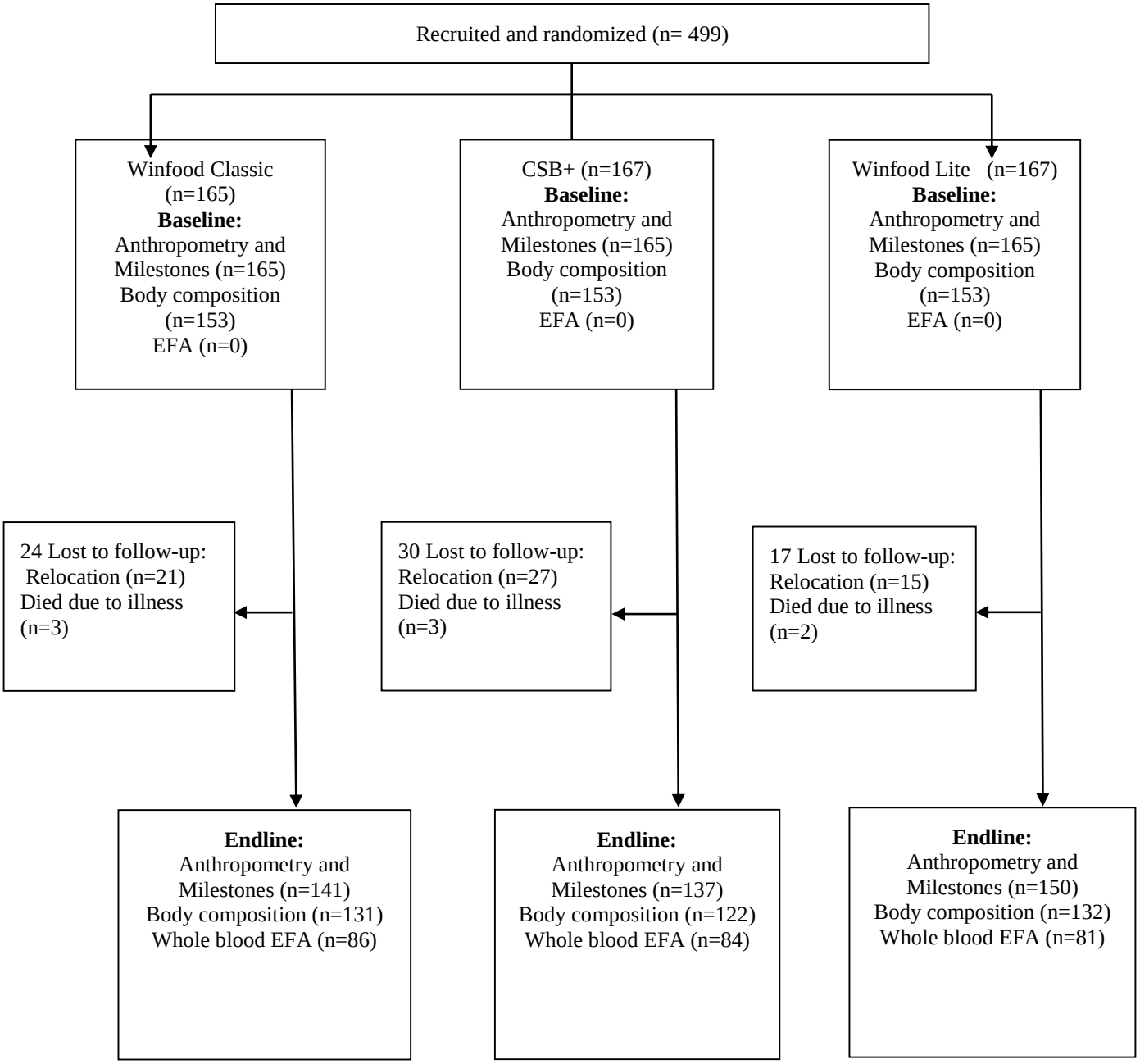


Table 4.1 shows the number of infants allocated per arm of the intervention and their baseline characteristics. The WFC group had 165 infants while the CSB+ group and WFL group both had 167 infants each. Girls were more in number in each of the cohorts, making up over 50% of infants in each group. Prevalence of breastfeeding was high in all groups: 98.2% in the

WFC group; 99.4% in the CSB+ group and 99.4% in the WFL group. The age of introduction to solid foods was approximately 3 months for all groups.

The weight at recruitment was also similar: 7.6 kg for the WFC group; 7.4 kg for the CSB+ group and 7.3 kg for the WFC group while all groups had a length of 65 cm. Haemoglobin levels were similar at approximately 10.7 g/dl in each group. Randomization resulted in baseline equivalence, although the WFL group had a lower weight-for-length z score (WLZ); a higher proportion of children with length-for-age z score (LAZ) <-2.0; and higher proportion of children with weight-for-age z score (WAZ) <-2.0.

Over 98% of caregivers were the biological mothers of the infants. The average age of the mothers was 26.4 years in the WFC group, 25.6 years in the CSB+ group and 25.3 years in the WFL group. The education level was similar in all groups with majority of mothers having 'primary incomplete' or 'primary completed' education level. Use of insecticide treated nets was high: 97% in the WFC group, 95% in the CSB+ group and 96% in the WFL group. The households had an average of 5 members and 1.8 children under the age of five years. The main source of income was farming and less than half of the households had access to safe water.

Table 4.1 Baseline characteristics of study participants by food group

	Winfood Classic	CSB+	Winfood Lite	P-value
Number of children	165	167	167	0.80
Child characteristics				
Sex, girls, n (percent)	89 (53.9)	84 (50.3)	86 (51.5)	0.22
Child age, months	6.0±0.2	6.1±0.2	6.0±0.2	0.24
Currently breastfeeding, n (percent)	162 (98.2)	166 (99.4)	166 (99.4)	0.53
Age introduced to solid foods, age in months, n (percent)	3.1±2.1	3.1±2.0	3.4±2.0	0.87
Weight, kg	7.6±1.0	7.4±1.0	7.3 ± 1.1	0.12
Length, cm	65.9 ± 2.9	65.3 ± 2.8	65.1 ±3.0	0.05
Haemoglobin, g/dl	10.8 ± 1.5	10.7±1.3	10.7±1.4	0.81
Weight-for-Length Z-score (WLZ)	0.25±1.2	0.25±1.2	0.16±1.7	0.75
Children with Z-score <-2, n (percent)	5 (3)	6 (3.5)	5 (3)	0.24
Length-for-age Z-score	-0.47±1.26	-0.76±1.13	-0.85±1.33	0.02
Children with Z-score <-2, n (percent)	14(8.5)	17(10.2)	34(20.4)	0.01
Weight-for-age Z-score	-0.18±1.16	-0.35±1.09	-0.48±1.26	0.07
Children with Z-score <-2, n (percent)	9(5.5)	10(6)	18(10.8)	0.85
Caretaker characteristics				
Age of main caretaker	26.4±6.9	25.6±6.7	25.3±5.4	0.3
Education level				
Unable to read and write, n (percent)	14 (8.5)	15 (9.0)	7 (4.2)	0.12
Primary incomplete, n (percent)	62 (37.6)	73 (43.7)	83 (49.7)	0.44
Primary completed, n (percent)	68 (41.2)	50 (29.9)	66 (39.5)	0.53
High school completed, n (percent)	18 (10.9)	26 (15.6)	8 (4.8)	0.17
University/ College graduate, n (percent)	3 (1.8)	3 (1.8)	3 (1.8)	0.86
Household characteristics				
Number of household members	5.9±2.2	5.6±2.2	5.4±2.1	0.18
Number of children <5 years	1.8±0.7	1.9±0.8	1.9±0.7	0.48
Access to protected well, n (percent)	77 (48.4)	75 (46.6)	68 (41.2)	0.77
Use of insecticide treated net, n (percent)	161 (97.6)	159 (95.2)	161 (96.4)	0.76
Primary income				
Farming, n (percent)	90 (55.6)	76 (47)	72 (44)	0.51

Data are mean±SD, n (percent) or median (range)

4.2 Effects of 9 months complementary feeding on FFM, FM and anthropometry

At all follow-up visits, all caregivers reported that the distributed foods had only been eaten by the infant and not shared with the household.

Table 4.2 shows the effects of 9 months complementary feeding on FFM, FM and weightfor all participants. During the nine month intervention period, overall mean weight increased from 7.45 kg to 9.60 kg. At 15 months, the weight increase was 2.15 (95% CI 1.95 to 2.31) kg. The increase in weight was due to a 2.3 (95% CI 2.02 to 2.45) kg increase in FFM and a 0.13 (95% 0.04 to 0.27) kg decline in FM.

The WFC group gained a weight 2.4 (95% CI 2.04 to 2.51) kg. The CSB+ group gained a weight of 2.2 (95% CI 2.01 to 2.75) kg. The WFL group gained a weight of 2.4 (95% CI 1.86 to 2.22) kg. The WFC group had 2.3 (95% CI 2.03 to 2.40) kg increase in FFM and 0.12 (95% CI 0.05 to 0.21) kg decrease in FM. The CSB+ group had 2.4 (95% CI 2.01 to 2.53) kg increase in FFM and 0.13 (95% CI 0.05 to 0.16) kg decrease in FM. The WFL group had 2.4 (95% CI 2.2 to 2.51) kg increase in FFM and 0.3 (0.01 to 0.40) kg decrease in FM.

The WFC group gained a weight difference of +0.13 (95% CI -0.09 to 0.34) kg from the CSB+ group (p=0.16); while the WFL group gained a weight difference of 0.04 (-0.17 to 0.25) kg from the CSB+ group (p=0.64). These differences were not statistically significant (p>0.05). The differences in length were 0.36 (95% CI -0.25 to 0.96) cm and 0.17 (95% CI -0.43 to 0.77) cm for the WFC and WFL groups respectively compared to the CSB+ group (p=0.17 and p=0.51) respectively. These differences in length (when compared with the CSB+ group) were not statistically significant (p>0.05).

The WFC group had +0.14 (95% CI -0.19 to 0.46) kg difference in fat free mass (FFM) from the CSB+ group (p=0.42) which is not statistically significant. The WFL group had 0.11

(95% CI -0.13 to 0.51) kg difference in FFM from the CSB+ group (p=0.23) which is also not statistically significant (p>0.05). Differences in fat mass (FM) were -0.03 (95% CI -0.03 to 0.15) kg in the WFC group and 0.13 (95% CI -0.1 to 1.26) kg in the WFL group compared to the CSB+ group (p=0.78 and p=0.28) respectively, which is also not statistically significant (p>0.05).

Table 4.2 Effects of 9 months complementary feeding on body composition and anthropometry, all participants.

	Winfood Classic	CSB+	Winfood Lite
Fat-free mass, kg			
Baseline	5.98 [5.86;6.11] (152)	5.89 [5.76;6.02] (147)	5.80 [5.65;5.94] (143)
Endpoint	8.24 [8.03;8.45] (113)	8.28 [8.06;8.50] (110)	8.16 [7.97;8.35] (122)
Difference from CSB+	0.14 [-0.19;0.46] (113)	Ref.	0.11 [-0.13;0.51] (122)
	p=0.42		p=0.23
Fat mass, kg			
Baseline	1.58 [1.49;1.68] (152)	1.57 [1.46;1.68] (147)	1.55 [1.44;1.65] (143)
Endpoint	1.46 [1.28;1.64] (113)	1.44 [1.27;1.61] (110)	1.27 [1.14;1.41] (122)
Difference from CSB+	-0.03 [-0.03;0.15] (113)	Ref.	0.13 [0.1;1.26] (122)
	p=0.78		p=0.28
Weight, kg			
Baseline	7.58 [7.42;7.74] (165)	7.43 [7.27;7.59] (167)	7.34 [7.18;7.50] (167)
Endpoint	9.66 [9.46;9.86] (141)	9.66 [9.45;9.86] (137)	9.50 [9.31;9.69] (150)
Difference from CSB+	0.13 [-0.09;0.34] (141)	Ref.	0.04 [-0.17;0.25] (150)
	p=0.16		p=0.64
Length, cm			
Baseline	65.9 [65.5;66.3] (165)	65.3 [64.9;66.8] (167)	65.1 [64.7;65.6] (167)
Endpoint	75.3 [74.8;75.8] (141)	75.3 [74.7;75.8] (137)	74.6 [74.1;75.1] (150)
Difference from CSB+	0.36 [-0.25;0.96] (141)	Ref.	0.17 [-0.43;0.77] (150)
	p=0.17		p=0.51

Data are presented as mean difference [95% confidence interval] (n)

To assess the growth over time, the changes in mean anthropometric indices were plotted as a function of the monthly follow-up visits period (see figures 4.2, 4.3 and 4.4). For WAZ, the pattern showed that the WFL group had an earlier decline compared to the WFC and CSB+ groups (p=0.51). The WFC group showed similar patterns with the CSB+ group in WHZ, LAZ and WAZ over the entire intervention period. No differences were found in the change of z-scores over time between the WFC group and the reference group (p=0.62). However,

the WFL group had lower anthropometric indices compared to the reference group LAZ (p=0.02). Difference in WAZ was not statistically significant (*p*=0.07).

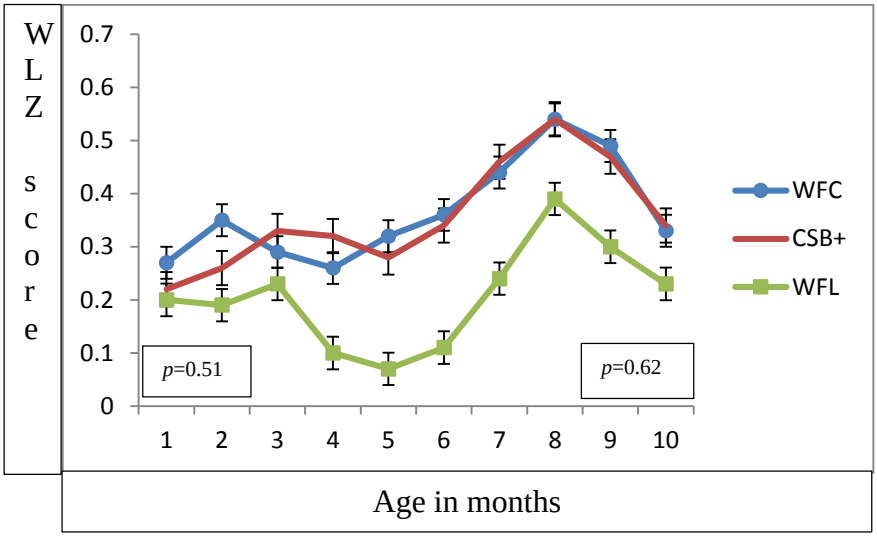


Figure 4.2: Mean WLZ score from baseline

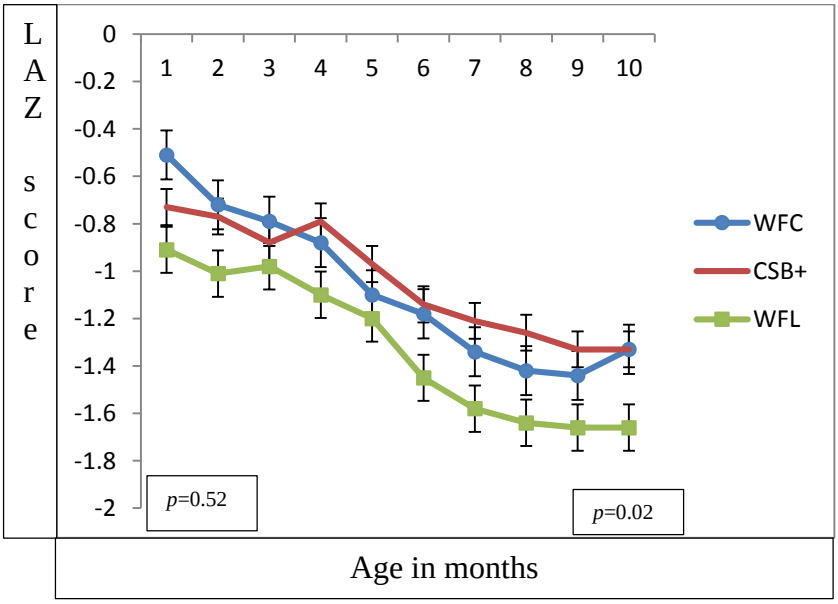


Figure 4.3: Mean LAZ score from baseline

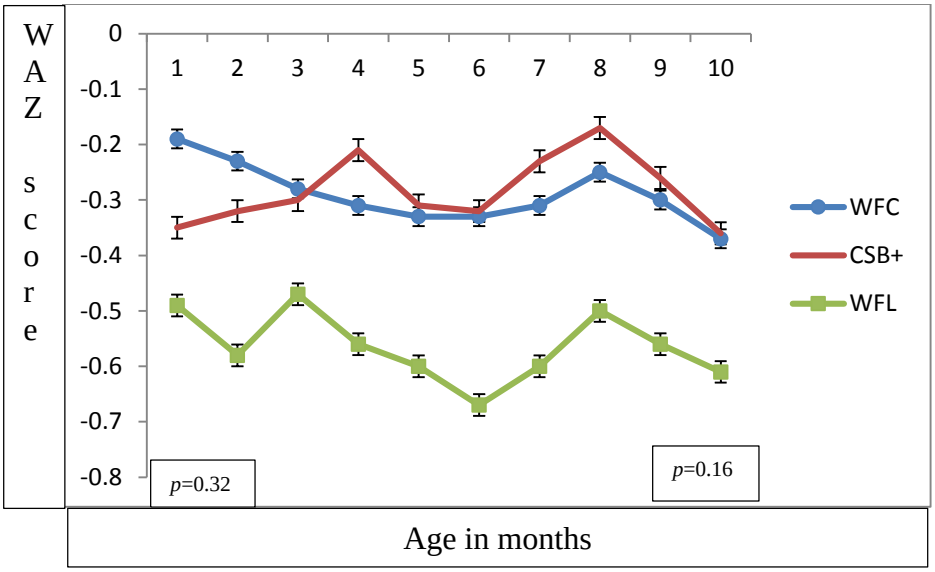


Figure 4.4: Mean WAZ score from baseline

Figure 4.5 shows total weight (kg), FFM (kg), FM (kg) and %FM by sex at baseline (6 months). Males had total weight of 7.17 (95% CI 7.57 to 7.84) kg. Females had total weight of 7.22 (95% CI 7.10 to 7.38) kg. Males had FFM of 6.15 (95% CI 6.03 to 6.26) kg, while females had FFM of 5.74 (95% CI 5.61 to 5.87) kg. FM was 1.59 (95% CI 1.50 to 1.68) kg for males and 1.46 (95% CI 1.34 to 1.56) kg for females. Males had 20.28 %FM, while females had 20.3% FM at baseline.

Figure 4.6 shows total weight (kg), FFM (kg), FM (kg) and %FM by sex at endline (15 months). Males had a total weight of 9.87 (95% CI 9.70 to 10.04) kg. Females had total weight of 9.35 (95% CI 9.21 to 9.50) kg. Males had FFM of 8.48 (95% CI 8.30 to 8.67) kg, while females had FFM of 7.99 (95% CI 7.84 to 8.14) kg. FM was 1.39 (95% CI 1.25 to 1.53) kg for males and 1.39 (95% CI 1.26 to 1.52) kg for females. Males had 13.83 %FM, while females had 14.57 %FM at endline.

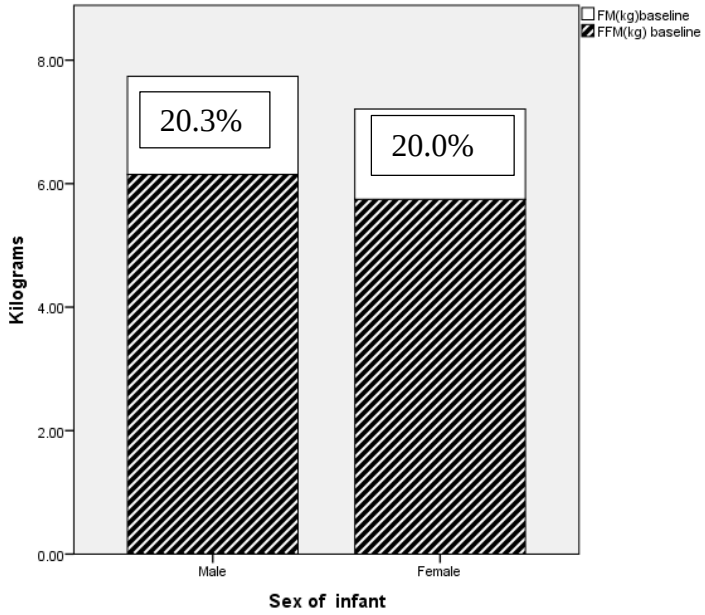


Figure 4.5: Total weight (kg), FFM (kg) FM (kg) and FM% by sex at baseline

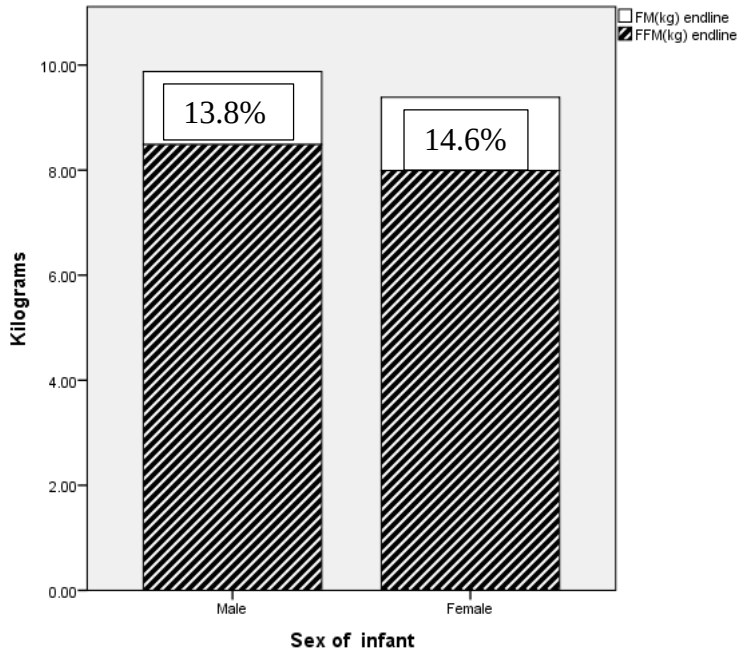


Figure 4.6: Total weight (kg), FFM (kg) FM (kg) and FM% by sex at endline

Table 4.3 shows the growth and body composition of infants according to sex. At six months, boys weighed 0.5 (95% CI 0.07 to 0.68) kg more than girls (p<0.001) due to 0.45 (95% CI 0.01 to 0.77) kg more FFM and 0.1 (0.05 to 0.21) kg more FM. Similarly, boys were 1.24 (95% CI 0.02 to 2.03) cm longer than girls at 6 months (p<0.001). Furthermore, boys had greater Mid Upper Arm Circumference (MUAC) at six months by 0.33 (0.07 to 0.59) cm (p<0.01) compared to girls. A similar pattern was seen when the children reached the age of 15 months. Boys weighed 0.52 (95% CI 0.12 to 0.69) kg more than girls (p<0.001) due to 0.49 (0.1 to 0.68) kg more FFM. There was no difference in FM between the sexes (p=0.98). Boys had a 0.28% FM higher than girls at six months (p=0.77) but at 15 months, girls had 0.74% FM higher than boys (p=0.41). There was therefore no statistically significant difference in FM between the boys and the girls at both 6 months and 15 months (p>0.05). At 15 months, boys were also longer by 1.24 (95% CI 0.09 to 2.74) cm (p<0.001) and had greater MUAC by 0.83 cm (95% CI 0.01 to 0.95) (p=0.88). However, the difference in MUAC between the boys and the girls at 15 months was not statistically significant (p>0.05).

At 6 and 15 months respectively, boys had a 0.36 (95% CI 0.31 to 0.62) kg/m^2 and 0.33 (95% CI 0.02 to 0.51) kg/m^2 higher FFMI compared to girls respectively; (p=0.055) and (p=0.63) respectively, which was not statistically significant. There was no difference in FMI between the sexes at neither 6 nor 15 months; (p=0.91) and (p=0.38) respectively. There were significant differences between the sexes in Weight-for-length z scores at both 6 months and 15 months (p=0.02 and p=0.046) respectively.

Table 4.3: Growth and body composition indices by sex

	Male	**Female**	**P-value**
Weight, kg			
Baseline	7.71 [7.57;7.84] (240)	7.22 [7.10;7.38] (259)	***p*<0.001**
Endpoint	9.87 [9.70;10.04] (204)	9.35 [9.21;9.50] (224)	***p*<0.001**
Length, cm			
Baseline	66.20 [65.84;66.55] (240)	64.76 [64.41;65.10] (259)	***p*<0.001**
Endpoint	75.70 [75.29;76.1] (204)	74.46 [74.08;74.84] (224)	***p*<0.001**
MUAC, cm			
Baseline	14.36 [14.20;14.52] (240)	14.03 [13.89;14.18] (259)	***p*<0.001**
Endpoint	14.86 [14.69;15.63] (204)	14.03 [13.89;14.18] (224)	*p*=0.88
Fat free mass, kg			
Baseline	6.15 [6.03; 6.26] (211)	5.74[5.61;5.87] (231)	***p*<0.001**
Endpoint	8.48 [8.30;8.67] (162)	7.99 [7.84;8.14] (183)	***p*<0.001**
Fat mass, kg			
Baseline	1.59 [1.50;1.68] (211)	1.46 [1.34;1.56] (231)	*p*=0.10
Endpoint	1.39 [1.25;1.53] (162)	1.39 [1.26;1.52] (183)	*p*=0.98
% Fat mass			
Baseline	20.28 [19.30;21.27] (211)	20.0 [18.50;21.51] (231)	*p*=0.77
Endpoint	13.83 [12.56;15.11] (162)	14.57 [13.37;15.77] (183)	*p*=0.41
Fat free mass index			
Baseline	14.03 [13.80;14.26] (211)	13.67 [13.40;13.95] (231)	*p*=0.055
Endline	14.74 [14.48;14.99] (162)	14.41 [14.18;14.64] (183)	*p*=0.63
Fat mass index			
Baseline	3.62 [3.42;3.82] (211)	3.47 [3.21;3.73] (231)	*p*=0.91
Endpoint	2.44 [2.21;2.68] (162)	2.46 [2.24;2.68] (183)	*p*=0.38
WLZ			
Baseline	0.19 [0.03;0.35] (240)	0.24 [0.11;0.38] (259)	***p*=0.02**
Endpoint	0.23 [0.09;0.38] (204)	0.36 [0.24;0.48] (224)	***p*=0.046**

Data are presented as mean [CI 95%] (n)

4.3 Effect of 9 months complementary feeding on whole blood EFA

The four essential fatty acids of public health importance are 18:2n-6 Linolenic acid (LA), 18:2n-3 Arachidonic acid (AA), 20:4n-6 Alpha linolenic acid (ALA) and 22:6n-3 Docosahexaenoic acid (DHA). Whole blood was collected at endline only, following 9 months of intervention.

Table 4.4 shows the EFA and head circumference per food group. The WFC group had 17.2% LA; the CSB+ group had 16.9% LA and the WFL group had 16.8% LA in whole blood. There was no difference in relative % LA in whole blood in the WFC group compared to the CSB+ group (p=0.50). Similarly, there was no difference in relative % LA in whole blood in the WFL group compared to the CSB+ group (p=0.83).

The WFC group had 7.3% AA; the CSB+ group had 7% while the WFL group had 7.3% AA in whole blood. There was significant difference in relative % AA in whole blood in the WFC group compared to the CSB+ group (p=0.004) but no difference in relative % AA in whole blood in the WFL group compared to the CSB+ group (p=0.79).

The WFC group had 0.2% ALA, while the CSB+ group and WFL group both had 0.2% and 0.2% respectively. There was no difference in relative % ALA in whole blood in the WFC group compared to the CSB+ group (p=0.46), neither was there a difference in relative % ALA in whole blood in the WFL group compared to the CSB+ group (p=0.37).

The WFC group had 2.4% DHA, the CSB+ group had 2.2% while the WFL group had 2.4% DHA in whole blood. There was no difference in relative % DHA in whole blood in the WFC group compared to the CSB+ group (p=0.30) neither was there any difference in relative % DHA in whole blood in the WFC group compared to the CSB+ group (p=0.52).

Despite not being of public health importance, there were significant differences in 10:0 decanoic acid between WFL and CSB+ (p=0.04). Similarly, there was marked difference between 23:0 tricosanoic acid and AA between WFC and CSB+ (p=0.02) and (p=0.004) respectively.

Compared to the reference group (CSB+ group), differences in the head circumference after 9 months intervention were not statistically significant (p>0.05) for both WFC and WFL groups.

Table 4.4: Essential Fatty Acids and head circumference per food group

	WFC	CSB+	WFL
Whole-blood Essential Fatty Acids at Endpoint, (Relative %)			
18:2n-6 Linolenic acid	17.19 [16.5;17.9] (86)	16.89 [16.2;17.5] (84)	16.80 [16.23;17.4] (81)
Difference from CSB+	-0.3 [-1.17;0.58]	Ref.	0.1 [-0.8;1.0]
	p=0.50		p=0.83
18:3n-3 Arachidonic acid	7.25 [6.8;7.7] (86)	7.00 [6.6;7.4] (84)	7.30 [6.8;7.82 (81)
Difference from CSB+	**0.05 [0.02;0.08]**	Ref.	0.03 [-0.01;0.06]
	***p*=0.004**		p=0.79
20:4n-6 Alpha linolenic acid	0.19 [0.2;0.2] (86)	0.24 [0.2;0.3] (84)	0.21 [0.2;0.2] (81)
Difference from CSB+	-0.25 [-0.9;0.4]	Ref.	-0.3 [-0.96;0.36]
	p=0.46		p=0.37
22:6n-3 Docosahexaenoic acid	2.43 [2.3;2.6] (86)	2.23 [2.1;2.5] (84)	2.38 [2.2;2.6] (81)
Difference from CSB+	-0.14 [-0.41;0.13]	Ref.	-0.09 [-0.36;0.18]
	p=0.30		p=0.52
10:0 Decanoid acid	0.03 [0.02;0.04] (86)	0.5 [0.03;0.06] (84)	0.03 [0.02;0.03] (81)
Difference from CSB+	0.01 [-0.01;0.02]	Ref.	**0.02 [0.01;0.04]**
	p= 0.4		***p*=0.04**
23:0 Tricosanoic acid	0.20 [0.19;0.22] (86)	0.19 [0.17; 0.20] (84)	0.2 [-0.18;0.23] (81)
Difference from CSB+	**-0.06 [-0.11;-0.01]**	Ref.	-0.03 [-0.08;0.02]
	***p*=0.02**		p=0.24
Head circumference, cm			
Baseline	43.45 [43.2;43.7] (165)	43.55 [43.3;43.8] (167)	43.12 [43.9;43.5] (167)
Endpoint	46.99 [46.7;47.3] (141)	46.82 [46.1;47.6] (138)	46.79 [46.6;47.0] (150)
Difference from CSB+	-0.25 [-0.82;0.32]	Ref.	0.12 [1.09;0.30]
	p=0.37		p=0.63

Data are presented as mean percentage [CI 95%] (n)

4.4 Effects of 9 months complementary feeding on gross motor milestone attainment

Table 4.5 shows gross motor milestones attainment in age in months per food group.

Table 4.5: Gross motor milestone attainment in age in months by food group

	WFC	CSB+	WFL
Sitting without support	4.9 [4.7;5.0] (165)	4.8 [4.6;5.0] (165)	4.8 [4.7;5.0] (166)
Difference from CSB+	-0.07 [-0.3;0.17]	Ref.	-0.04 [-0.28;0.20]
	p=0.57		*p*=0.74
Hands and knees crawling	6.8 [6.5;7.0] (159)	6.9 [6.6;7.2] (162)	7.9 [6.8;7.4] (161)
Difference from CSB+	0.16 [-0.25;0.56]	Ref.	-0.17 [-0.57;0.23]
	p=0.45		*p*=0.41
Standing with assistance	7.6 [7.3;7.9] (161)	7.6 [7.4;7.9] (162)	7.84 [7.5;8.1] (160)
Difference from CSB+	0.01 [-0.39;0.40]	Ref.	-0.23 [-0.63;0.17]
	p=0.97		*p*=0.25
Walking with assistance	8.5 [8.1;8.8] (156)	8.6 [8.2;8.9] (158)	8.6 [8.2;9.0] (160)
Difference from CSB+	0.1 [-0.39;0.59]	Ref.	-0.07 [-0.56;0.42]
	p=0.69		*p*=0.77
Standing without assistance	9.8 [9.47;10.19] (150)	10.1 [9.7;10.4] (155)	10.5[10.1;10.8] (156)
Difference from CSB+	0.25 [-0.24;0.74]	Ref.	-0.37 [-0.86;0.12]
	p=0.32		*p*=0.13
Walking without assistance	11.5 [11.2;11.9] (138)	11.8 [11.6;12.0] (144)	12.1 [11.7;12.4] (141)
Difference from CSB+	0.28 [-0.16;0.71]	Ref.	-0.26 [-0.69;0.17]
	p=0.21		*p*=0.23

Data are presented as mean [CI 95%] (n)

<u>Sitting without support</u>

The intervention began at 6 months of age. According to caretaker reports; The WFC group attained sitting without support at 4.9 [95% CI 4.7; 5.0] months. The CSB+ group attained sitting without support at 4.8 [95% CI 4.6; 5.0] months. The WFL group attained sitting without support at 4.8 [95% CI 4.7; 5.0] months. There was no significant difference in the WFC and WFL groups compared to CSB+ (p=0.57 and p=0.74) respectively.

Hands and knees crawling

The WFC group attained hands and knees crawling at 6.8 [95% CI 6.5; 7.0] months. The CSB+ attained hands and knees crawling at 6.9 [95% CI 6.6; 7.2] months. The WFL group attained hands and knees crawling at 7.9 [95% CI 6.8; 7.4] months. There was no significant in the WFC and WFL groups compared to CSB+ (p=0.45 and p=0.41) respectively.

Standing with assistance

The WFC group attained standing with assistance at 7.6 [95% CI 7.3; 7.9] months. The CSB+ group attained standing with assistance at 7.6 [95% CI 7.4; 7.9] months. The WFL group attained standing with assistance at 7.8 [95% CI 7.5; 8.1] months. There was no significant difference in the WFC and WFL groups compared to CSB+ (p=0.97 and p=0.25) respectively.

Walking with assistance

The WFC group attained walking with assistance at 8.5 [95% CI 8.1; 8.8] months. The CSB+ group attained walking with assistance at 8.6 [95% CI 8.2; 8.9] months. The WFL group attained walking with assistance at 8.6 [95% CI 8.2; 9.0] months. There was no significant difference in the WFC and WFL groups compared to CSB+ (p=0.69 and p=0.77) respectively.

Standing without assistance

The WFC group attained standing without assistance at 9.8 [95% CI 9.5; 10.1] months. The CSB+ group attained standing without assistance at 10.1 [95% CI 9.7; 10.4] months. The WFL group attained standing without assistance at 10.5 [95% CI 10.1; 10.8] months. There

was no significant difference in the WFC and WFL groups compared to CSB+ (p=0.32 and p=0.13) respectively.

Walking without assistance

The WFC group attained walking without assistance at 11.5 [95% CI 11.2; 11.9] months. The CSB+ group attained walking without assistance at 11.8 [95% CI 11.6; 12.0] months. The WFL group attained walking without assistance at 12.1 [95% CI 11.7; 12.4] months. There was no significant difference in the WFC and WFL groups compared to CSB+ (p=0.21 and p=0.23) respectively.

There were no significant differences between WFC and WFL from CSB+ in gross motor milestones. However there were significant differences between the two Winfood products in standing without assistance (p=0.01) and walking without assistance (p=0.02), the WFC cohort peforming better than WFL cohort.

CHAPTER FIVE

DISCUSSION

This study aimed to assess the efficacy on lean mass accrual, essential fatty acids profile and gross motor development among Kenyan infants receiving a daily portion of either two versions of locally produced complementary foods based on maize and germinated amaranth grains with (Winfood Classic [WC]) or without (Winfood Lite [WL]) animal source foods namely edible termites and small fish compared to a standard food aid product ('Corn-Soy-Blend plus [CSB+]).Contrary to the hypothesis, the WFC and WFL cohorts did not have higher lean mass accrual than the CSB+ cohort. There was significant difference in relative % AA in whole blood in the WFC group compared to the CSB+ group. There were no differences in levels of LA, ALA and DHA among the three foods. There were significant differences in decanoic acid between WFL and CSB+. Similarly, there was marked difference in tricosanoic acid between WFC and CSB+. There were no differences in head circumference. Contrary to the hypothesis, the WFC cohort and the WFL cohort did not have any different gross motor milestone acquisition that the CSB+ cohort.

The strength of this study was the use of FFM as the primary outcome which was proved as a sensitive measure of growth. Comparing the body composition data to reference data based on healthy infants from the US (Fomon, 1985) the infants in the present study at six months of age, had similar FFM, but lower FM. At endpoint all groups had a lower FFM and FM compared to this reference group (calculated by interpolating data from 12 to 18 months). Thus, body fat in this Kenyan population is low, compared to healthy American children, and intervention with daily diet of nutritious complementary food did not contribute to increased fat deposition.

The first limitation of the study was that there was no non intervention control group since it would be unethical to deny infants foods that are perceived to have nutritional and health benefits. The trial foods were therefore compared against CSB+. The second limitation was that compliance and breastfeeding were primarily self-reported by the caregiver. The third limitation of the study was the lack of adequate benchmark figures for comparison, especially those from developing countries.

Based on a large longitudinal sample measured with senstitive methods, this study presents the effect of improved complementary foods in a randomized control trial in a resource poor setting. This study represents a first step toward providing a useful reference tool on FM and FFM from a rural sub-Saharan African context, important for clinical care and research.

5.1 Baseline characteristics of the study population

Infants were recruited at 6 months of age in accordance with the WHO recommendations for inception of complementary feeding. Girls were a higher number than boys constituting 51.9% of infants randomized. Breastfeeding practice was high throughout the intervention, with prevalence of over 90% for all groups. However, the prevalence of exclusive breastfeeding was low since introduction to other foods was approximately 3 months of age for all groups. Family planning was widely accepted in the community, which led to birth spacing and longer duration of breastfeeding.

Infants had similar weight, length and haemoglobin levels at baseline. The WFL group had a disadvantage in a lower WLZ<-2; higher proportion of infants with LAZ<-2 and higher proportion of infants with WAZ<-2.

The mean age of mothers was 25 years (range 15 to 42). Generally, participants were of low socio-economic status, had low levels of education. The average number of household

members was 5, and average number of children under 5 in the household was 1.8. More than half the households did not have access to safe drinking water. Use of insecticide treated nets was high (over 95%). The primary income was farming, albeit as farm hands in sugarcane plantations

In this study, out of the 499 randomized, 428 (86%) were followed to completion. Of the 71 (14%) children lost to follow up, 63 (89%) were relocated and 8 (11%) died due to illness. Relocation was observed to be due to lack of employment, leading to high mobility and migration as men work outside the area and the married women accompany their husbands. Equally important was cases of domestic disputes; causing the mother to move back with her parents, often with the infant, until the dispute was resolved. A contributor to low follow up was travel for funeral ceremonies out of the study area that would take weeks and sometimes months.

Deaths were caused by high fever and vomiting which was diagnosed as malaria by clinical staff. Initially there was shortage of anti-malarial drugs in the clinics. Mothers were required to purchase anti-malaria drugs at commercial pharmacies, of which they could not afford. Subsequently, the study budget was reviewed and a provision made to purchase medicines for the study participants. This action halted cases of deaths among the infants in the study.

Low socio-economic status has been linked to high levels of acute and chronic malnutrition (UNICEF, 2010). Growth faltering is prevalent in developing countries where children are susceptible to infection and malnutrition. Children less than 2 years of age are particularly vulnerable due to their increased demand for nutrients as they transition from breastfeeding exclusively to consuming complementary foods. Limited variety of complementary foods has been associated with low nutrient density adequacy of the foods, which may cause malnutrition and developmental delays (UNICEF, 2010).

Ensuring optimal complementary feeding practices for young children living in developing countries is a global public health priority because of their overwhelming importance for optimal growth, development, and well-being of infants and young children. In this respect, the WHO and UNICEF provide, as a high priority action in their Global Strategy for Infant and Young Child Feeding, guidance on appropriate complementary feeding, with an emphasis on the use of suitable locally available foods (UNICEF/WHO, 2002).

Several approaches exist for designing population-specific recommendations that are based on locally available foods. These approaches usually involve expert consultation that takes into account the most common nutritional problems, as well as such factors as cultural food consumption patterns, acceptable foods (available, affordable, and regularly consumed), realistic food portion sizes, and the impact of recommendations on other nutrients and the environment (Ferguson *et al*, 2006).

Breastmilk intake continues to make a substantial contribution to the energy and nutrient intakes of infants and young children in developing countries after the age of 6 months, but nutrient needs from complementary foods increase as breastmilk intake declines with age (PAHO/WHO, 2002). Dewey and Brown (2003) have reviewed the amounts of energy and other nutrients needed from complementary foods, taking into account the average breastmilk intake and its nutrient composition during each age interval among children in developing countries (Dewey & Brown 2003).

5.2 Effect of improved complementary foods on lean body mass

Contrary to the hypothesis, the WFC and WFL cohorts did not have higher lean mass accrual than the CSB+ cohort. However, the Winfoods were equal in lean mass accrual. At all

follow-up visits, all caregivers reported that the distributed foods had only been eaten by the infant and not shared with the household. It was difficult to verify compliance to the regime.

The results are similar to those from Cambodia (Skau, 2014). There was no difference in the primary outcome of FFM. The lack of difference in FFM in the current study could be due to the food composition, for example high fibre content, presence of antinutrients and low bioavailability. In addition, the lack of difference in FFM could have been due to due to contextual factors such as sanitation and hygiene, which were not measured in the study.

To assess the growth over time, the changes in mean anthropometric indices were plotted as a function of the monthly follow-up visits period. For WAZ, the pattern showed that the WFL group had an earlier decline compared to the WFC and CSB+ groups. The WFC group showed similar patterns with the CSB+ group in WHZ, LAZ and WAZ over the entire intervention period. No differences were found in the change of z-scores over time between the WFC group and the reference group. However, the WFL group had lower anthropometric indices compared to the reference group. WFL group had a lower WAZ score at baseline and the trend continued throughout the intervention.

At six months, boys weighed significantly more than girls due to higher FFM and FM. Similarly, boys were significantly longer than girls at 6 months. A similar pattern was seen when the children reached the age of 15 months. Boys weighed notably more than girls due to higher FFM. There was no difference in FM between the sexes at endline. There was no difference in FMI between the sexes at neither 6 nor 15 months. There were significant differences between the sexes in Weight-for-length z scores at both 6 months and 15 months. This confirms what is known concerning the different growth velocities among the sexes, hence the different growth and reference charts for the sexes (WHO, 2006).

The primary outcome in this study was lean mass (FFM) measured by deuterium dilution

technique. The strengths of this technique are that it provides data on both lean mass and fat mass, and is easy to use in infants. The limitations are that a proportion of dosings are unsuccessful due to spilling of the isotope, and that the analysis using mass spectrophotometry is time-consuming and expensive. A second limitation of the technique is that total body water must be converted to lean mass using an assumed value for the hydration of lean tissue (Wells, 2000).

Very limited data on body composition during infancy are currently available. Furthermore, those data that do exist tend to come from industrialized populations, and have often been collected in the context of concern over macrosomic infants and the risk of later obesity or diabetes. These studies use %FM as the primary outcome. There are no universal standards for FFM, FM or %FM that have been established and accepted.

Adu-Afarwuah *et al* (2007) conducted a randomized comparison of 3 types of micronutrient supplements for home fortification of complementary foods in Ghana in order to determine effects on growth body composition and motor development. They compared the home fortification of complementary foods with Sprinkles (SP) powder, crushable Nutritabs (NT) tablets, or energy-dense (108 kcal/d), fat-based Nutributter (NB) and randomly assigned 313 Ghanaian infants. Body composition was measured by anthropometric techniques. All 3 supplements had positive effects on motor milestone acquisition by 12 months compared with no intervention, but only NB affected growth (Adu-Afarwuah et al, 2007).

A study in Peru found that addition of zinc to prenatal folic acid_iron supplementation neither had any effect on birth anthropometric measures nor on body composition of the newborn (Caulfield *et al*, 1999). However, maternal zinc supplementation in this population was associated with better growth of the offspring beginning at month 4 and continuing through to month 12 (Iannotti *et al*, 2008). A recent randomized antenatal micronutrient trial in Nepal

(Stewart *et al*, 2009) found that maternal supplementation with folic acid_iron_zinc resulted in an increased mean height and a reduction in the mean triceps skinfold thickness, subscapular skinfold thickness, and arm fat area; however, no significant differences among the groups were found in the mean weight or BMI-for-age z-scores, waist circumference or arm muscle area at 6_8 years of age. Prenatal dietary supplementation to rural Gambian women did not affect body composition of the offspring at 11to 17 years of age (Hawkesworth *et al*, 2008).

In Bangladesh, the effects of maternal food and micronutrient supplementation and exclusive breastfeeding counseling on growth of offspring aged 0-54 months and their body composition at 54 months of age were studied. Body composition was assessed by leg-to-leg bioelectrical impedance analysis (BIA). Early food supplementation during pregnancy reduced the occurrence of stunting among boys but not girls, while prenatal MMS increased the proportion of stunting. Food and micronutrient supplementation or EBF intervention did not affect body composition of offspring at 54 months of age (Kahn, 2013).

A study from Ethiopia showed that infants with low birth weight and who had a low weight gain until 6 months of age maintained a deficit in fat-free mass (FFM). If the infants grew fast, they resolved the FFM deficit but at the cost of greater fatness at 6 months of age (Andersen, 2013). Another study found that Gambian infants had less FFM and fat mass (FM) than UK infants at 4 months of age and the FFM deficit increased by age, whilst the FM decreased (Wells, 2009).

Results from the Winfood Cambodia trial (Skau, 2014) show that infants at six months of age had similar FFM, but lower FM compared to the body composition data to reference data based on healthy infants from the US (Fomon, 1982). At endpoint all groups had a lower FFM and FM compared to this reference group.

5.3 Effect of improved complementary foods on whole blood EFA

The four essential fatty acids of public health importance are 18:2n-6 Linolenic acid (LA), 18:2n-3 Arachidonic acid (AA), 20:4n-6 Alpha linolenic acid (ALA) and 22:6n-3 Docosahexaenoic acid (DHA). Whole blood was collected at endline only, following 9 months of intervention.

There was significant difference in relative % AA in whole blood in the WFC group compared to the CSB+ group. Levels of LA, ALA and DHA were similar among the three foods. Despite not being of public health importance, there were significant differences in 10:0 decanoic acid between WFL and CSB+. Similarly, there was marked difference between 23:0 tricosanoic acid and AA between WFC and CSB+. There were no differences in head circumference.

Dietary essential fatty acids (EFA) have long been considered part of the lipid supply necessary for energy, growth, cellular metabolism, and muscle activity. The fact that some EFAs serve as indispensable dietary precursors for eicosanoid formation has provided greater significance to the study of their role in health and disease. During the 1980s and 1990s, attention was placed on the effect of n-3 and n-6 EFAs in normal fetal and infant development (Sprecher, 1981; Bazan, 1989; Willis, 1984; Uauy and Hoffman, 1991; Simopoulos, 1991; Innis, 1991; Uauy et al, 1989). Extensive research has been carried out in industrialized countries documenting sources and benefits of EFAs. There is however limited data on the same in low-income countries.

Michaelsen and colleagues conducted a review of the available data on dietary intake of fatty acids in low-income countries, with special focus on pregnancy and the first years of life. The review includes data on the fatty acid content of relevant foods, the amount of long-chain polyunsaturated fatty acids (LCPUFA) in breast milk, fatty acid intake during the

complementary feeding period, and data on fatty acid availability in selected low-income and middle-income countries based on Food and Agriculture Organization (FAO) food balance data (Michaelsen *et al*, 2011).

The report emphasized the importance of breastfeeding during the complementary feeding period; breast milk being a key source of PUFA because complementary foods in low-income countries are typically cereal-based with low fat content, and therefore contribute very little PUFA.

In all the 13 countries included in the food balance calculations, there was a strong positive association between the economic status of the country (GDP) and the supply of total fat and n-3 fatty acids. Most of the fat and PUFA intake comes from vegetable oils and cereals among families living on a primarily plant-based diet. Fat content of cereals is low and mainly located in the outer layers of the kernels, so whole grains are a better source of fatty acids than refined cereals. A very important source of PUFA in low-income countries is vegetable oils, but they differ considerably in PUFA content and especially in the LA/ALA ratio. Soybean oil and canola/ rapeseed oil have the highest ALA content (Michaelsen *et al*, 2011).

Furthermore Michaelsen (2011) identified fish and seafood as the most important sources of n-3 LCPUFA, with marine fish generally a better source than freshwater fish. 'For poor rural populations in particular, farming of small fish in rice fields and lakes could provide an important extra supply of n-3 fatty acids. Other potentially sustainable and cheap sources of LCPUFA are indigenous foods (e.g. amphibia, worms and insects) which are underutilized in many populations' (Michaelsen *et al*, 2011).

5.4 Effect of improved complementary foods on gross motor milestones

Six milestones were selected for study: sitting without support, hands-and-knees crawling, standing with assistance, walking with assistance, standing alone and walking alone. These milestones were considered to be universal, fundamental to the acquisition of self sufficient erect locomotion, and simple to test and evaluate (WHO, 2006). There were two independent sources of information about the achievement of motor milestones in the study. The first, by the caretaker, provided the actual date when the milestone was first observed and/or tested. The second, by the fieldworker, provided a date when the performance was first demonstrated on a scheduled visit.

Contrary to the hypothesis, the WFC cohort and the WFL cohort did not have any different gross motor milestone acquisition that the CSB+ cohort. Instead all intervention arms performed equally well. The attainment of sitting without support was especially difficult to verify because the study began when infants were already 6 months of age. The average reported age of sitting without support was 4.8 months in all groups. There were no infants with delayed developmental milestones during the study period.

Limited variety of complementary foods has been associated with low nutrient density adequacy of the foods, which may cause malnutrition and developmental delays (Siegel *et al*, 2005). Animal protein intake was positively associated with earlier walking acquisition among Guatemalan infants (Kukhlina *et al*, 2004). Anemia and iron deficiency were independently associated with walking in a cohort of Zanzibari infants (Kariger *et al*, 2005). Delays in motor milestone acquisition, including walking, were found among cohorts of stunted, underweight Indonesian (Pollit *et al*, 1994), Zanzibari (Kariger *et al*, 2005), and Guatemalan infants (Kukhlina *et al*, 2004), and among stunted and wasted Pakistani infants (Cheung *et al*, 2001).

The current study followed the protocol outlined in The Motor Development Study (Wijnhoven *et al*, 2004), aimed to describe the acquisition of six universal gross motor milestones, and thereby fill an existing gap in knowledge. By having caregiver records of the exact dates of milestone achievements facilitated internal cross validation with fieldworker's records; and comparison with previuos studies that relied on parental reporting alone.

The information on complementary feeding will also permit studies of associations between child feeding and motor development.

The limitations inherent in the Motor Development Study similarly apply to this study. Information on stimulation and child rearing practices that might influence milestone acquisition was not collected. Despite this limitation, this study provides an important addition to the literature on gross motor development in a resource poor setting.

5.5 Summary of discussion

Food products distributed for complementary feeding in food insecure populations should not only be fortified, but also contain ASF. In Kenya, small fishes and edible termites have potential as a cheap and sustainable local ASF source, which can contribute to improve the nutritional quality of local processed fortified complementary foods and food aid products.

Better understanding of FFM and FM in the early age and how body composition impacts the onset of healthy growth in infants and young children in low income settings can contribute to shaping of nutrition interventions in the future. Linking these findings from this thesis, to the consequences in adult life and risk of overweight and obesity, and related non-communicable diseases, can assist policy-makers in the challenging task of preventing the emergence of a double-burden of malnutrition in Kenya, and other food insecure settings facing a future of nutrition transition with unknown health consequences.

This study has provided important evidence on nutrition interventions during the crucial complementary feeding period. Traditionally, assessments of growth has largely been based on anthropometric measurements such as body weight and length, with less attention to the quality of growth and relative partitioning of nutrients to fat-free mass or fat mass. Currently, the amount and distribution of body fat and the amount and composition of lean mass are understood to be very important for long term health prospects of infants and children.

This study also demonstrates the effect of use of traditional plant and animal source foods in complementary feeding. It shows the benefits gained from combining customary knowledge and modern scientific methodology. The use of maize, grain amaranth, edible termites and small fish may be exploited in provision of high quality diets especially in the developing countries. These approaches, when combined with other essential components for improving infant and young child nutrition; such as promotion of continued breastfeeding, dietary diversity, responsive feeding practices, and attention to hygiene and sanitation; would greatly improve the nutrition status of infants and young children in resource poor settings.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Complementary feeding interventions encompass a wide variety of approaches, including education/ counselling about child feeding, food supplementation, fortification or home fortification of complementary foods, and food processing techniques to increase energy density or enhance nutrient quality of prepared complementary foods. Because there is no single, universal package of components in such interventions, it is difficult to generalize about the impact of efforts to improve complementary feeding. Furthermore, there are many different outcomes that can be assessed when evaluating the impact of complementary feeding interventions.

No significant differences in impact on lean mass accrual, essential fatty aacids profile and gross motor miletone attainment were observed from feeding locally produced complementary foods with or without animal source foods (termites and small fish) compared to a standard plant-based product. Since the WFC and WFL have similar health outcomes to CSB+, they may be utilized as improved complementary foods in populations where they are culturally aceptable.

6.2 Recommendations

1. WFC and WFL have similar health outcomes to CSB+ and can therefore be utilized as improved complementary foods in populations where they are culturally acceptable

2. There is need for increased documentation of the EFA content of local foods

3. Further studies on the bioavailability of nutrients from edible insects are needed

6.2.1 Implementation vision, expected benefits and potential uses of the study results

The vision of the study is to generate a simple, versatile and easy-to use tool specifically adapted for the situation in Kenya, for the development of 'Winfoods'. The WinFood CF and WinFood Lite developed for this study are to be viewed as models for how complementary foods can be made from local foods. Future 'Winfoods' are envisaged to be a range of energy-dense, high-nutrient complementary foods, whose formulation can be adapted to population and/or program and/or production requirements, and which make optimal use of locally available foods. Winfood-products can be produced on a local level or by (small) enterprises. Potential uses can be in (national) nutrition programs aimed at food-insecure populations, for production by NGO's working in the mother-child health field and/or income-generating projects, programs targeting especially vulnerable population groups and also commercial production by larger local food companies. The composition of WinFoods can also be adapted in recipes for homemade improved complementary foods. The study will assess the effects of different complementary foods on health and growth based on the most recent nutritional knowledge as to what constitutes optimal growth and beneficial health effects, thereby assessing the quality of the complementary foods in the most relevant way possible by using end-point functional outcomes, to ensure short- and long term benefits for Kenyan children. The study results will contribute to the effective implementation of the guidelines by providing the scientific documentation for the nutritional quality of a complementary food based on local indigenous Kenyan food stuffs.At a policy level, these results from the Winfood project can act as good evidence-based guidance for policy-makers to develop guidelines which can improve and strengthen the national policies on nutrition in Kenya in the future.

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ANNEXES

ANNEX 1: DATA COLLECTION PROCESS DURING THE INTERVENTION STUDY

All eligibleHH for the MCHclinics will be identified in collaboration with the Health Centre in the Operational District.
Responsible: Winfood Team andstaff,DHMT of Kisumu West and MCH in charge St. Mary's Hospital

⇩

Winfood staff identifies the eligible HHs to the Winfood study (HH with a household member at 6-7 month of age).
Responsible: Winfood staff (2 people)

⇩

Estimated time: 30 min
Winfood staff (data collectors) visit the eligible HH to inform the caretaker about the Winfood study and get a written consent from the caretaker.
Responsible: Winfood staff (3 data collectors)

⇩

Baseline data collection: (**Estimated time: 4 hours and 10 min)**

- Anthropometry and clinical examination (20 min)
  - (Children with a WHZ< -3, will be referred to treatment for severe malnutrition at the hospitals)
- Saliva sample (10 min)
- Giving the Deuterium solution – 10 ml of total liquid (10 min)
- Three hours of waiting hours at the clinical
  - Questionnaire-interviews: general baseline (socio-economic) and developmental milestones
  - Dietary assessment
- Second saliva sample (10 min)
- Blood sampling (20 min)

Responsible: Winfood staff (3 clinical staff and 2 data collectors)

⇩

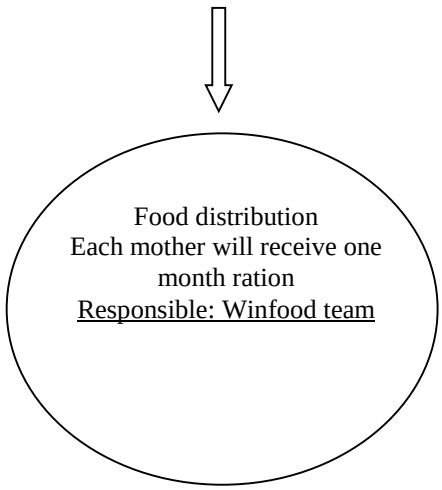
Food distribution
Each mother will receive one month ration
Responsible: Winfood team

⇩

8 x Follow-up: Estimated time: 55 min

- Anthropometry and clinical examination (20 min)
- General Questionnaire (10 min)
- Developmental milestones (10 min)
- Dietary assessment (15 min)

Responsible: Winfood staff (2 clinical staff and 2 data collectors)



9th follow-up: (**Estimated time: 4 hours and 10 min)**

- Anthropometry and clinical examination (20 min)
  - (Children with a WHZ< -3, will be referred to treatment for severe malnutrition at the hospitals)
- Saliva sample (10 min)
- Giving the Deuterium solution – 10 ml of total liquid (10 min)
- Three hours of waiting hours at the clinical
  - Questionnaire-interviews: general baseline (socio-economic) and developmental milestones
  - Dietary assessment
- Second saliva sample (10 min)
- Blood sampling (20 min)
- Physical activity

Responsible: Winfood staff (3 clinical staff and 2 data collectors)

ANNEX 3: TIMELINE TABLE FOR THE INTERVENTION STUDY

	Q2/2011			Q3/2011			Q4/2011			Q1/2012			Q2/2012			Q3/2012			Q4/2012			Q1/2013		
	April	May	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	March	April	May	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	March
Preparation	X	X	X	X	X	X	X																	
Acceptability study	X																							
Recruitment								X	X	X														
1st month of follow –up									X	X	X													
2nd month of follow –up										X	X	X												
3rd month of follow –up											X	X	X											
4th month of follow –up												X	X	X										
5th month of follow –up													X	X	X									
6th month of follow –up														X	X	X								
7th month of follow –up															X	X	X							
8th month of follow –up																X	X	X						
9th month follow-up																	X	X	X					
Laboratory work											X	X	X	X	X	X	X	X	X	X	X	X	X	
Data entry										X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Data Analyses Preliminary findings dissemination Dissemination														X						X X	X X	X X X	X X X	X X

ANNEX 4: GENERAL DATA COLLECTION FORM – BASELINE

(Interviewer ID): ☐ **(Child´s ID):** ☐☐☐☐

The Winfood Intervention Study

(Village): _____*(Date of data collection)* _____ / _____ / _____

(No of data coll: BASELINE)

Verbal consent obtained from primary caretaker Yes☐ No☐

(First, I would like to ask some general questions about the child)

1. *(CHILD´S NAME)* **:** _____ *(FATHER'S NAME)***:** _____ *(MOTHER'S NAME)***:** _____	
2. *(CARE TAKER´S NAME)* **:** _____ *(HEAD OF HOUSEHOLD NAME)* **:**_____	
3. *(HAVE THE CARETAKER PICKED UP THE FOOD RATION?)* *(Circle ONLY ONE answer)*	*(Yes)* ..... *(No)* ..... *(Answer refused)* ..... *(Don't know)* .....
4. *(WHAT IS YOUR RELATIONSHIP TO (name)?* *(Circle ONLY ONE answer)*	*(Biological mother)* ..... *(Grandmother)* ..... *(Sister)*..... *(Stepmother)*..... *(Aunt)* ..... *(Other female relative)*..... *(Brother)* ..... *(Father)*..... *(Other (specify)* ..... *(Answer refused)* ..... *(Don't know)* .....
5. *(HAVE YOU HAD PRIMARY RESPONSIBILITY FOR TAKING CARE OF (name) FOR AT LEAST THE LAST TWO WEEKS?)* *(Circle ONLY ONE answer)*	*(Yes)* ..... *(No)* ..... *(Answer refused)* ..... *(Don't know)* .....
6. *(IS (name) A BOY OR GIRL?)* *(Circle ONLY ONE answer)*	*(Boy)*..... *(Girl)* .....
7. *(WHAT IS (name)´S DATE OF BIRTH?)* *(Even if the mother knows the exact date of birth, ask if she has an immunization card or other document and check that the date is correct.)* *(mark 01.01.2099 if not known)*	☐☐ ☐☐ ☐☐☐☐ *(Day)(Month)(Year)*
8. *(SOURCE OF DATE OF BIRTH INFORMATION)* *(Circle ONLY ONE answer)* *(If a date of birth is available, jump to question 10.)*	*(immunization or vaccination card)* ..... *(Birth certificate)*..... *(Caretaker's recall)*..... *(Other (specify)* ..... _____
9. *(HOW OLD IS (name)?*	☐☐ *(Age in months)* .....

(<u>Demographic and socio-economic variables</u>)

I will now ask you about the people who live here and other things about your household.

10. *(WHAT IS THE HIGHEST LEVEL OF SCHOOLING the caretaker HAVE COMPLETED?)* *(Write in NUMBER OF YEARS of school.)* *From the DHS survey*	*(Number of years of school)* ☐☐ *(Informal schooling)* ..... 66 *(Answer refused)* ..... 88 *(Don't know)* 99
11. *(WHAT IS THE HIGHEST LEVEL OF SCHOOLING the household head HAS COMPLETED?)* *(Write in NUMBER OF YEARS of school)* *From the DHS survey*	*(Number of years of school)* ☐☐ *(Informal schooling)* ..... 66 *(Answer refused)* ..... 88 *(Don't know)* 99
12. *(HOW MANY PEOPLE USUALLY LIVE IN THIS HOUSEHOLD, THAT IS,* *HOW MANY PEOPLE USUALLY SLEEPS IN THE HOUSE DURING NIGHT TIME?)*	*(Number of people)*: ..... ☐☐
13. *(HOW MANY CHILDREN UNDER 5 YEARS OF AGE USUALLY LIVE IN THIS HOUSEHOLD?)* *Including the study child*	*(Number children under 5 years)* ..... ☐☐
14. *(WHAT IS THE MAIN SOURCE OF DRINKING WATER DURING DRY SEASON FOR MEMBERS OF YOUR HOUSEHOLD?)* *(Circle ONLY ONE answer)* *From the DHS survey*	*(Piped water)* *(Piped into dwelling)* ..... 1 *(Piped to yard/plot)* ..... 2 *(Public tap/standpipe)* ..... 3 *(Tube well or borehold)* ..... 4 *(Dug well)* *(Protected well)* ..... 5 *(Unprotected well)* ..... 6 *(Rainwater)* *(Surface source)(river/dam/stream/lake pond/canal/irrigation channel)* ..... 12 *(Tanker truck or water vendor)* ..... 13 *(Bottled water)* ..... 14 *Other (specify)* ..... 7 *(Answer refused)* ..... 8 *(Don't know)* 9
15. *(DURING THE WET SEASON, IS THE MAIN SOURCE OF DRINKING WATER* *FOR MEMBERS OF THE HOUSEHOLD THE SAME AS DURING DRY SEASON?)* *From the DHS survey*	*(Yes)* ..... 1 *(No)* ..... 2 *(Answer refused)* ..... 8 *(Don't know)* 9
16. *(WHERE IS THE WATER SOURCE LOCATED)* *From the DHS survey*	*(In own dwelling)* ..... 1 *(In own yard/plot)* ..... 2 *(Other (specify)* ..... 7 *(Answer refused)* ..... 8 *(Don't know)* 9
17. *(DO YOU TREAT YOUR WATER IN ANY WAY TO MAKE IT SAFER TO DRINK?)* *(Circle ONLY ONE answer)* *From the DHS survey*	*(Yes)* ..... 1 *(No)* ..... 2 *(Answer refused)* ..... 8 *(Don't know)* 9

18. *(WHAT DO YOU DO TO THE WATER TO MAKE IT SAFER TO DRINK?)* *(Circle ALL applicable answers)* *From the DHS survey*	*(Boil)*.....1 *(Add bleach, chlorine or Agar)*.....2 *(White alum)*.....3 *(Strain it through a cloth)*.....4 *(Use a water filte)*.....5 *(Solar disinfection)*.....6 *(Let it stand and settle)*.....10 *(Other (specify)*.....7 _____ *(Answer refused)*.....8 *(Don't know)*.....9
19. *(WHERE DO MEMBERS OF YOUR HOUSEHOLD USUALLY GO TO RELIEVE THEMSELVES?)* *(Circle ONLY ONE answer)* *From the DHS survey*	*(Flush or pour flush toilet)* *(Flush to piped sewer system)*.....1 *(Flush to septic tank)*.....2 *(Flush to pit latrine)*.....3 *(Flush don't know where)*.....4 *(Pit latrine)* *(Ventilated improved)*.....5 VIP.....6 *(Pit latrine with slab)*.....10 *(Pit latrine without slab/open pit)*.....11 *(Composting toilet)*.....12 *(Bucket toilet)*.....13 *(Toilet over water)*.....14 *(No Toilet/field/forest)*.....15 *(Other (specify)*.....7 _____ *(Answer refused)*.....8 *(Don't know)*.....9
20. *(DO YOU SHARE THIS TOILET FACILTY WITH OTHER HOUSEHOLDS?)* *From the DHS survey*	*(Yes)*.....1 *(No)*.....2 *(Answer refused)*.....8 *(Don't know)*.....9
21. *(HOW MANY HOUSEHOLDS USE THIS TOILET FACILITY?)* *From the DHS survey*	*(No of household)*..... ☐☐ *(Answer refused)*.....88 *(Don't know)*.....99
22. *(WHAT IS THE PRIMARY SOURCE OF INCOME FOR THIS HOUSEHOLD?)* *Circle ONLY ONE answer)*	*0 = None* *1= Farming* *2=Self Employed* *3=Salaried* *4=Not applicable(dependant)* *5= Remittance*
23. *(DOES THIS HOUSEHOLD OWN ANY LIVESTOCK, HERDS OR FARM ANYMALS?)* *From the DHS survey*	*(Yes)*.....1 *(No)*.....2 *(Answer refused)*.....8 *(Don't know)*.....9
24. *(I WILL NOW MENTION SOME ANIMALS, AND I WOULD LIKE YOU TO TELL ME HOW MANY ANIMALS OF EACH TYPE YOU HAVE.)* *(Fill in NUMBER of each type of animal)* *From the DHS survey*	*(Cows/bulls)* :..... ☐☐☐ *(goats)* : ..... ☐☐☐ *(pigs)* : ..... ☐☐☐ *(Chicken)* :☐☐☐ *(ducks)* :..... ☐☐☐

25. *(DOES ANY MEMBER OF THIS HOUSEHOLD OWN ANY LAND THAT CAN BE USED FOR AGRICULTURE?)* *From the DHS survey*	*(Yes)* ..... 1 *(No)* ..... 2 *(Answer refused)* ..... 8 *(Don't know)* 9
26. *(HOW MUCH LAND DOES YOUR HOUSEHOLD OWN?)* *Write in number of local units and the name of the local unit* *From the DHS survey*	*(sq meters)* ..... ☐☐☐ *(a)* : ..... ☐☐☐ *(Hectare)* : ..... ☐☐☐ *(ray)* : ..... ☐☐☐ *(don't know)* : 999
27. *(DOES YOUR HOUSEHOLD HAVE)* : *(Circle 1 or 2 for each item)* *From the DHS survey*	Yes / No *(Electricity/generator)* : ..... 1 2 *(A sewing machine)* : ..... 1 2 *(A bicycle)* : ..... 1 2 *(A radio)* : ..... 1 2 *(A television)* : ..... 1 2 *(A mobile telephone)* : ..... 1 2 *(A non-mobile telephone)* : ..... 1 2 *(A refrigerator)* : ..... 1 2 *(A table)* : ..... 1 2 *(A chair)* : ..... 1 2 *(A bed)* : 1 2
28. *(DOES YOUR HOUSEHOLD HAVE ANY MOSQITO NETS THAT CAN BE USED WHILE SLEEPING?)*	*(Yes)* ..... 1 *(No)* ..... 2 *(Answer refused)* ..... 8 *(Don't know)* 9
29. Religion of Household head *Circle only one*	*0=None* *1=Protestant* *2=Catholic* *3=Indigenous Church* *4=Muslim* *5=OthersSpecify*
Housing *type*	*1. Permanent* *2. Semi Permanent* *3. All temporary materials* *7 =Others specify*
Group belonging of the household head	*0=None* *1 Women/ Men grps* *2=Church* *3=Clubs/ Clan groups* *5= Youth* *6=Under 6 years*

Thank you very much for taking your time for this interview.

(Signature of team Interviewer): _____

ANNEX 5: GENERAL DATA COLLECTION FORM – FOLLOW-UP

Interviewer ID: ☐ **Child´s ID:** ☐☐☐☐

The Winfood Intervention Study

(Village): _____ *(Date of data collection)* _____ / _____ / _____

(No of data coll) : 1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9

(Verbal consent obtained from primary caretaker) Yes ☐ No ☐

1. ***(CHILD´S NAME)* :** _____ ***(FATHER'S NAME)* :** _____ ***(MOTHER'S NAME)* :** _____	
2. ***(CARETAKER´S NAME)* :**_____ ***(HEAD OF HOUSEHOLD NAME)* :**_____	
3. *(WHAT IS YOUR RELATIONSHIP TO (name)*? *(Circle ONLY ONE answer)*	*(Biological mother)* 1 *(Grandmother)* 2 *(Sister)* 3 *(Stepmother)* 4 *(Aunt)* 5 *(Other female relative)* ..... 6 *(Brother)* 10 *(Father)* 11 *(Other (specify)* ..... 7 *(Answer refused)*..... 8 *(Don't know)* 9
4. *(HAVE YOU HAD PRIMARY RESPONSIBILITY FOR TAKING CARE OF (name) FOR AT LEAST THE LAST TWO WEEKS)***?** *(Circle ONLY ONE answer)*	*(Yes)* 1 *(No)* 2 *(Answer refused)* 8 *(Don't know)* 9

*(**Child morbidity**)*

(I will now ask you some questions about any illnesses (name) has had during the last 7 days.)

5.*(IN THE PAST 7 DAYS, HAS (NAME) BEEN)* **:** *(Circle ONLY ONE answer)*	*(Well)* ……..…..1 *(Mild illness but recovered without treatment)*……..2 *(Moderate illness which needed drugs or Clinical visit)* *(Serious illness requiring doctor)* ..... 4 *(Answer refused)* ……8 *(Don´t know)*..... 9

(IN THE PAST 7 DAYS, HAS) **(***NAME***) HAD:**

6.

		1=yes	2= no
A	*(Convulsions)*		
B	*(loose watery diarrhea)*		
C	*(diarrhea with blood or mucus)*		
D	*(coughing)*		
E	*(running nose)*		
F	*(fever)*		
G	*(difficulty in breathing)*		
H	*(vomiting)*		
I	*(eye problem (redness, discharge)*		
J	*(ear problem (discharge) /pain)*		
K	*(feeding poorly)*		
L	*(lethargic child)*		
M	*(skin rashes)*		
N	*(constipation)*		
O	*(Other (specify)* _____		
N	*(medicine taken)*		

*(**Medicine codes**)* : 0=*(nothing)* ; 1=*(vitamins)* , *(tonics)* ; 2=*(anti-cough)* , *(anti-vomiting)* , *(anti-diarrhea)* ; 3=*(painkillers)* , *(anti-inflammatories)* ; 4=*(antibiotics)*; 5=*(other medicine supplied by health professionals)* ; 6=*(other medicine supplied by non-health professionals)*

(Thank you very much for taking your time for this interview.)

Signature of interviewer: _____

ANNEX 6: FOOD RECALL FORM

Interviewer ID: ☐ Child´s ID: ☐☐☐☐

THE WINFOOD INTERVENTION STUDY

Village _____No of data collection: Baseline / 1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9

Date of data collection _____ / _____ / _____, Day of week: _____ ,
Time of Recall:_____ / _____

Time	Place	Food & Description	Serving Size	Raw ingredients		Weight of total cooked ingredients (g)	Amount Eaten (g)
				Foods items	Weight (g)		

Additional questions

WHO Breastfeeding (*from WHO standard question on breastfeeding*)

I will now ask you some questions about breast feeding your child

1 **HAS *(name)* EVER BEEN BREASTFED?** *Circle ONLY ONE answer*	Yes 1 No 2 Answer refused .....8 Don´t know 9
2. **SINCE THIS TIME YESTERDAY, HAS *(name)* BEEN BREASTFED?** *Circle ONLY ONE answer*	Yes 1 No 2 Answer refused .....8 Don´t know 9
3.**AT WHAT AGE DID *(name)* START EATING COMPLEMENTARY FOOD?** **COMPLEMENTARY FOOD IS ANY FOOD WHICH IS NOT BREASTMILK EXCEPT MEDICATIONS.**	Number of months ..... ☐☐

Compliance

(I will now ask you some questions about the utilization of the complementary food in the household you have received from Winfood.)

4**. CAN I PLEASE SEE THE EMPTY RATIONS BAGS FROM THE MONTHLY FOOD RATION?**	No. of empty packets ..... ☐☐ Answer refused ..... 88 Don't know 99
5. **YESTERDAY, DID YOU PREPARE AND SERVE ANY OF THE WINFOOD FOOD YOU RECEIVED?**	Yes 1 No 2 Answer refused .....8 Don´t know 9
6**.HOW MANY PACKETS DID YOU USE TO PREPARE THE FOOD YESTERDAY?**	No. of used packets ..... ☐☐ Answer refused ..... 88 Don't know 99
7. **YESTERDAY, WHO IN THE HOUSEHOLD ATE THE PREPARED WINFOOD FOOD YOU PREPARED?** *Circle ALL applicable answers*	(name) 1 Another child in the HH under 5 years of age .....2 Another child in the HH 5 years or older .....3 Father 4 Mother 5 Pregnant/lactating woman in the household .....6 Elderly member .....10 Other member of household .....11 Other (specify).....7 Answer refused .....8 Don't know 9
8. **YESTERDAY, WHAT PROPORTION OF THE PREPARED WINFOOD FOOD YOU PREPARED WAS EATEN BY *(name)*?** *(Circle ONLY ONE answer)*	*(None)* 0 *(Less than ½)* .....1 *(About half)* 2 *(Most)* 3 *(All)* 4 *(Answer refused)* .....8 *(Don't know)*.....9

ANNEX 7: THE MORBIDITY – ROUTINE CLINICAL EXAMINATION

Health professional ID: ☐ **Child´s ID:** ☐☐☐☐

The Winfood Intervention Study

(Morbidity – Routine Clinical Examination)

(Village): _____ *(Date of data collection)* _____ / _____ / _____

(No of data coll: Baseline)/ **1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9**

1.	*(GENERAL CONDITION)*	*(Well/alert)* 1 *(Restless/irritable)* ..... 2 *(Abnormally sleepy)* ..... 3 *(Sleeping, could not be assessed)* 4
2.	*(RESPIRATORY RATE/MINUTE)* *(count for 60 sec and repeat)*	1: .....☐☐ 2: .....☐☐
3.	*(COUGHING)*	*(Yes)* .....1 *(No)* 2
4.	*(RUNNING NOSE)*	*(Yes)* .....1 *(No)* 2
5.	*NASAL FLARING*	*(Yes)* .....1 *(No)* 2
6.	*AUDIBLE WHEEZING OR GRUNTING*	*(Yes)* .....1 *(No)* 2
7.	*SEVERE CHEST INDRAWING*	*(Yes)* .....1 *(No)* 2
8.	*BULGING FONTANELLE*	*(Yes)* .....1 *(No)* 2
9.	*(EYE PROBLEM) (redness, discharge)*	*(Yes)* .....1 *(No)* 2
10.	*(EAR PROBLEM) (e.g. discharge)*	*(Yes)* .....1 *(No)* 2
11.	*(SKIN RASH OR PUSTULES)*	*(Yes)* .....1 *(No)* 2
12.	*(JAUNDICE)*	*(Yes)* .....1 *(No)* 2
13.	*(TREATMENT REQUIRED)*	*(None)* ..... 1 *(Home care without medicine)* ..... 2 *(Home care with medicine)* ..... 3 *(Hospitalization)* 4

ANNEX 8: THE ANTHROPOMETRIC AND CLINICAL EXAMINATION

Responsible Data collector ID: ☐ Child´s ID: ☐☐☐☐

The Winfood Intervention Study

(Anthropometric and clinical examination)

(Village): _____*(Date of data collection)* _____ / _____ / _____

(No of data coll: Baseline) / 1 / 2 / 3 / 4 / 5 / 6 / 7 / 8 / 9

---

2. *(Anthropometry)*

1.1 *(Weight (record three measurements))*		
1.1.1 I__I__I•I__I__I kg	1.1.2 I__I__I•I__I__I kg	1.1.3 I__I__I•I__I__I kg
1.2 *(Length (record three measurements))*		
1.1.1 I__I__I__I•I__I cm	1.2.1 I__I__I__I•I__I cm	1.2.3 I__I__I__I•I__I cm
1.3 *(Knee-heel (record three measurements))*		
1.3.1 I__I__I•I__I__I cm	1.3.2 I__I__I•I__I__I cm	1.3.3 I__I__I•I__I__I cm
1.4 (MUAC*(record three measurements))*		
1.4.1 I__I__I•I__I cm	1.4.2 I__I__I•I__I cm	1.4.3 I__I__I•I__I cm
1.5 *Head circumference (record three measurements)*		
1.5.1 I__I__I•I__I cm	1.5.2 I__I__I•I__I cm	1.5.3 I__I__I•I__I cm
1.6 *TricepsSkinfold Thickness (TSF) (record three measurements)*		
1.6.1 I__I__I•I__I cm	1.6.2 I__I__I•I__I cm	1.6.3 I__I__I•I__I cm
1.7 *Subscapular, Triceps, Biceps and Suprialiac Skinfold Thickness (TSF) (record three measurements)*		
1.7.1 I__I__I•I__I cm	1.6.2 I__I__I•I__I cm	1.6.3 I__I__I•I__I cm

2. *(Clinical Examination)*

2.1 *(Temperature)*		
I__I__I•I__I C^{o}		
2.2 *(Blood pressure)*		
Diastolic: I__I__I mmHg	Systolic: I__I__ImmHg	
2.3 *(Signs of undernutrition related diseases)*		
(Oedema: yes no)	*(Vitamin A deficiencies)*: yes no	
2.4 *(Hemoglobin)*		
I__I__I•I__Ig/L		
Lipid Profile HDL_____ LDL_____ TC_____ EFA_____ _____		

ANNEX 9: ANTHROPOMETRY SOP

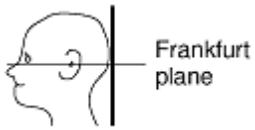
The Winfood Intervention Study

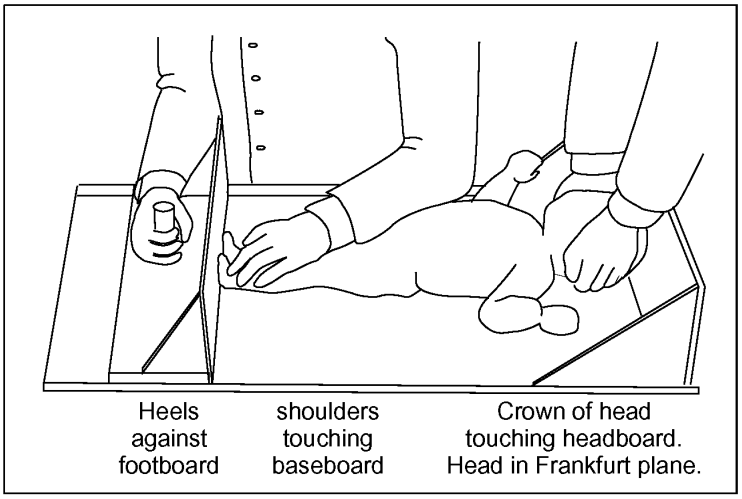
Measuring Recumbent length

Only children less than 24 months will be enrolled in the Winfood study, therefore always the recumbent length will be measured with a measuring board. Two examiners are required to correctly position the subject and to ensure accurate and reliable measurements of length.

1. Place the subject, face upward, with the head at the fixed end of the board and the body parallel to its long axis. The shoulders should rest against the surface of the board.
2. Apply gentle traction to bring the crown of the subject's head into contact with the fixed headboard and simultaneously position the head so that it is in the Frankfurt plane.
3. Hold the subject's feet, without shoes, toes pointing directly upward, while keeping the subject's knees straight by placing one hand on the knees. Then bring the movable footboard to rest against the heels.
4. Record the length to the nearest millimeter.
5. Repeat 1-4 two times, repositioning the subject between measurements.
6. Record all three measurements on the form.

Note: If the subject is restless, only the left leg should be positioned for the measurement.





Measuring weight with Uniscale

1. Put the scale on the floor. Choose the flattest, most level surface you have.
2. Do not stand on the scale yet.
3. Turn on the scale. Move your foot across switch window **(Figure a)**
4. Ask the mother to step on the scale by herself. She can give her child to you or another person to hold **(Figure b)**
5. Make sure her feet or clothes do not cover the switch window. You will see the mother's weight in the display, for example: 52,4
6. With the mother on the scale pass your foot slowly across the switch window **(Figure c)**. Then wait a couple seconds and you will see: 0,0
7. Ask the mother to step off the scale. You should see: --.-
8. Ask the mother to step back on the scale with her child **(Figure d and e)**. You should see the child's weight: 5.4
9. Ask the mother to step off the scale. You should see: --.-
10. Repeat 4-9 two times more.
11. Record all three measurements on the form.


Figure a


Figure b


Figure c

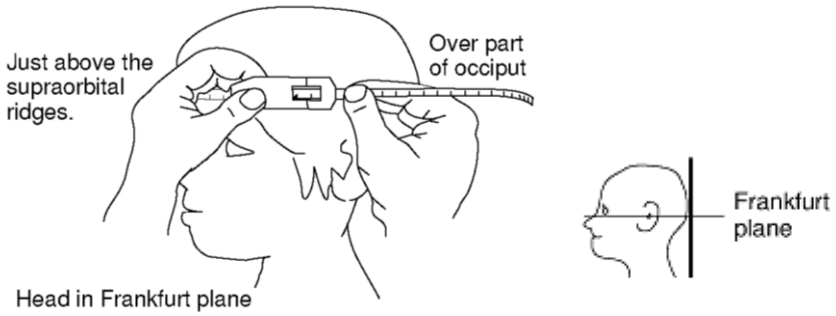

Figure d


Figure e

Measure head circumference

For the measurement, a fiberglass insertion tape. Any added objects in the hair such a hair pins should be removed for the measurement.

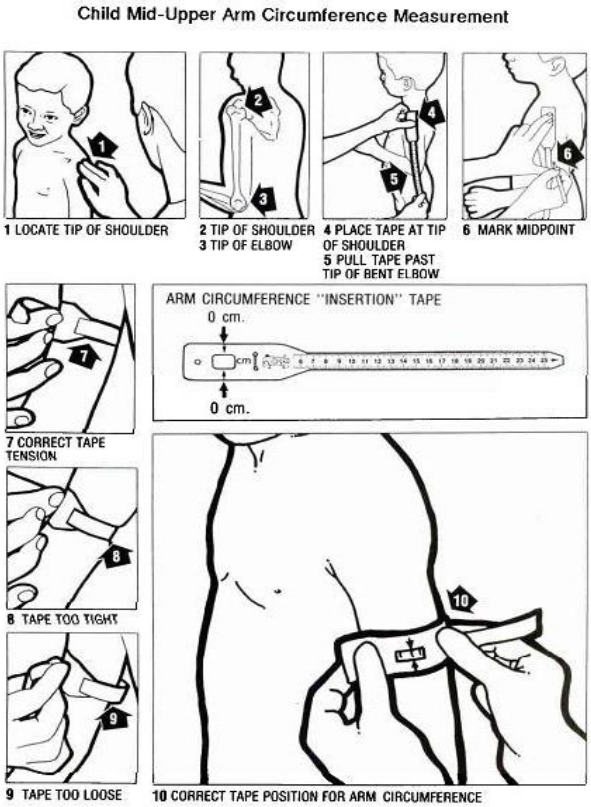
1. The measurer should stand facing the left side of the subject. Gently, place the subject, face upward, on an examination table, allowing the shoulders to rest against the surface of the table.
2. Position the head so that it is in the Frankfurt plane i.e., an imaginary plane which passes through the external auditory meatus (the small flap of skin on the forward edge of the ear) and over the top of the lower bone of the eye socket immediately under the eye, is vertical.
3. Place the tape just above the supraorbital ridges covering the most prominent part of the frontal bulge and over the part of the occiput that gives the maximum circumference. Care must be taken to ensure that the tape is at the same level on each side of the head and pulled tightly to compress the hair.
4. Measure the circumference to the nearest millimeter.
5. Repeat 1-4 two times more.
6. Record all three measurements on the form.



Measure mid-upper arm circumference

Obtain this measurement with the mother or caregiver seated and holding the infant in her lap. The infant should be wearing loose clothing without sleeves to allow exposure of the shoulder area. Use a fiberglass insertion tape.

1. Gently bend the left arm through 90 degrees at the elbow, and then place the forearm with the palm down across the body.
2. Locate and mark the tip of the shoulder.
3. Locate the tip of the elbow.
4. Measure the distance between these two points using a fiberglass insertion tape, and mark the midpoint with a soft pen or indelible pencil, directly in line with the point of the elbow and shoulder.
5. Relax the arm so that the elbow is extended and hanging just away from the side of the trunk, with the palm facing the thigh. Then wrap the tape gently but firmly around the arm at the midpoint, care being taken to ensure that the arm is not squeezed. Measurements are taken to the nearest mm.
6. Repeat 1-5 two times more.
7. Record all three measurements on the form.

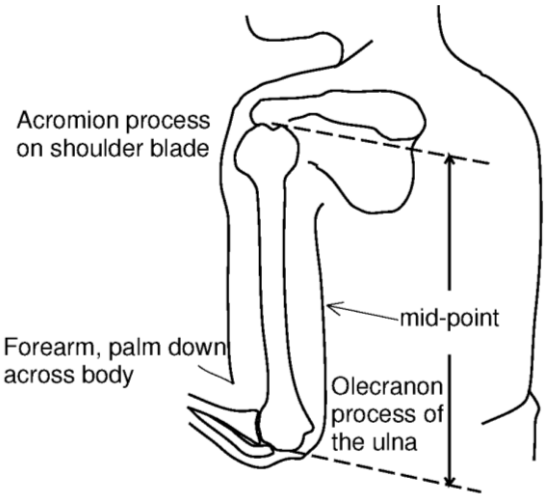


Measure skinfold

Obtain these measurements with the mother seated and holding the infant in her lap.
Alternatively, children may be measured lying down. It is helpful to demonstrate the caliper on the hand of the measurer and on the hand of the infant, measuring total palm thickness, before beginning to measure skinfold thickness.

Measure the tricepskinfold

1. Gently bend the left arm through 90 degrees at the elbow, and then place the forearm with the palm down across the body.
2. Locate and mark the tip of the acromion process of the shoulder blade at the outermost edge of the shoulder.
3. Locate the tip of the olecranon process of the ulna.
4. Measure the distance between these two points using a fiberglass insertion tape, and mark the midpoint with a soft pen or indelible pencil, directly in line with the point of the elbow and shoulder.

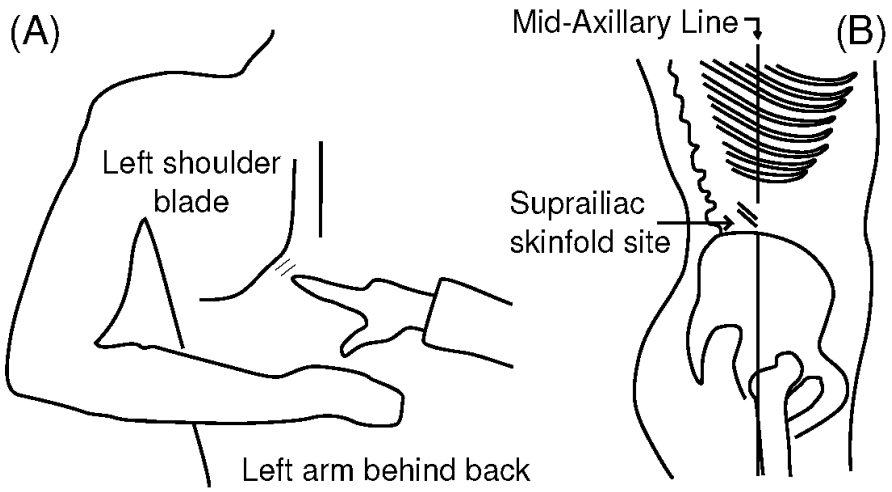


5. Extend the infant's arm so that it is hanging loosely by the side.
6. Grasp a vertical fold of skin plus the underlying fat, 1cm above the marked midpoint, in line with the tip of the olecranon process, using the thumb and forefinger.
7. Gently pull away the skinfold from the underlying muscle tissue, and apply the caliper jaws at right angles, exactly at the marked midpoint.
8. Hold the skinfold between the fingers while measuring.
9. Repeat 5-8 twice more and write the measure in the form

Measure subscapular skinfold

The site is just inferior to the inferior angle of the shoulder, and can be identified more readily by placing the child's arm behind the back (see figure A).

1. To locate the site, the health professional will run a finger along the shoulder blade until the inferior angle is identified.
2. Relax the shoulder and the arm, and pick up a skinfold on a 45^0 angle from horizontal, in the same direction as the inner border of the shoulder (i.e., medially upward and laterally downward) (See Figure A).
3. Skinfolds should be recorded to 0.2 mm on the Harpenden skinfold calipers three times. Skinfold measurements made with precision calipers should normally agree to within 1mm.
4. Record all 3 measurements on the form.


(A)
Left shoulder blade
Left arm behind back
Mid-Axillary Line
(B)
Suprailiac skinfold site

ANNEX 10: BLOOD SAMPLE COLLECTION, HANDLING AND STORAGE SOP
The Winfood Intervention Study

Venous blood sample collection

Preparatory

1. Clean dedicated white coat, powder-free vinyl or nitrile gloves, tourniquet and indelible black pen to be used
2. Sharps box and clinical waste bag are available
3. Supplies of cling film, cotton wool, vacutainers (red top fro serum), butterfly needles (23G Becton Dickinson), cotton wool, ethanol, plasters
4. Racks for holding blood samples stored in the refrigerator
5. Pack for transporting samples to lab is refrigerated. NOTE: Samples must be kept chilled at all times, preferably with 'blue ice' or ice packs from blood collection to arrival at lab, *but not frozen*. For this, a large coolbox, with the icepacks on the bottom is preferred. Direct contact between icepacks and blood tubes should be avoided.

Taking samples

1. Samples to be taken in a private room, each child individually. Hands to be washed between children and a new pair of gloves to be used for each child
2. Apply a small amount of ethanol to the site of venipuncture
3. Apply tourniquet above site of venipuncture
4. A 23G butterfly needle is used to take the blood sample. Insert needle and insert trace vacutainer into collection system barrel when blood is drawn
5. The tourniquet should be loosened during blood collection, *before* the needle is removed from the child's arm
6. The ideal volume of blood to be collected is 3mls. When sample collected removed vacutainer from barrel first and then needle from child's arm. Apply cotton wool immediately and gentle pressure to the site of venipuncture.
7. Dispose of blood collection system into sharps box
8. Remove cotton wool and apply plaster to site of venipuncture
9. Label tubes with subject ID, visit of collection and date of collection
10. Invert tube 8 times and place immediately in the refrigerator in an upright position
11. Note the subject details and collection of blood in log book.
12. Samples are transported to the University of Nairobi Institute of Tropical and Infectious Diseases, where it will be kept cold until it is sent to the Laboratory. The samples will be transported in the refrigerated cool pack in an upright position. If frozen ice-packs are used, wrap the sample tubes in cotton wool to prevent them directly touching the frozen ice-pack.
13. The blood samples obtain the current day is copied to the drivers log book.
14. A lab sheet accompanying the sample must display the subject ID, the visit of the blood sample, and the time of blood collection. The sheet must be signed by the nurse in charge. NOTE: The name of the child must **not** be displayed.

B: Venous blood samples processing

Preparatory

1. Dedicated lab coat for sample handling – to be worn at all times in lab, and stored in laboratory only
2. Powder-free vinyl or nitrile gloves (a new pair between laboratory protocols) should be used at all times for the preparation and handling/processing of the blood samples
3. Dedicated cold-pack rack for holding tubes is stored in –20 freezer
4. Ice box with polystyrene tube inserts ready for the vacuutainers when they arrive with the sample
5. Pre-printed labels and fine, indelible black pen available

6. All labelling to be done using the ‘permanent’ indelible, freezer and water-proof, black marker pens provided
7. Used gloves and used plastic ware in laboratory to be discarded and autoclaved
8. Keep a clear log book of all the activities.

Final check after venous blood processing

1. Log book filled in completely
2. Sample location entered on databases
3. Rack replaced in –20 freezer
4. Tubes and pipette tips replaced in designated cupboard
5. Blood collection tubes, waste tubes and pipette tips disposed of for autoclaving
6. Bench wiped clean with disinfectant

ANNEX 11: THE DEUTERIUM DILUTION METHOD - SOP

A. Materials for deuterium dilution outcome measure
B. Deuterium dilution
C. Extra information on the procedure for Saliva sampling
D. Extra information on the procedure for Dosing

A . <u>Materials fordeuteriumdilution</u>

Preparation of dose bottle:

Mixing bottles (sterile)
Syring (fitting the micropore filter) for adding the deuterium
0.2 microlitremicropore filter
Deuterium
Sterile water
Pastette to withdraw approx 1 ml of the solution
Weight (sensitivity of 0.01g)
Syringe + needle for adding the water

Deuterium dosing kit:

Sealable plastic bag
Dose bottle labelled with subject ID
Dosing syring (10 ml or 20 ml)
Scalp vein butterfly needle (with the needle removed) (make sure that the catheter is long enough for reaching the bottom of the bottle, so it is possible to withdraw the hole dose)
Scalpel (for removing the butterfly needle from the catheter)
Tissue 1 + sealable plastic bag (pre-weighed)*
Tissue 2 + sealable plastic bag (pre-weighed)
Tissue 3 + sealable plastic bag (pre-weighed)

*tissue+ plastic bag could be weighed and registered on a separate list with consecutive numbers.

2 x Saliva sampling:

2 x 5 mLsyringe
2 x2ml plastic vial labelled with subject ID (id + 0h or 3h)
2 x swab and cotton balls
Cool bag

*repeat measurements should be done only after the tracer from a previous measurement has cleared the body, typically 10-14 days for infants

Analysis of saliva samples:

FTIR. (100ppm deuterium oxide enrichment of body water)
Sample volume (minimum 2 mL)
-20 Celsius degrees storage for samples

B. Deuterium Dilution in infants (FTIR)

1) Preparation of the 99.9% deuterium oxide solution (day -1)

a) The doses are prepared the day before the measurement.
b) Prepare each dose in each bottle with 3 g 99.9% deuterium measured at the weight
c) The 99.9% deuterium will be poured through a 0.2 microlitremicropore filter into the sterile dose bottle. This process is for sterilising by filtering out micro organisms.
d) Add sterile water to a total volume of 10 mL, put cap on bottle, and mix gently for at least one minute.
e) The sterile dose bottles will be kept cold (4ºC)
f) Put dose bottle in sealable plastic bag, which must also contain everything to be used in giving the dose to the infant (e.g. dosing syringe in packaging; pre-weighed tissues in small sealable plastic bags). Seal the bag, **tare scales** and record the weight: WEIGHT BEFORE DOSE GIVEN.

Direction for use of microlitremicropore filter: First draw the amount of deuterium into the syringe, and then apply the filter and ad the deuterium to the dose bottle through the filter. **NB!** It is important that the filter is not applied when drawing the deuterium. If so any possible micro organisms will be on the wrong side of the filter and will enter the dose bottle when the deuterium is push back through the filter.

2) Pre dose Saliva sampling (day 0)

a) Make sure that the infant is fasting for min. 20 min prior to "pre dose" saliva sampling
b) Pre dose saliva sampling (20 min pre sample fasting). Minimum 2 mL of saliva is required.
c) Wrap extra piece of cotton around a swab. Collect saliva by moving the swab around the infant's mouth until the cotton wool is sodden. The time required for this will vary between infants. Be subject. It may take several attempts to collect the 2 ml of saliva.
d) Remove the plunger from a new 20 ml disposable syringe. Remove the cotton wool from the swab and place it in the barrel of the 20 ml syringe. Replace the plunger in the body of syringe.
e) Label a sample storage vial with participants ID, Date and Time of collection.
f) Remove the lid from the vial and use the syringe plunger to extract saliva from the cotton wool into the sample storage vial. Replace the lid to avoid evaporation and subsequent isotope fractionation.
g) IF there is not at least 2 ml saliva repeat step c-f with a new cotton ball or swab.
h) Discard swab, syringe, cotton wool and gloves. Do not reuse sample vials or syringes.
i) Keep sample cold after collection (4ºC) and freeze (-20º to -80ºC) as soon as possible. Saliva samples should be stored frozen (-20ºC) until analysis to minimise bacterial growth.
j) Note the time of sampling.
k) Make sure the pre dose saliva sample is sealed and put away before any handling of the deuterium oxide dose

3) Deuterium dosing (day 0)

a) Gentle shake and stir the deuterium solution for at least 1 min, just prior drawing the dose into the syringe. REMEMBER TO STIR THE DEUTERIUM MIX BEFORE DRAWING IT IN THE SYRINGE. ALL EQUIPMENT USED FOR PREPARING DOSES MUST BE COMPLETELY DRY TO AVOID CONTAMINATION BY WATER!
b) Weight the syringe that will use and note it in the form
c) Dry the infant around the mouth with a paper tissue before dosing is initiated.
d) Administer the dose to the infant using the syringe and catheter. Be prepared for collecting any spillage during administration of the dose. It can take as long as the infant needs – e.g. 15 minutes is fine. Collect all spillages on pre-weighed tissues. The critical thing is that by the end of the procedure, you should know that the entire dose

is either (1) back in the bottle; (2) collected as saliva/dribble on the pre-weighed tissues; (3) inside the dosing syringe, tube or whatever else you used to allow the infant to drink; or (4) inside the baby.
If possible, it is good also to weigh the pre-weighed tissue bags separately before and after dosing. Because babies sometimes dribble saliva without actually drooling the dose, you need to consider whether you are collecting lost dose or just dribbled saliva. Then decide whether the collected on the tissues should be included in the 'lost dose' or actually ignored, by adding the value for DOSE IN BABY!

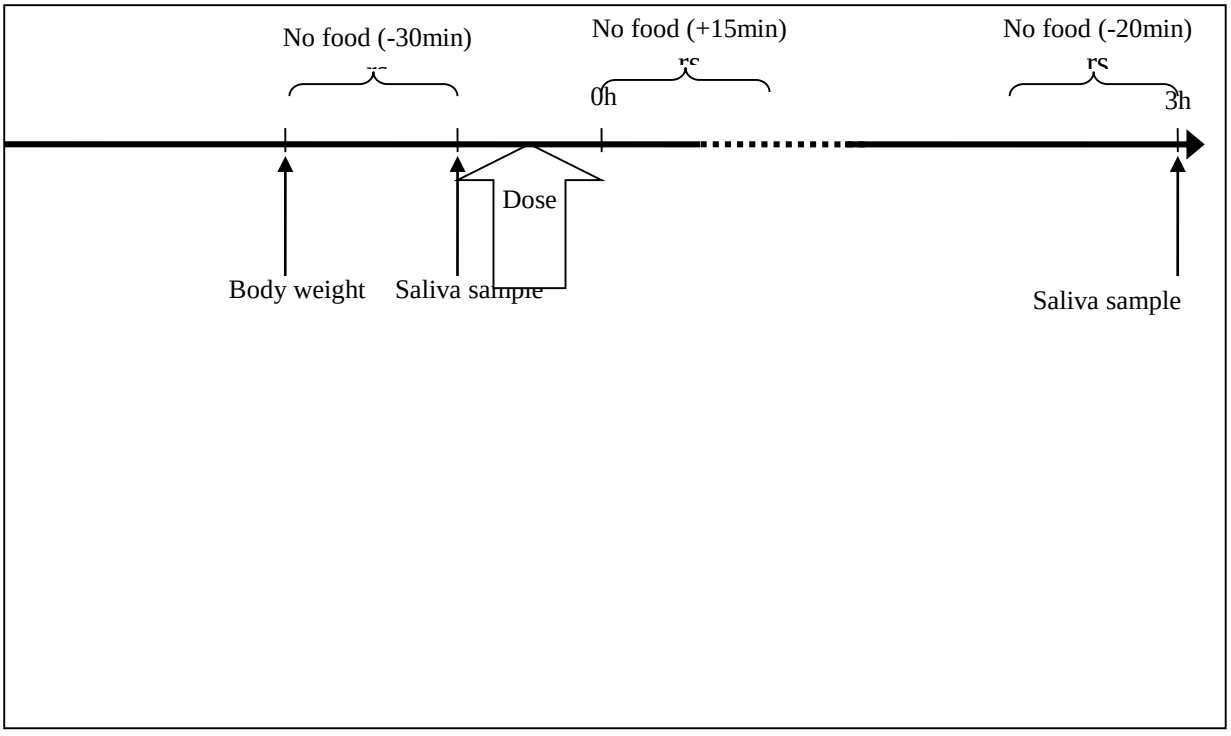
e) At end of dosing every thing is placed in the plastic bag. **Tare scales**, reweight the bag: WEIGHT AFTER DOSING. The difference between weights is then DOSE IN BABY!
f) Note the exact time of end of dosing in the form.
g) 15 min post dose fasting. In this time period the infant will be supervised toassure that the dose isn't regurgitated.

4) ~3 hours of spare time until the final saliva sample will be taken:

a) It is important that the baby is fasting in the given period before and after sampling and dosing. The baby can be breastfed in the time in between but it is important that the fluid intake is recorded. Estimation of the amount of fluid intake will be done by weighing the baby prior to and after breastfeeding.
b) The child will then be getting an accelerometer for the physically test and will be based in the play zone. The child will be observed, and if the child is getting breastfed the child will be weighted before and after the breastfeeding.

5) Post dose saliva sampling

a) 3 hours after completion of dosing a post dose saliva sample is taken. The infant should be fasting for 20 minutes pre sampling. Minimum 2 mL of saliva is required. Keep sample cold after collection (4ºC) and freeze (-20º to -80ºC) as soon as possible.
b) Repeat step **c-j** described in the **pre-dose saliva sampling. Section 2**
c) Note the time of sampling



D. Saliva sampling – practical notes/ experiences

Note: It may be helpful to have two people involved in the collection process. One person (preferably the mother to make the infant comfortable) can hold the infant, and the second person (nurse) can collect the sample.

The amount of saliva and the ease of collecting it may vary with the infant's age. Saliva flow may be stimulated by gently stroking the catheter over the tongue /playing/sucking/being close to the breast. Repositioning the infant may also help, if the infant is lying down the saliva may be more difficult to collect.

The time required for this will vary between infants. Be subject. It may take several attempts to collect the required volume

E. Dosing – practical notes/ experiences

The most important thing is to know exactly how much of the dose is in the infant.

The exact amount administered is obtained by weighing everything used for administration before administration of the dose and again after dosing. The difference in weight is equivalent to the amount of dose consumed by the infant. It is very important that nothing is spilled during administration of the dose. Working with infants this is a big challenge. So it is important to be prepared for colleting any spillage of the dose. This could be done by holding a tissue close to the infant's mouth (under the chin). If there is any uncertainty about the amount consumed (ex. spillage during dosing) by the infant the infant cannot be included in the study. Before administering the dose the infant should be dried around the mouth with a paper tissue.

During dosing the infant should be held in the mother's arms. Slowly and gently push the plunger down to gently squirt the water into the inside of the infant's cheek. Allow it to swallow it. DO NOT forcefully push down the plunger, or squirt the water to the back of the infant's mouth or throat, as the infant may choke.

The baby should be in a position so that it is easy for it to drink the dose without the dose being spilled (sitting or half-upright).

Dose preparation for infants (FTIR)

Table 1 show the standardized dose to give children from IAEA.

Table 1: Standardized doses of ^2H_2O for the estimation of TBW in children (from unpublished document by C. Slater, 2009 - IAEA)

Body weight (kg)	Weight of ^2H_2O required (g)
<10	3
10-20	6
20-30	10
30-50	20
>50	30

ANNEX 12: DEUTERIUM TEST - TOTAL BODY WATER IN THE ENROLLED CHILDREN

Responsible Data collector ID: ☐ Child´s ID: ☐☐☐☐

The Winfood Intervention Study

(DETERIUM TEST - TOTAL BODY WATER IN THE ENROLLED CHILDREN)

A: ***(BASIC INFORMATION)***

A1. *(Name of infant)*: _____

A2. *(Body weight at the anthropometric measurement)* _____

B: (SALIVA SAMPLING)

B1. *Time of pre dose saliva sample (local time)* _____

B2. *(Time of post dose saliva sample (local time)* _____

C: ***(Dose administered to infant)***

C1.*(WEIGHT BEFORE (weight of deuterium kit before dosing)*?_____g (X.XX)

C2.*(WEIGHT AFTER (weight of deuterium kit after dosing)?* _____g (X.XX)

C3.*(Extra tissues)*:

1- "*(weight before)*_____g" – "*(weight after)*_____g"= "*(dose spillage)* _____g"

2- "*(weight before)* _____g" – "*(weight after)* _____g"= "dose spillage_____g"

C4. *(TOTAL DOSE IN BABY)* _____g (X.XX)

C5.*(TIME OF DOSING)(end of dosing)* (local time): _____

C6*(COMMENTS)*

(COMPLETED BY (NAME) _____

F: *(Estimate of Fluid Intake)*

F1.*(Please note how many times the infant was breastfed in the 3 hours between measurements?)* ____

(Time of BF (local time) _____

(Weight of infant before BF) _____g

(Weight of infant after BF) _____g

(Milk Intake) _____g

(Time of BF (local time) _____

(Weight of infant before BF) _____g

(Weight of infant after BF) _____g

(Milk Intake) _____g

(TOTAL MILK INTAKE) g

(NOTES) :

ANNEX 13: DATA COLLECTION FORM TO MEASURE THE CHILD DEVELOPMENT MILESTONE – CARETAKER REPORT

The Winfood Intervention study

Responsible Data collector ID: ☐ Child´s ID: ☐☐☐☐

(Village): _____

(The tool for measuring milestone (ref. WHO – the Multicentre Growth Reference Study) – (The Caretaker report)

(Test items)	
1. (Sitting without support) *(Tested and recorded)* *(Recalled)*	☐☐ ☐☐ ☐☐☐☐ *(Day)(Month)(Year)*
2.*(Hands-and-knees crawling)* *(Tested and recorded)* 1 *(Recalled)* ……...2	☐☐ ☐☐ ☐☐☐☐ *(Day)(Month)(Year)*
3.*(Standing with assistance)* *(Tested and recorded)* 1 *(Recalled)*… ……...2	☐☐ ☐☐ ☐☐☐☐ *(Day)(Month)(Year)*
4.*(Walking with assistance)* *(Tested and recorded)* 1 *(Recalled)*… ……...2	☐☐ ☐☐ ☐☐☐☐ *(Day)(Month) (Year)*
5.*(Standing alone)* *(Tested and recorded)* 1 *Recalled)*… ……...2	☐☐ ☐☐ ☐☐☐☐ *(Day)(Month)(Year)*
6.*(Walking alone)* *(Tested and recorded)* 1 *(Recalled)*… ……...2	☐☐ ☐☐ ☐☐☐☐ *(Day)(Month)(Year)*

ANNEX 14: DATA COLLECTION FORM TO MEASURE THE CHILD DEVELOPMENT MILESTONE – STAFF REPORT

Responsible Data collector ID: ☐ Child´s ID: ☐☐☐☐

The Winfood Intervention Study
(Village): _____

(Date of data collection) **_____ / _____ / _____**

(No of data coll: BASELINE), **1, 2, 3, 4, 5, 6, 7, 8, 9**

(The tool for measuring milestone (ref. WHO – the Multicentre Growth Reference Study)(The Staff data collection form)

(Test items)	
1.*(Sitting without support)*	*(No (inability)* ..... 1 *(No (refusal)*..... 2 *(Yes)*..... 3 *Unable to test)*..... 9
2.*(Hands-and-knees crawling)*	*No (inability)*..... 1 *(No (refusal)*..... 2 *(Yes)*..... 3 *(Unable to test)* 9
3.*(Standing with assistance)*	*(No (inability)* ..... 1 *(No (refusal)*..... 2 *(Yes)*..... 3 *(Unable to test)* 9
4.*(Walking with assistance)*	*(No (inability)* ..... 1 *(No (refusal)*..... 2 *(Yes)*..... 3 *(Unable to test)* 9
5.*(Standing alone)*	*(No (inability)* ..... 1 *(No (refusal)*..... 2 *(Yes)*..... 3 *(Unable to test)* 9
6.*(Walking alone)*	*(No (inability)* ..... 1 *(No (refusal)*..... 2 *(Yes)*..... 3 *(Unable to test)* 9
7a. *(Child's emotional state)* *(Rate the child's emotional state during the testing of all the milestones)*	*(Drowsy)*..... 1 *(Awake and alert)*..... 2 *(Unable to test)* 9
7b. *(Child's emotional state)* *(Rate the child's emotional state during the testing of all the milestones)*	*(Calm)*..... 1 *(Fussy)* ..... 2 *(Crying)* ..... 3 *(Unable to test)* 9
(Remarks)	

ANNEX 15: INFORMATION AND CONSENT FORM – IN ENGLISH

The Winfood Intervention Study

INFORMATION FOR PARTICIPANTS IN A STUDY TITLED

Alleviating Childhood Malnutrition by Improved Utilization of Traditional Foods (WinFood)

Hello, my name is _____. I work with the University of Nairobi Institute of Tropical and Infectious Disease (UNITID).

The most vulnerable age group for lack of good nutrition (undernutrition) as well as lack of vitamins and minerals (micronutrient deficiencies) is children from 6 to 24 months of age, which is the time the child receives new foods in addition to breast milk (complementary feeding) period. At about 6 months, when breastmilk must be complemented with other foods, these foods must have high contents of energy and nutrients to maintain the high growth velocity. We are doing a study, where we will compare four types of complementary foods given to children in terms of effects on growth, nutritional status and wellbeing. Two of the complementary foods are similar to the usual cereals used to wean off infants from six months but with added termites and omena which all are very rich in iron and zinc. The other version has added the fish and a premix of vitamins and minerals. The last two complementary foods are Corn-Soy-Blend (CSB+), which is normally distributed by World Food Programme (WFP). The CSB product contains the important vitamins and minerals. If you choose to join the study, we will ask you to feed your child with the given complementary food in pre-prescribed amounts depending on age in the next 9 months. We cannot inform you

which of the foods you will be given, because you and all the other participants will be chosen randomly. We don't know which kind of complementary foods works best and therefore we do this study. During the 9 months period you will receive a monthly ration and we will ask you to bring your child to each monthly food distribution and permit for the following examinations of your child as outlined below by our trained staff.

Questionnaires

You will be asked questions about family situation, your child's health and development, breastfeeding pattern and your child dietary intake at beginning of the study and continuously until the end of the study. You do not have to answer any questions that you do not want to answer.

Clinical examination

Every month the child will be examined by a health professional, where temperature and blood pressure will be measured. The health professional will look for signs of symptoms of diseases or malnutrition. If your child is severely anaemic or malnourished, you and your child will be referred for treatment.

Body size

Weight, height, arm circumference, head circumference and thickness of fat at several places on the body will be measured at each month of the study.

At the beginning and the end of the study, we will measure the child body size by staple isotope deuterium-labelled water. First we will sample saliva from your child, about one teaspoon (max. 2 ml), then give your child staple isotope deuterium-labelled water ($2H_2O$) which is diluted in normal drinking sterile water (about one spoon = 10 mL). We need you to wait at the mobile-clinic for three hours, where we again will sample one teaspoon saliva from your child. The staple isotope deuterium-labeled water is naturally occurring, non-radioactive and no health risk at all. Deuterium does already exist naturally in your body fluid.

Blood samples

At the beginning and at the end of the study, we will take about one teaspoon of blood from your child's inner elbow. We will test your child's blood for anaemia, the iron and zinc status other nutrient deficiencies, general infection and measure the amount of growth hormones. The blood will not be used for anything else.

Your participation is entirely your choice. Whether you choose to participate or not, it will not affect the other services you and your family receive from health centers or other government authorities. Although we hope you will continue with the study for 9 months, you can stop participating with the study any time during the study.
Only minimum and absolutely necessary invasive procedures will be carried out your infant during this study and this will be no more than would be done during a normal routine examination. The amount of blood to be drawn on the two occasions in little (3cc) and will not to cause any harm to the subjects. Standard operating procedures of handling potentially infectious blood and blood products will be strictly followed during the study.
The wound inflicted on the infants during invasive procedures will be sterilized and dressed. No medical findings of a subject will be communicated to other subjects. Infants that show poor growth during the study will be referred for care.Any unanticipated discovery of a subject's unknown condition (disease, etc) as a result of study procedures will be communicated to the parent/legal guardian of the subjects. If necessary, the infants will be referred for treatment and/ or counselling.There will be no immediate direct benefit to the study participants apart from the high nutrient dense foods that will be given to the infants which are likely to enhance their nutritional status and improved knowledge to mothers on infant and young children feeding. On regional and national scale, the study aims to generate a simple adaptable method for developing complementary foods from locally available but neglected foods specifically adapted for the situation in Kenya. The complementary foods developed for this study are to be viewed as models for how complementary foods can be made from local foods. Potential uses of the developed foods will be in health and nutrition programs aimed at food-insecure and vulnerable populations and/or income-generating projects. The proposed intervention foods compare favourably with the currently distributed Corn Soy Blend by the World Food Program thus no adverse events expected. The children will however be continuously observed and parents/guardians advised to seek care incase of any unexpected observation. Incase of any, the subject will be referred and transported to the nearby appropriate health facility if need be. University of Nairobi Institute of Tropical and Infectious Diseases will be responsible (through the principal investigator) for the treatment of physical injuries resulting from infants participation in study procedures.
I do understand that the aim of this study is to understand the amounts of different foods and their effects on infants, that is, the food that will be provided by the study project, foods that are traditionally used by mothers to feed children and the con-soy blend which are the gold standards for malnourished children and supplied by World Food Program.

I have been fully informed of this study and I am aware that should I not wish to participate in this study it will not affect the treatment of myself or my child. Equally should I consent to participation I will not be given any special services or be given payment or gifts.

I agree to allow a visit to my home for interview and that I will allow the study group to ask me the types and amounts of foods I prepare for my baby. I will allow the study group to ask me how much of each food my baby will actually eat.

I agree to to allow the special water to be given to my child to drink when provided on selected days over one day on two occassions. I agree that I will allow the study group to collect saliva/urine from my child and myself over the selected days on both occassions.

I agree to allow the study group to collect blood (about a table spoonful-twice during the study) from my child so that they can measure the fat in the blood.

I agree to allow the study group to measure my child's weight, length, head circumference and skinfolds

I agree to allow the study group to measure my weight.

I agree to receive the packet of food that will be provided by the study team after all the measurements
This consent is only valid for this study. I hereby consent to participate.
Do you have any questions? If at any time during the study you have any questions, you can contact: The researcher or any of the other parties whose contacts are given below:

Researcher (Principal investigator): Sellina Ayoma Omollo Institute of Tropical and Infectious Diseases, University of Nairobi P.O. Box 19676-00202, NAIROBI. Tel.:0202726765 Mobile: 0729243930 Email: - selinaayoma@gmail.com	OR The Director, Institute of Tropical and Infectious Diseases, University of Nairobi P.O. Box 19676-00202, **NAIROBI**. Tel.:0202726765, Email: unitid@uonbi.ac.ke Attn : Prof B.B.A. Estambale	OR The Secretary, KHN/UON Ethics Reseach Committee, Kenyatta National Hospital, Hospital Road, P.O Box 20723, **NAIROBI** Tel 726300-9 Fax: 725272 Telegrams: MEDSUP, Nairobi Email:KHNplan@Ken.Healthnet.org

We would like to ask for your participation in the study now. If you agree to participate with your child in the study, please sign or mark in the box below.

Signature or thumbprint of the caregiver:

For study fieldworker
I have read the consent form in its entirety to the caregiver of the child.
Signature of study fieldworker:_____
Name (in print)
Date (day/month/year): _____/_____/_____
Leave a copy of the consent form with the caregiver. Circle the telephone number on the page to ensure that they understand they can call for more information.

MAELEZO NA KANDARASI KWA KISWAHILI

Utafiti wa Winfood

Maelezo kwa washirika katika utafiti uitwayo

KUKABILIANA NA LISHE DHAIFU KWA WATOTO KWA UTENGENEZAJI WA VYAKULA BORA

Jambo, Jina langu ni _____. Nafanya kazi na Cuo Kikuu Cha Nairobi.

Watoto wa umri wa kati ya miezi sita na ishirini na nne wanaweza kudhoufika kiafya wasipopata lishe bora. Haswa wanapofika miezi sita na kuhitaji vyakula vingine pamoja na maziwa ya mama.

Tunafanya utafiti wa vyakula vitatu tofauti na kuona jinsi ambavyo vinaimarisha kukuwa na afya ya watoto wa miezi sita hadi kumi na tano. Chakula moja inaviungo ya mahindi, unga ya terere pamoja na omena na tchiswa anbavyo vina madini ya zinc na iron. Chakula cha pili kina viungu vya mahindi, unga ya terere na madini. Chakula cha tatu kina viungu vya mahindi, soya na madini.

Iwapo utakubali mtoto wako ashiriki katika utafiti huu, utahitajika kumlisha chakula kwa kipimo utakayoelezewa kulingana na umri wake kwa muda wa miezi tisa. Hatutakuambia ni chakula kipi mwanawe atapewa. Kwa muda wa miezi tisa, utapokea vipimo vya chakula kila mwezi.

Utahitajika kumleta mtoto cliniki mara moja kila mwezi kupokea chakula na pia kupimwa na wauguzi kama ifuatovyo:

Kujaza fomu

Utaulizwa maswali juu ya jamii yako, afya na kukua kwa mtoto, jinsi anavyo anakunywa maziwa ya mama, vyakula anavyukula, na kadhalika. Haupaswi kujibu swali lolote usilotaka kijibu.

Kupimwa kwa hali ya afya

Kila mwezi, mtoto atapimwa na muuguzi. Atapimwa joto ya mwili, presha, pia dalili ya kudhoufika afya. Iwapo anakosa damu au kudhoufika kwa hali yoyote, atapewa matibabu.

Vipimo vya mwili

Mtoto atapimwa urefu, kimo, mkono, kichwa na mafuta ya mwili.

Mwanzo na mwisho wa utafiti, tutapima mafuta ya mwili kwa kutumia mbinu ya kisayansi ya maji maalum yaitwayo ‘stable isotope deuterium labeled water’ ambayo yatachanganywa na maji ya kawaida. Haya maji hayana madhara yoyote na yamo katika mwili wa binaadam kwa kiwango. Tutapima mafuta kwa kuchukuwa mate ya mtoto kutumia pampa, mara tatu kwa siku. Utahitajika kukaa kwa cliniki kwa muda wa masaa matatu.

Kupimwa damu

Mwanzo na mwisho wa utafiti, damu ya mtoto itapimwa. Itachukuliwa kwa mkono wake. Tutapima hali ya damu, madini ya iron na zinc na homoni. Damu haitatumika kwa hali nyingine ila utafiti.

Ushirikiano wako katika utafiti huu ni kwa hiari yako. Unaweza kuchagua kushiriki au la. Chaguo lako halite kuzuia tupata huduma katika cliniki hii au mashirika ya serikali. Unaweza kukatisha ushirika hata kabla ya miezi tisa kuisha, ingawaje tungependa umalize miezi yotr tisa.

Ni vipimo vichache tuu na vya maana ambavyo vitafanyiwa mtoto wako kama ilivyo desturi ya utafiti wa kiafya. Tutachukua damu kidogo sana (3ml) na hatutamdhuru mtoto wako.

Maneno yote utakayosema juu yako na jamii yako hayatafunuliwa. Yatawekwa siri.

Watoto ambao wanaonyesha dalili ya kudhoufika watapewa matibabu.

Hamna malipo yoyote kwa washiriki ila kupewa chakula ambayo kitawajenga watoto miili.

Tunatarajia ya kwamba utafiti huu utawezesha kuundwa kwa vyakula maalum ya watoto wa miezi sita kuendelea, ambavyo vina viungu vya kitamaduni vya Kenya.

Vyakula hivi vitapewa wakaaji wa maeneo yanayokumbwa na baa la njaa.

Naelewa ya kwamba utafiti huu ni ya kulinganisha vyakula vitatu tofauti na kupima nguvu ya kila chakula kwa afya na kukuwa kwa watoto wa miezi sita hadi kumi na tano. Vyakula hivi vimetengenezwa na viungu vya kitamaduni ya Kenya.

Naelewa kuwa sio lazima nishiriki katika utafiti huu. Kutoshiriki hautanitenga na matibabu ya cliniki hii au huduma yoyote ya serikali. Aidha, naelewa ya kwamba kushiriki hauna malipo yoyote.

Nakubali watafiti kuzuru nyumba yangu kuniuliza maswali kuhusu vyakula tunavyokula, jinsi ambavyo tunapika, na kupima viungo na pia jinsi ambavyo mtoto anakula.

Nakubali maji maalum yapewe mtoto wangu mwanzo na mwisho wa utafiti. Nakubali mate ya mtoto ipimwe.

Nakubali watafiti watoe na kupima damu ya mtoto wangu.

Nakubali watafiti wapime kimi, urefu, kichwa, mkono na mafuta ya mwili wa mtoto wangu.

Nakubali kupimwa kimo.

Nakubali kupokea pakiti ya chakula kila mwezi baada ya vipimo vyote.

Kandarasi hii ni ya utafiti huu pekee. Nakubali kushiriki.

Unayo maswali? Iwapo unamaswali kwa wakato wowote unaweza kuwasiliana na maafisaa wafuatao:

Mtafiti Mkuu, Sellina Ayoma Omollo UNITID Chuo Kikuu Cha Nairobi Simu: 0729243930 Barua pepe: selinaayoma@gmail.com	Au, Mkurugenzi Mkuu, Prof. B. Estambale UNITID, Chuo Kikuu Cha Nairobi SLP 19676-00202, Nairobi. Simu: 0202726765 Barua pepe: unitid@uonbi.ac.ke	Au, Katibu Mkuu, KNH/UON Ethics Research Committee, SLP 20723, Nairobi Simu: 726300-9 Barua pepe: KNHplan@Ken.Healthnet.org

Tungependa kukuomba uwe mshiriki katika utafiti huu. Iwapo unakubali, pamoja na mtoto wako, tafadhali weak sahihi au alama yako katika sanduku ifuatayo:

Sahihi au alama

Kwa afisaa wa utafiti
Nimesmsomea mzazi wa mtoto kandarasi hili kwa kina.
Sahihi ya afisaa_____
Jina_____ **Tarehe**_____
Mpe mzazi fomu moja. Andika nambari yako ya simu na umuelezee ya kwamba anaweza kuwasiliana nawe.

Annex 16: Budget

Budget Line Items	Year 1 (US$)	Year 2 (US$)	Year 3 (US$)	Year 4 (US$)	Total	% of total
Personnel						
Laboratory Assistant (1)	0.00	2,500.00	5,000.00	2,500.00	10,000.00	
Senior Nurses (2)	0	3,000.00	7,000.00	3,500.00	13,500.00	
Senior Nutritionist (2)	0.00	4,000.00	8,000.00	4,000.00	16,000.00	
Total Personnel	**0.00**	**9,500.00**	**20,000.00**	**10,000.00**	**39,500.00**	**15.97**
Complementary blend Randomised study					0.00	
Food production	0.00	3,000.00	10,000.00	5,000.00	18,000.00	
Field staff and baseline recruitment	0.00	4,000.00	4,000.00	4,000.00	12,000.00	
9 month subject follow-up	0.00	0.00	5,000.00	5,000.00	10,000.00	
Biochemical, food, and child development analyses including the stable isotope analyses	0.00	15,000.00	20,000.00	30,000.00	65,000.00	
Data double entry and checking	0.00	2,000.00	5,000.00	3,500.00	10,500.00	
Statistical analyses	0	0	7,000.00	4,000.00	11,000.00	
Preparation of reports and papers	0	0.00	0	2,500.00	2,500.00	
Oral and written dissemination of results	0	0	0	10,000.00	10,000.00	
Total randomized trial Study	**0.00**	**24,000.00**	**71,000.00**	**64,000.00**	139,000.00	**64.27**
Equipment					0.00	
Laptop	1,000.00	0	0	0	1,000.00	
Desktop computer	800	0	0	0	800.00	
Printer	400	0	0	0	400.00	
Digital camera	200	0	0	0	200.00	
cooler box	1,000.00	0	0	0	1,000.00	
Total Equipment	**3,400.00**	**0.00**	**0.00**	**0.00**	3,400.00	**1.37**
Recurrent costs					0.00	
Transport	0.00	4,000.00	5,000.00	4,000.00	13,000.00	
Office rent	0.00	3,000.00	3,000.00	3,000.00	9,000.00	
Communication	0.00	3,600.00	3,600.00	3,600.00	10,800.00	
Stationery	500.00	1,000.00	1,000.00	1,000.00	3,500.00	
Total Recurrent costs	**500.00**	**12,200.00**	**12,200.00**	**12,200.00**	37,100.00	**15.00**
SUBTOTAL	**500.00**	**45,700.00**	**103,200.00**	**86,200.00**	215,600.00	
CONTINGENCIES (5% of Total)	**25.00**	**2,285.00**	**5,160.00**	**4,310.00**	10,780.00	
GRAND TOTAL	**525.00**	**47,985.00**	**108,360.00**	**90,510.00**	226,380.00	

ANNEX 17: FOOD RECIPES & INGREDIENTS NUTRITIONAL COMPOSITION SAMPLE EXCEL SHEET

Source: http://www.nal.usda.gov/fnic/foodcomp/search/index.html

Ingredients (%)		**66.00%**	**15.00%**	**3.00%**	**15%**	**0.6%**	**0.4%**					
Wts in 50kgs		**33.00%**	**7.50%**	**1.50%**	**7.50%**	**0.30%**	**0.20%**					
Nutrient	**Units**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**	**Value per 100g**		**Nurient density value as eaten-porridge assuming 100ml consumption**		
Proximates		**Amaranthus**	**Maize**	**Omena (Dagaa)**	**Insect (Termite)**	**Oil (soy bean)**	**Sugar**	**Product**	**Value on 40g serve/day**		**RDI**	**% RDI met by 40g serve**
Water	G	2.37	15.2	15.35	1.7	0	0.3	**4.6**	1.8	0.9	1100.0	0.2
Energy	kcal	402.44	1476	359	656	884	387	**603.0**	241.2	120.6	686.0	35.2
Protein	G	16.68	12.4	50.5	35.7	0	0	**19.7**	7.9	3.9	9.6	82.2
Total lipid (fat)	G	6.96	3.6	14.4	54.3	100	0	**14.3**	5.7	2.9		
Ash	G	2.58	1.5	7.2	4.8	0	0	**2.9**	1.1	0.6		
Carbohydrate, by difference	G	68.27	68.6	33.67	3.5	0	99.98	**57.3**	22.9	11.5		
Fiber, total dietary	G	3.14	0	9.7	3.4	0	0	**2.9**	1.1	0.6		
Sugars, total	G				0	0	99.91	**0.4**	0.2	0.1		
Minerals												
Calcium	Mg	189.14	8.2	33.3	22			**130.4**	52.1	26.1	525.0	9.9
Phoshorus	Mg	322.75	231	277	182.3			**283.3**	113.3	56.7	80.0	141.7
Magensium	Mg	219.45	92.13	86	42.63			**167.6**	67.1	33.5	80.0	83.8
Potassium	Mg	324.39	287	461	259.6			**309.9**	124.0	62.0	400.0	31.0
Sodium	Mg	7.99	35	55	123.6			**30.7**	12.3	6.1	320.0	3.8
Iron	Mg	15.8	2.62	1.3	11.52			**12.6**	5.0	2.5	11.0	45.8
Zinc	Mg	5	1.8	0.4	10.23			**5.1**	2.0	1.0	2.8	73.1
Copper	Mg	1.7			1.7			**1.4**	0.6	0.3	0.3	183.6
Manganese	Mg	2.5			3.29			**2.1**	0.9	0.4	0.2	428.7
Lipids												
Fatty acids, total saturated	G		0.5	0.3	14.7	14.7	0.0	**2.4**	1.0	0.5		
18:2 undifferentiated	G		0	0	6.2	53.3	0.0	**1.2**	0.5	0.2		
18:3 undifferentiated	G		0	0	0.7	7.1	0.0	**0.2**	0.1	0.0		
Vitamins*												
Vitamin A	Mcg		0	43	2.41	3		**1.7**	0.7	0.3	350.0	0.2
Vitamin D	Mcg			12				**0.4**	0.1	0.1	7.0	2.1

ANNEX 18: SAMPLE REFERRAL LETTER

The Winfood Intervention Study

PRESCRIPTION AUTHORIZATION/LETTER OF MEDICAL NECESSITY OF A STUDY SUBJECT

Child Name	
Child Study ID	

ATTENTION FOR CLINICAL REVIEW:

This letter provides a medical necessity for a clinical review of -_____(child name) hereby accompanied by the mother/guardian/care giver following _____(reason for referral e.g. poor growth e.t.c) observed in the last _____months in an intervention study we are currently undertaking in Western Kenya and in which the referred infant(s) is a participant. Accept this notification as such from Dr/Mr_____(print physician name) of _____health facility who has examined/observed/prescribed and recommended that the prescription/recommendations are necessary for _____ below for purposes referenced. This document does not guarantee coverage for the listed item(s)

Diagnosis or reason for medical necessity_____

Physician Signature_____Date_____

Diagnosis and reason for medical necessity_____

For more information do contact any the following in that order:

TO The researcher (PI) Attn: (Sellina Ayoma Omollo), Institute of Tropical and Infectious Diseases, University of Nairobi P.O. Box 19676-00202, NAIROBI. Tel.:0202726765/0729243930 Email: selinaayoma@gmail.com.com	OR The Director, Institute of Tropical and Infectious Diseases, University of Nairobi P.O. Box 19676-00202, NAIROBI. Tel.:0202726765, Email: unitid@uonbi.ac.ke Attn : Prof B.B.A. Estambale	OR The Secretary, KHN/UON Ethics Research Committee, Kenyatta National Hospital, Hospital Road, P.O Box 20723, NAIROBI Tel 726300-9 Fax: 725272 Telegrams: MEDSUP, Nairobi Email:KHNplan@Ken.Healthnet.org

ANNEX19: KENYA BUREAU OF STANDARDS FOODS ANALYSIS REPORT

Kenya Bureau of Standards

Fax: +254 (0) 20 604031/609660
E-Mail:info@kebs.org
Website: www.kebs.org

Laboratory Test Report

KEBS Centre, Popo Road
P.O. Box 54974, 00200 Nairobi
Tel.: (+254 020) 605490, 605550

Report Ref: KEBS/TES/**1181/M/11**

Date: **02 March 2011**

PRIVATE SAMPLE

1. Description of Sample: **Amaranth Based Flours**
2. Sample Submitted by: **Jomo Kenyatta University of Agriculture & Tech.**
3. Customer Contact: **John Ndungu**
4. Customer's Ref. No:
5. Customer's Address: **P.O. BOX 62000-00200, Nairobi Kenya**
6. KEBS Sample Ref.No: **BS/03532/11**
7. Date of Receipt : **18 February 2011**
8. Date Analysis Started: **21 February 2011**
9. Sample Submission Form No: **47608**
10. Additional information provided by the customer:
Winfood Lite
11. Acceptance criteria-title and number of specification against which it is tested:
Not Applicable
12. Parameters tested and Method(s) of test: as listed in the report below

LABORATORY TEST REPORT

No.	Parameters		Results	Requirements	Test Method No	LOD
1.	E. coli	cfu/g	Not detected	Not Specified	TES/MIC/TM/17	10
2.	Salmonella	/30g	Not detected	Not Specified	TES/MIC/TM/08 *	1

Please note that tests marked with an * are covered by our current UKAS accreditation scope.

COMMENTS/REMARKS:
The sample performed as shown

Clarkson Agembo - Manager, Microbiology Laboratory
FOR: MANAGING DIRECTOR

02 March 2011
Date of Issue

ANNEX 20: APPROVAL BY KNH/UON-ERC (P334/08/2011)

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke
Link:www.uonbi.ac.ke/activities/KNHUoN

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/59

20th March 2012

Sellina Ayoma Omollo
UNITID
University of Nairobi

Dear Sellina

RESEARCH PROPOSAL: "EFFECT OF IMPROVED COMPLEMENTARY FOODS ON LEAN BODY MASS, LIPID PROFILE AND GROSS MOTOR DEVELOPMENT OF KENYAN CHILDREN" (P334/08/2011)

This is to inform you that the KNH/UoN-Ethics & Research Committee (ERC) has reviewed and **approved** your above revised research proposal. The approval periods are 20th March 2012 to 19th March 2013.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN -ERC website **www.uonbi.ac.ke/activities/KNHUoN**

"Protect to Discover"