

EFFECT OF IMPROVED COMPLEMENTARY
FOODS ON GROWTH AND IRON STATUS OF
KENYAN INFANTS

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University of Nairobi Institute of Tropical and Infectious Diseases

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November, 2014

DECLARATION

This thesis is my original work and has not been submitted for an award of a degree in any other University and that all sources of materials used for the thesis have been correctly acknowledged.

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DEDICATION

This thesis is dedicated to my beloved soul mate and lovely wife Annemarie, our children Adrienne, Audrey and those to come, to my grandmother Odilia and to all the mothers and their children in resource limited settings to whom feeding is a daily struggle.

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ACRONYMS AND ABBREVIATIONS

ACC: Administrative Committee on Co-ordination (ACC) of the United Nations

AOAC: Association of Official Analytical Chemists.

ASFs: Animal Source Foods

CODESRIA: Council for Development of Social Science Research in Africa

CONSORT: Consolidated Standards of Reporting Trials

CRP: C-Reactive Protein

CSB: Corn Soy Blend

CSB+: Corn Soy Blend Plus

CSB++: Corn Soy Blend Double Plus

DANIDA: Danish International Development Agency

DPT: Diphtheria, Pertussis, Tetanus.

FAO: Food and Agricultural Organization

GSIIYCF: Global Strategy for Infant and Young Children Feeding

HAZ: Height-for-Age Z score

Hb: Hemoglobin

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

ID: Iron Deficiency

IGF: Insulin-like Growth Factor

IYCF: Infant and Young Child Feeding

IZNCG: International Zinc Nutrition Consultative Group

KDHS: Kenya Demographic Health Survey

KIRDI: Kenya Industrial Research and Development Institute

KNH ERC: Kenyatta National Hospital Ethics Review Committee

LVEMP: Lake Victoria Environment Management Program

MAFA: Mid Arm Fat Area

MAM: Moderate Acute Malnutrition

MAMA: Mid Arm Muscle Area

MDGs: Millennium Development Goals

MGRS: Multicenter Growth Reference Study

MUAC: Mid Upper Arm Circumference

NCHS: National Centre for Health Statistics

RBP: Retinol Binding Protein

SCN: (United Nations) Standing Committee on Nutrition

SOP: Standard Operating Procedure

sTfR: Soluble Transferrin Receptor

STIs: Sexually Transmitted Infections

TfR: Transferrin receptors

TM: Trade Mark

TMB: Tetramethylbenzidine Dihydrochloride

UNICEF: United Nations Children's Fund

UNITID: University of Nairobi Institute of Tropical and Infectious Diseases

VAD: Vitamin A Deficiency

WAZ: Weight-for-Age Z score

WFC: WINFOOD Classic

WFL: WINFOOD Lite

WFP: World Food Programme

WHO: World Health Organization

WHZ: Weight-for-Height Z score

WLZ: Weight-for-Length Z score

DEFINITION OF OPERATIONAL TERMS

Anaemia: Haemoglobin concentration below 11.5 g/dl of blood

Animal Source Foods: Nutrient dense foods of animal origin that provide protein of high biological value, energy, and fat and are likely to be the only unfortified foods that can provide enough calcium, iron, and zinc for infants and children

Complementary foods: Foods other than breast milk or infant formula (liquids, semisolids, and solids) introduced to an infant to provide nutrients.

Entomophagy: The practice of eating insects.

Food fortification: “The addition of one or more essential nutrients to a food, whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups” (FAO, 1996).

Indigenous foods: Foods known to be grown in a particular area that cannot be grown elsewhere

Low Body Mass Index: Body mass index below 18.5 kg/m²

Malnutrition: A condition that results from eating a diet in which nutrients are not enough or are too much such that it causes health problems. Often used specifically to refer to undernutrition where there are not enough calories, protein or micronutrients; however, it also includes overnutrition.

Moderate Acute Malnutrition (MAM): A weight-for-age between -3 and < -2 Z the median of the WHO Child Growth Standards which can be due to wasting or stunting or to a combination of both

Morbidity: Abnormality observed in the study participants as a result of infections

Pathology: Deviation from a healthy or normal condition

Study participants: Children who participated in the study

Traditional foods: Includes wild indigenous plants and animals collected from land and aquatic environments, semi domesticated indigenous plants and animals, locally available staple foods processed using indigenous techniques

PREAMBLE

The primary aim of this thesis was to comparatively assess the effect of three improved complementary foods namely, 1) maize-germinated grain amaranth-edible termite- *dagaa* small fish complementary food (WINFOOD Classic-(WFC)), 2) multi-micronutrient (with vitamins and minerals) fortified maize-germinated grain amaranth complementary food (WINFOOD Lite-(WFL)) and 3) multi-micronutrient fortified Corn Soy Blend plus (CSB+) on linear growth (stunting), weight gain, haemoglobin and iron status of Kenyan infants 6-15 months. To achieve the primary objective, the study had the following secondary objectives;

1. To assess the acceptability of the improved complementary foods(WFC and WFL) and CSB+ among the study population prior to the randomized controlled trial (RCT)
2. To determine the prevalence and severity of malnutrition among the study population prior to the RCT
3. To assess the effect of the improved complementary foods (WFC and WFL) compared to CSB+ on linear growth (stunting) and body mass accrual (weight gain) of infants 6-15 months old
4. To assess the effects of the improved complementary foods (WFC and WFL) compared to CSB+ on iron status and anaemia prevalence among infants 6-15 months old

This thesis presents results of work done in three sub-studies and organized into eight chapters. **Chapters 1 and 2** provide the background information for the study with literature review on key components of the study. In **Chapter 3**, in order to fulfill specific objective number one, the sensory attributes of the three improved complementary foods namely, WFC,CSB+ and WFL were assessed and the factors influencing consumption identified for

improvement. This work has since been published in a peer reviewed journal. In **Chapter 4**, the prevalence and severity of malnutrition among the study population were assessed. This was to achieve the second specific objective where a food consumption and nutrition assessment was done in a cross-sectional survey. **Chapter 5** reports on the findings of the RCT in which the effect of the three improved complementary foods on growth (both linear growth and weight gain) was assessed. **Chapter 6** explores if enriching amaranth grain porridge with *dagaa* small fish and edible termites as animal source foods for enhanced protein and minerals intake or with a multi-micronutrient fortification can improve iron status and reduce iron deficiency among 6-15 months old infants and young children. The effect of the adequacy of iron intake was estimated and their potential in reducing iron deficiency prevalence investigated. Finally, in **Chapter 7** the main findings and conclusions as well as the implications for public health policy and future research of this thesis are discussed.

LIST OF PUBLICATIONS

1. Oiye SO, **Konyole S** and Ngala SN. (2012) Effects of Rosemary Spice (*Rosmarinus Officinalis* L.) and Nitrite Picking Salt Combination on Keeping and Organoleptic Quality of Beef Sausages *J. Basic. Appl. Sci. Res.*, **2(4)**4008-4015.
2. Okeyo NO.,**Konyole SO.**,Okeyo LA.,Abongo BO.,&Onyango RO.(2012) Characteristics of caregivers and households practicing bottle-feeding in Kisumu East District. *African Journal of Food Agriculture, Nutrition and Development*, **12 (7)**, 6868-6880.
3. John N. Kinyuru, **Silvenus O. Konyole**, Glaston M. Kenji, Christine A. Onyango,Victor O. Owino, Bethwell O. Owuor, Benson B. Estambale, Henrik Friis & Nanna Roos (2012) Identification of Traditional Foods with Public Health Potential for Complementary Feeding in Western Kenya. *Journal of Food Research* **1(2)**148-158
4. **Silvenus O. Konyole**, John N. Kinyuru, Glaston M. Kenji, Christine A. Onyango, Bethwell O. Owuor, Benson B. Estambale, Henrik Friis, Nanna Roos and Victor O. Owino (2012) Acceptability Of Amaranth Grain-Based Nutritious Complementary Foods With Dagua Fish (*Rastrineobola Argentea*) And Edible Termites (*Macrotermes Subhylanus*) Compared To Corn Soy Blend Plus Among Young Children/Mothers Dyads In Western Kenya. *Journal of Food Research* **1(3)**111-120
5. John N. Kinyuru, **Silvenus O. Konyole**, Glaston M. Kenji, Christine A. Onyango,Victor O. Owino, Bethwell O. Owuor, Benson B. Estambale, Henrik Friis & Nanna Roos(2013).Nutrient Composition of Four Species of Winged Termites Consumed in Western Kenya. *Journal of Food Composition and Analysis* **30(2)**, 120-124.

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ABSTRACT

Background: Infant growth faltering is usually associated with an increased risk of morbidity and mortality. It is common in resource limited settings and constrains infants' full development resulting in reduced physical capacity and overall productivity later in life. Major causes are inappropriate feeding practices and poor quality complementary foods with low energy density and micronutrients deficiency. Interventions to improve the quality of complementary foods exist like use of fortified blends among others but with mixed results/outcomes. Among the interventions is also the use of animal source foods (ASFs) to improve the quality of the blends. With increasing attention to ASFs and especially those that are locally available, easily accessible, cheaper and acceptable in the localities, the effects of the same on infant nutrition, growth and development need special attention. The impact of incorporating indigenous nutrient rich and relatively cheap, locally available and readily acceptable but underutilized/neglected ASFs such as edible termites and *dagaa* small fish and micronutrient fortification of complementary foods on growth of infants from resource limited settings has been inadequately studied.

Objectives: To test the acceptability and efficacy of improved complementary food blends named WINFOOD Classic ((WFC)- with 10% edible termites, 3% *dagaa* small fish and germinated grain amaranth), WINFOOD Lite ((WFL)-with germinated grain amaranth fortified with micronutrients at the rate of Corn Soy Blend Plus (CSB+)) and CSB+ (Corn and Soya fortified with micronutrients) on growth (measured as length and weight gain), haemoglobin concentration, anaemia prevalence and iron status of infants 6 to 15 months from a resource limited rural setting in Kakamega County, Western Kenya.

Study implementation: Three-stage study comprising assessment of: 1) acceptability of improved-animal source foods enhanced-fortified complementary blends 2) prevalence of malnutrition in the study population among infants 6-23 months and, 3) the effect of the complementary foods on linear growth and weight gain, haemoglobin concentration, anaemia prevalence and iron status among infants 6 -15 months of age in a randomized controlled trial (RCT).

Design: The acceptability study was a cross over design study with wash out periods while the nutritional status study prior to the intervention study was determined using a cross section design. The intervention study was a community-based double blind randomized trial in which infants were individually randomized at 6 months of age to receive one of the three study complementary foods: WFC, WFL and CSB+ for 9 months.

Methods: Acceptability to mothers and infants of the improved-ASFs enhanced and fortified complementary blends WFC, WFL or CSB+ porridge recipes was assessed prior to the industrial production of the blends among fifty seven (57) infant-caregivers dyads.

A cross sectional anthropometric, dietary and morbidity data among six hundred and eighteen (618) infants and young children aged 6-23 months old was also collected prior to the randomized controlled trial (RCT).

In the intervention study, Four hundred and ninety nine (499) infants were randomized to receive WFC (n= 165), WFL (n= 167) or CSB+ (n=167) from 6–15 months of age. The primary outcome was linear growth while secondary outcomes included weight gain, haemoglobin concentration, iron status (determined as ferritin and soluble transferrin receptor

cells), reported morbidity and serum concentration of acute phase reactants. Anthropometric measurements were taken monthly. Dietary intake and morbidity were determined by mother reported monthly recall. Haemoglobin concentration and iron status and related biomarkers were also assessed at baseline (6 months) and endline (15 months).

Results: The acceptability study showed that all the three foods (WFC, WFL and CSB+) were acceptable to the study population with no adverse effects.

The cross section study to help interpret the results of the intervention study showed that malnutrition was highly prevalent. Despite high breastfeeding rates of about 83%, complementary feeding was introduced earlier than recommended at an average age of 3.8 (SD 0.3) months. The prevalence of stunting (Z-scores for height-for-age [HAZ] <-2), wasting (Z-scores for weight-for-height [WHZ] <-2) and underweight (Z-scores for weight-for-age [WAZ] <-2) was 35.7%, 4.1%, and 13.4%, respectively in the study population.

The intervention study revealed a mean length gain from 6 to 15 months of 9.5 (SD 2.4) cm, 9.8 (SD 2.0) cm and 9.7(SD 2.2) cm for infants in the WFC, CSB+ and WFL groups respectively (p = 0.39). The mean weight gain between 6 and 15 months was 2.0 (SD 0.8) kg, 2.2 (SD 0.7) kg and 2.2 (SD 0.7) kg for infants in the WFC, CSB+ and WFL groups respectively (p=0.35). Infants in WFC, CSB+ and WFL had significantly higher haemoglobin concentration (p < 0.001) at 15 months compared to haemoglobin concentration at 6 months [-0.3(SD1.8) g/dl, 0.6 (SD 1.6) g/dl and 0.2(SD 2.0) g/dl respectively].

The mean transferrin receptors were significantly different between 6 and 15 months: 1.9 (SD 5.2), -1.4 (SD 3.5) and 0.3 (SD 3.6) (mg/l) (p<0.001) for WFC, CSB+ and WFL respectively. The iron body stores (mg/kg body weight) at 15 months was also significantly different from those at six months: -2.5 (SD 4.2), -0.7 (SD 3.8) and -1.7 (SD 4.0) (p=0.001) for WFC, CSB+ and WFL respectively.

The daily energy intake from traditional complementary foods for infants 6-8, 9-11 months and young children ≥ 12 months old was 1.6 \pm 0.7, 2.4 \pm 0.5 and 2.2 \pm 0.5 (MJ/day) respectively while the Infants 6-8, 9-11 and ≥ 12 months consumed about 60g, 142g and 150g respectively. The daily iron, calcium and zinc intake from the complementary foods was 0.4 \pm 0.2 mg, 130.0 \pm 90.0mg and 2.0 \pm 0.6 mg respectively.

Throughout the 9 months study period and across the food groups, the infants were well/alert and the caregivers reported no major symptoms of disease except for coughing, audible wheezing and running nose (76.3%, 61% and 80.5% respectively) which were significantly different across all the food groups in the first 2 months of the study (p=0.02, 0.01 and 0.01 respectively).

Conclusion: All the foods were acceptable with no adverse effects. The prevalence of malnutrition in the study area was high. The complementary food blends had a similar effect on infant and young children growth (weight and length gain) but had significant improvement in haemoglobin concentration, body iron stores and transferrin receptors. These blends may therefore be used to improve the micronutrient status and health of infants in resource limited settings. More research to investigate the bioavailability mechanisms of the nutrients from the edible termites is however needed to help create evidence based and enabling policy environment to support the use of the edible insects in related interventions.

CHAPTER ONE

1. INTRODUCTION

1.1Background

Growth retardation of infants and young children (IYC) constrains their full development and is associated with a higher risk of morbidity and mortality (WHO, 2003; UNICEF, 2014). The result is delayed motor development, reduced physical capacity and overall productivity later in life (UNICEF, 2014; World Bank, 2012). Together, Africa and Asia account for 94 % of all underweight children below five years in developing countries (UNICEF, 2014). In Kenya about 35% of children are stunted, 16 % underweight, 7% are wasted and only 32% of children below six months are exclusively breastfed (UNICEF, 2014; KDHS, 2008-09).

Growth faltering is a major public health problem in resource limited settings (Shrimpton et al., 2001; UNICEF, 2009) and rapidly accelerates between 3-12 months of life (Shrimpton et al., 2001) which coincides with the introduction of complementary foods when breast milk alone is insufficient to meet entire nutritional requirements of IYC (WHO, 2002; Dewey, 2005). Moreover, inadequate food intake and infections especially in situations where nutritionally adequate complementary foods are limited, hinders optimum feeding and growth of IYC after 6 months of age when breastfeeding alone becomes inadequate (Dewey, 2005). Multiple micronutrient deficiencies result in such circumstances (UNICEF, 2014) since the commonly used complementary foods often fail to meet the nutritional requirements of IYC (Dewey, 2005). This is because the complementary foods are often plant foods with low energy, low nutrient density and anti-nutrient factors that limit the absorption of essential micronutrients in addition to inappropriate complementary feeding practices (Gibson et al., 1998; Lartey et al., 1999; WHO, 2003; Mamiro et al., 2005).

Food fortification has been used to increase micronutrient density. Energy density can be improved by the addition of oil and sugar or by the reduction of the amount of water added during complementary food preparation (Dewey and Brown, 2003). According to Piwoz &

Prebble (2000), there is little data on energy and nutrient intake from complementary foods while dietary intake data can be used to determine the level of nutrient addition during fortification of complementary foods. Dietary data can also serve as the basis for the recommendation of daily rations useful in the education of caregivers on sound feeding practices to help prevent early growth faltering (Lutter, 2003; Owino et al., 2008). Enzymes like α -amylase which reduce the bulk density may also be used to increase food intake and industrial processing techniques such as extrusion cooking may be used to ensure good hygiene (Dewey and Brown, 2003).

The most frequently used strategies to correct iron deficiencies include food fortification in various forms and/or supplementation (WHO, 2008) and plant-breeding techniques/biofortification (Caulfield et al., 2006). The effectiveness of large-scale fortification programs is however reduced due to cost, challenges in ensuring constant and continuous availability, timely distribution of fortificants, and compliance with the prescribed fortificant (Schultink and Gross, 1996; Thu et al., 1999). The limitations of fortification and supplementation for micronutrients deficiency correction underline the importance of other strategies employing the food-based approach (WHO, 2008).

The World Health Organization (WHO) (2003) recommends use of nutritious indigenous foods together with traditional processing technologies such as fermentation and germination among others to yield nutrient and energy-dense complementary foods for IYC. Due to the limitations of the other strategies, food based approaches using indigenous, acceptable, nutrient-rich, but neglected foods together with simple home based processing techniques to combat micronutrient deficiencies and improve the energy/growth of infants deserve more attention. This is because such foods and therefore the strategies are likely to be more sustainable in the long run (WHO, 2008). Use of indigenous foods and more so the animal source foods (ASFs) and especially edible insects like termites combined with processing

techniques such as germination to enhance nutrient availability has not been tested among the infants mainly in resource limited settings (WHO, 2008) though they are widely consumed in these settings (Van Huis et al., 2013; Bauserman et al., 2013). Moreover, the results of various processing techniques like germination on growth and micronutrients status have been mixed resulting in gaps such as no growth, increased iron stores and haemoglobin concentrations and increased cognitive functions and physical activity among others (WHO, 2008).

Recently in Africa, supplementation of cereals with locally available legumes such as soy beans, groundnuts (Martin et al., 2010), cowpeas (Muhimbula et al., 2011), pigeon peas (Asma et al., 2006) and bambara nuts (Owino et al., 2007b) among others as a protein source has been tried with mixed results. In addition, World Food Programme (WFP) has promoted corn-soy-blend plus (CSB+) and corn-soy-blend plus plus (CSB++), food supplements utilized in management of moderate acute malnutrition (WFP, 2010) with mixed results. In Kenya, although Grillenberger et al., (2006) showed that school going children consuming diets high in ASFs grew better, studies on complementary foods development with ASFs and especially those of indigenous sources such as edible termites and *dagaa* small fish as ingredients have not been reported. Existing work elsewhere have been based on the possible use of fish (Lartey et al., 1999; Haug et al., 2010) while Bwibo and Neumann (2003) observed underutilization of ASFs in complementary foods and recommended an increase among Kenyan children. Foods made from some ASFs are better sources of minerals such as calcium, iron and zinc, vitamins and lipids (Lartey et al., 1999; Haug et al., 2010).

According to Van Huis et al. (2013), demand for insects as food and feed is increasing due to the rising cost of animal protein. Since food and feed insecurity among others have led to increasing demand for protein, alternative solutions to conventional livestock and feed sources urgently need to be found. With the need for alternatives, there has been increased

appeal for utilization of edible insects to enhance food and nutrition security in Kenya (Ayieko et al., 2010). Utilization of edible insects like termites in complementary feeding may therefore be of interest. Indeed one study so far has tried the same with caterpillars in the Democratic Republic of Congo (Bauserman et al., 2013). Entomophagy (the eating of insects) contributes positively to the environment and to health and livelihoods with an estimated insect's consumption as part of the traditional diets of at least 2 billion people (FAO, 2008; Van Huis et al., 2013). Insects such as termites are a highly nutritious and healthy food source with high fat, protein, vitamin, fibre and mineral content (Kinyuru et al., 2013; Van Huis et al., 2013; Bauserman et al., 2013).

According to Abila, (2003), *dagaa* small fish has been used for human consumption as a source of protein mainly in the Lake Victoria region and surroundings by many low and medium income fish consumers. Although a significant proportion goes into making fishmeal as most of the Nile perch is exported, *dagaa* remains the "staple fish" to many households. Despite the use of *dagaa* in fishmeal industry leading to increases in its price, it has a strong demand with between 89-95 % of rural households being ready consumers (Abila and Jansen, 1997, Abila et al., 1998). Small quantities of *dagaa* can be bought for \$ 0.1-0.3 at a time and the fish contributes an important source of animal protein and mineral rich skeleton composition is an advantage to the consumers and especially children who are at higher risk of malnutrition (Abila, 2003).

Amaranth, a pseudo-cereal grain, is a subtropical gluten-free crop originally cultivated in ancient Mexico. The grain is a nutritionally promising choice to enrich maize porridge usually taken by young children (Rastogi et al., 2011). It contains high quality protein with lysine and sulphur-containing amino acids normally limiting in maize and pulse crops respectively (Escudero et al., 2004; Gorinstein et al., 2003; Alvarez-Jubete et al., 2009). The total mineral content in amaranth grain varies between and within species but is usually

higher than that observed in conventional grains with iron being shown to be at least two times more than in wheat (Pedersen et al., 1987).

Food interventions have thus included the use of cereal legume blends ranging from traditional ones to the commercially and centrally processed ones like CSB,CSB+ and CSB++ among others. Such foods have been modified through use of various strategies as germination, soaking, fermentation, extrusion cooking etc and addition of animal source foods such as meat, milk among others. Despite the increasing interest in entomophagy and the clear advantages both nutritional and otherwise, use of *dagaa* and edible termites has not been explored in IYC feeding and thus dearth of data on the likely benefits of using them for infants foods despite the wide consumption. This study therefore aimed to assess the acceptability and efficacy of improved complementary foods processed centrally by extrusion cooking, using edible termites (*Macrotermes subhylanus*) and *dagaa* small fish (*Rastroneobola argentea*) which are locally available, already acceptable and micronutrient rich but neglected/underutilized ASFs together with germinated grain amaranth (*Amaranthus cruentus*) and maize (*Zea mays*) on growth (primarily linear), haemoglobin concentration and iron status compared to standard CSB+ in Kenyan IYC 6-15 months.

1.2Rationale

There has been little success in the efforts to provide safe and nutritionally adequate complementary foods in many developing countries (WHO, 2003). This is despite the fact that complementary feeding determines a child's growth and survival with implications on work capacity in later life (WHO, 2003). Poor quality complementary foods due to high dietary bulk, low energy and nutrient density coupled with inappropriate feeding practices and poor hygiene are among the major causes of malnutrition in IYC (WHO, 2003) leading to a great risk of nutritional deficiencies during the second half of infancy (Pelto et al., 2003; Shrimpton et al., 2001). Food selection and preparation practices which affect food safety,

nutrient density and feeding practices and maternal characteristics such as education, and socio-economic status are known as integral components of care-giving in relation to nutrition of IYC (Pelto et al., 2003). Provision of safe and appropriate complementary foods processed using indigenous nutrient-rich foodstuffs have been emphasized (WHO, 2002) however, the availability of high-quality; low-cost fortified complementary foods on the commercial market are still low in the developing world (Lutter, 2003). The success in combating micronutrient deficiencies like for iron through complementary food fortification and addition of ASFs (especially milk) in industrialized countries (Darton-Hill, 1998; ILSI, 2003) may be applied in resource limited settings with relevant modifications to suit specific population needs (Bauserman et al., 2013). The need for nutritionally adequate complementary foods is especially relevant in circumstances where the HIV prevalence is high since HIV exposed infants often have lower birth weights and are more prone to growth faltering (Miller et al., 1993). Further, knowing that HIV-1 can be transmitted through breastfeeding might result in decreased initiation or duration of breastfeeding (Owino et al., 2007a; Mutie-Macharia et al., 2012). The dilemma of HIV and breastfeeding thus pose a challenge to address the needs of children beyond the period of exclusive breastfeeding through the development of improved complementary or replacement foods and adoption of better feeding practices (Piwoz et al., 2003; Owino et al., 2007b).

It has been shown that it is difficult to meet the micronutrient needs for example iron in infants beyond 6 months of age, especially non-breast infants, without food fortification (Dewey et al., 2004; Ruel et al., 2004; Hurrell, 2007). This justifies the need to explore the production of multi-micronutrient fortified complementary foods based on locally produced and widely used foods stuffs such as grain amaranth and maize. Evaluating complementary foods for acceptability is useful in their optimization before they are introduced for intervention programmes (Mensa-Wilmot et al., 2001). The quality of cereal-based

complementary foods may be improved to ensure hygiene (Dewey and Brown, 2003) and to enhance nutrient density and especially if centrally processed. A study from Bangladesh (Kimmons et al, 2004) found that although greater intakes of complementary foods were associated with higher total energy intake, micronutrient intake was inadequate due the low micronutrient density of the complementary foods consumed. Multi-micronutrient fortified, nutrient-dense complementary foods may partially displace less nutritious traditional complementary foods resulting in increased energy and nutrient intake. It is thus important to accurately measure the amounts of complementary foods consumed by IYC (Haisma et al, 2004).

WHO recommends use of indigenous foodstuffs in complementary feeding. Western Kenya has diverse indigenous plant and animal foodstuffs among them the edible termites and *dagaa* which are nutrient rich. The ASFs have high mineral content; the minerals are more bioavailable and have been found to improve bioavailability of minerals in plant foodstuffs (Bauserman et al., 2013). They are also rich in proteins of high quality and coupled with appropriate indigenous processing techniques will reduce anti-nutrients thus further improve mineral bioavailability. The foodstuffs are culturally acceptable and affordable in Western Kenya and acceptability of the foods is likely to be high. This is relevant for the current study, where ingredients that are not commonly used such as edible termites, *dagaa* and vitamin-mineral fortificants, were used to modify the foods. There is urgency and an opportunity to explore the possibility to produce nutrient adequate complementary foods based on locally available but neglected plant and ASFs (Bauserman et al., 2013). The study thus gives an opportunity to test the effect of the indigenous ASFs on linear growth and micronutrient status as only few other studies have done this before with mixed results (Lartey et al., 1999; Faber et al., 2005; Bauserman et al., 2013).

1.3 Objectives

1.3.1 Overall objective

1. The main objective of the study was to comparatively assess the impact of three improved complementary foods namely, 1) maize-germinated grain amaranth-edible termite-fish complementary food naturally enriched with iron and zinc (WINFOOD Classic-(WFC)), 2) multi-micronutrient (with vitamins and minerals) fortified maize-germinated grain amaranth complementary food (WINFOOD Lite-(WFL)) and 3) multi-micronutrient fortified corn soy blend plus (CSB+) on linear growth (stunting), iron status of Kenyan infants 6-15 months.

1.3.2 Specific objectives

1. To determine the prevalence and severity of malnutrition among the study population prior to the randomized controlled trial (RCT).
2. To assess the acceptability of the improved complementary foods (WFC and WFL) and CSB+ among the study population prior to the RCT.
3. To assess the effect of the improved complementary foods (WFC and WFL) compared to CSB+ on linear growth (stunting) and body mass accrual (weight gain) of infants 6-15 months old.
4. To assess the effects of the improved complementary foods (WFC and WFL) compared to CSB+ on iron status and anaemia prevalence among infants 6-15 months old.

1.3.3 Hypotheses

1.3.3.1 Primary hypothesis

1. Infants 6-15 months consuming the improved complementary foods (WFC and WFL) have greater linear growth than those consuming CSB+ .

1.3.3.2 Secondary hypotheses

1. The acceptability of the three intervention foods are similar and high among the study population.
2. Infants 6-15 months consuming the improved complementary foods (WFC and WFL) will have higher weight gain compared to those consuming CSB+ .
3. Infants 6-15 months consuming the improved complementary foods (WFC and WFL) will have higher haemoglobin concentration, higher serum ferritin and lower serum transferrin receptors levels compared to those consuming CSB+.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Growth retardation and complementary feeding

Growth faltering evidenced by increased stunting from birth together with underweight and wasting from 3 months of age, persisting for at least the first 18-24 months of life (Shrimpton et al., 2001), is a public health problem in developing countries (WHO, 2008; WHO, 2012). Growth faltering rapidly accelerates between 6-12 months of life (Shrimpton et al., 2001) when breast milk alone is inadequate to meet entire nutritional requirements of IYC and complementary foods are introduced (Gibson et al., 1998; WHO, 2002). Evidence show that exposure to undernutrition and the likely irreversible consequences of the same during the foetal and early life period coinciding with the first 1000 days provide the “window of opportunity” to optimally prevent undernutrition (WHO, 2008; UNICEF, 2014).

Complementary foods also called breast milk supplements are defined as any food, whether manufactured or locally prepared, suitable as a complement to breast milk or to infant formula, when either becomes insufficient to satisfy the nutritional requirements of the IYC (WHO, 2008). In resource limited settings, complementary foods often fail to meet the nutritional requirements of IYC (Owino et al., 2007a) because the foods are mostly plant based, have low energy and nutrient density and contain inhibitory ligands such as phytates that limit the absorption of essential micronutrients such as iron and zinc (Gibson et al., 1998; Hurrell et al., 2007). The problem is particularly serious for infants who may not breastfeed for various reasons including maternal HIV-infection, death or severe illness of the mother or lack of the desire for the mother to breastfeed (Dewey et al., 2004) and micronutrient deficiencies are likely to occur in these circumstances (Dewey, 2000; Zimmerman and Hurrell, 2007).

According to WHO (2003), exclusive breastfeeding means that an infant receives only breastmilk with no additional foods or liquids, not even water. The benefits of exclusive

breastfeeding on child survival, growth, and development are well documented. Exclusive breastfeeding also provides health benefits for mothers. For instance, WHO (2003) reports that exclusive breastfeeding is the single most effective intervention for preventing child deaths. Diarrhea and pneumonia are the leading causes of death among infants in developing countries and infants under 2 months old who are not breastfed are six times more likely to die from diarrhea or acute respiratory infections than those who are breastfed (Arifeen et al., 2001; WHO. 2003). Approximately 1.3 million deaths could be prevented each year if exclusive breastfeeding rates increased to 90 percent (Arifeen et al., 2001). Breastfeeding also protects against illness especially the colostrums which contain anti-bacterial and anti-viral agents and high levels of vitamin A that protect infants against disease and promotes recovery of the sick child as it provides a nutritious, easily digestible food when a sick child loses his or her appetite for other foods (Chisenga et al., 2005; Brown et al., 1989; Arifeen et al., 2001).

Continued breastfeeding during diarrhea reduces dehydration, the severity and duration of diarrhea, and the risk of malnutrition (Arifeen et al., 2001). Moreover, breastmilk also impacts on child nutrition and development by providing total food security since breastmilk is a hygienic source of food with the right amount of energy, protein, fat, vitamins, other nutrients and water for infants in the first six months and is the only safe and reliable source of food for infants in an emergency (Brown et al., 1989). Offering water before 6 months of age reduces breastmilk intake, interferes with full absorption of breastmilk nutrients, and increases the risk of illness from contaminated water and feeding bottles. Finally, infants fed breastmilk show higher developmental scores as toddlers and higher IQs as children than those who are not fed breastmilk (Brown et al., 1989; Arifeen et al., 2001). To the mothers, breastfeeding reduces the mother's risk of fatal postpartum hemorrhage and premenopausal breast and ovarian cancer (Chisenga et al., 2005). Frequent and exclusive breastfeeding

contributes to a delay in the return of fertility and helps protect women against anemia by conserving iron (Arifeen et al., 2001). Breastfeeding also provides frequent interaction between mother and infant, fostering emotional bonds, a sense of security, and stimulus to the baby's developing brain (Chisenga et al., 2005; Brown et al., 1989; Arifeen et al., 2001)

Use of indigenous foodstuffs and industrial processing procedures (WHO, 2002; Dewey and Brown, 2003) for the improvement of the quality of complementary foods has been proposed. Food fortification may be used to increase micronutrient density while addition of oil and sugar or reducing the amount of water added during complementary food preparation may improve energy density (Dewey and Brown, 2003). Alternatively, enzymes such as α -amylase which break down starch thus enabling the addition of more dry flour per unit amount of water may be used to increase food intake (Moursi et al., 2003; Owino et al., 2007b). Industrial processing techniques such as extrusion cooking can also be used ensure good hygiene (Dewey and Brown, 2003).

Previous studies assessing the effect of improved complementary foods on growth and micronutrient status carried out among IYC in resource limited settings have had mixed results thus further work being recommended (Dewey, 2000; Dewey and Brown, 2003; Bauserman et al., 2013). Deficiencies of essential vitamins and minerals for optimal functions of the human body are severe and widespread in many parts of the world causing an immeasurable burden on individuals, health services, education systems and families caring for disabled children (UNICEF, 2009; UNICEF, 2014). Iron deficiency is common in both developing and developed countries (Hascke, 1999) and may affect a much larger proportion of the population than those with clinical symptoms (Maberly et al., 1998). The World Bank (2004) observed that countries whose populations suffer from micronutrient deficiencies (as high as 40% for iron) encounter economic losses as high as 5% of the Gross Domestic Product while the solutions to control and prevent micronutrient deficiencies are available

and affordable. Nationally, micronutrient malnutrition can be addressed by implementing programmes designed to educate people to diversify their diets (where appropriate foods are available), or by fortifying commonly eaten foods with the missing micronutrients or providing nutrient supplements through targeted distribution programmes (Ruel et al., 2004; WHO, 2005). Deficiencies of iron, iodine and vitamin A are the biggest concern among the most vulnerable populations while others like zinc, calcium, thiamine, riboflavin and pyridoxine have also been identified as problem nutrients for IYC beyond 6 months in developing countries (Dewey and Brown, 2003; Owino et al., 2008). A multi-micronutrient intervention through supplementation, fortification or diet diversification has been stressed since micronutrient deficiencies occur together, especially in settings where diets are deficient in several nutrients (Piwoz and Prebble, 2000; Lutter and Dewey, 2003; Owino et al., 2008).

Low-quality complementary foods combined with inappropriate feeding practices put under-twos in developing countries at high risk for undernutrition and its associated outcomes (Pelto et al., 2003; WHO, 2003; UNICEF, 2009). Often, complementary foods are introduced too soon or too late and the frequency and amount of food offered may be less than required for normal child growth or their consistency or nutrient density may be inappropriate in relation to the child's needs (UNICEF, 2009; Owino et al., 2008). Even with optimum breastfeeding children will become stunted if they do not receive sufficient quantities of quality complementary foods after six months of age (Shrimpton et al., 2001). Quantity is equally of concern in infant feeding with most children only fed 2 or 3 times a day in Tanzania (Mamiro et al., 2004). Elsewhere, exclusive breastfeeding was predominant for the first 6 weeks of life in South Africa after which mixed feeding was predominantly practiced through to 6 months (Faber et al., 2005) while in South America 12% of infants aged one month were exclusively breastfed (Marquis et al., 1997; Pelto et al., 2003; Lindsay et al.,

2008). In Kenya, less than 32% of infants aged six months are exclusively breastfed and fluids other than breastmilk are introduced before 6 months doubling the prevalence of diarrhoea (UNICEF, 2014). Provision of safe and appropriate complementary foods processed from indigenous nutrient-rich foods is thus currently emphasized (WHO, 2008) with the common forms of processed complementary foods being semi-solid gruels (Pelto et al., 2003). An estimated 6% under-five deaths can be prevented by ensuring optimal complementary feeding (UNICEF, 2014). To ensure higher nutrient density therefore; often a major problem in complementary foods among others, different strategies either singly or in combination have been proposed. These include germination, soaking, fermentation, mechanical processing, incorporation of ASFs and a combination of the named strategies depending on the contextual settings.

2.2 Moderate Acute Malnutrition in children

The WHO (2008) defines Moderate Acute Malnutrition (MAM) as a weight-for-age between -3 and -2 Z the median of the WHO Child Growth Standards (2006) which can be due to wasting or stunting or to a combination of both. The ideal nutritional characteristics of foods to treat children with MAM in resource limited settings are not fully known (Golden, 2009; Michaelsen et al., 2009) and the WHO (2012) has issued recommendations on the nutritional composition of foods to rehabilitate children with MAM. These range from blended flours and lipid-based nutritional supplements (LNS) designed to provide large quantities of protein and energy, to multi-micronutrient powders or tablets designed to provide only some micronutrients. Key is that the composition of all food supplements given to MAM children should be examined along with their ability to provide the specific nutrients needed by MAM children and their ability to supply the nutrients missing in the family diet (Michaelsen et al., 2009).

2.3 Prevalence, causes and consequences of MAM

MAM affects around 10% (40 to 55 million) of children under five years in resource limited settings (Kerac, 2011). Although there is a variation in the prevalence on a regional basis within the same countries with South-Central Asia estimated to have the highest point prevalence (19%) and the highest absolute number of affected children at 30 million children (WHO, 2013). A prevalence rate above 15% has been recorded in several countries in East, Central, and West Africa, and rates of over 10% are reported in some countries in the Middle East (WHO, 2013). Time trends in the prevalence of MAM have shown improvements in some regions of the world, notably Latin America (Lima, 2010; Stevens, 2012), while in many other countries the prevalence still remains unacceptably high including Kenya (WHO 2013).

MAM is caused by poor nutritional quality of foods, infections, inadequate intake among others and strongly impacts on child mortality and morbidity. Children with MAM have an estimated three- to four-fold increased risk of overall mortality compared to well-nourished children (Caulfield, 2004; Black, 2008). Cause-specific mortality risk in resource limited settings for children with MAM is increased for common infections such as pneumonia (Black, 2008) and if not adequately supported; children with MAM can rapidly progress towards SAM, a life-threatening condition (Garenne, 2009). About 14% child deaths per year are attributable to acute malnutrition with 10.2% due to MAM (Pelletier, 1995; Black, 2008). In terms of disease-adjusted life years (DALYs), SAM and MAM together account for 14.8% of the total DALYs in children under five years of age (World Bank, 2006). Poor quality protein and micronutrient deficiency and particularly iron contribute enormously to the extent of MAM observed in the resource limited settings. It is for this reason that the current study set out to investigate the highlighted ASFs with special reference to their protein and iron content in addressing MAM.

2.4 Food based approaches to solve MAM

Currently, most food supplements given to MAM children are processed cereal-based foods for IYC. The Standard for formulated food supplement refers to "foods that provide those nutrients which either are lacking or are present in insufficient quantities in the basic staple foods" and may be more suitable than cereal-based foods for improving the diet of MAM children who usually have an excess of cereal in their diets (WHO, 2008). Family diets complying with current nutritional recommendations are often costly to many families (WHO, 2008) thus the justification to use a blend from locally available, acceptable and nutritious but neglected indigenous edible termites and *dagaa* to manage MAM.

2.4.1 *The grain amaranth*

Amaranth, a pseudo-cereal, is a subtropical gluten-free crop originally cultivated in ancient Mexico. Amaranth grain is a nutritionally promising choice to enrich maize porridge usually taken by young children (Rastogi et al., 2011). Amaranth grain is rich in high quality protein with lysine and sulphur-containing amino acids, which are normally limiting in maize and pulse crops respectively (Escudero et al., 2004; Gorinstein et al., 2003; Alvarez-Jubete et al., 2009). The total mineral content in amaranth grain varies between and within species but is usually higher than that observed in conventional grains with iron being shown to be at least two times more than in wheat (Pedersen et al., 1987). Amaranth grain grown in Kenya has been found to have a phytate content of 7.9 mg per 100g, with much lower levels that could not be detected after germination (Okoth et al., 2011). Levels of phytic acid have been found to be lower than that of corn and wheat but higher than that of rice and millet (Lorenz and Wright, 1984). Amaranth grain (*Amaranthus cruentus*) also contains polyphenols [for example, tannin content of 0.8% catechin equivalents (Okoth et al., 2011)] which are important as dietary anti-oxidants and their content has been found to be lower than those in sorghum and millet and other pseudo-cereals such as *quinoa*, (Lorenz and Wright, 1984;

Escudero et al., 2011; Paško et al., 2008). *Amaranthus cruentus* species has been shown to give high yields in arid and semi-arid areas thereby being suggested as a crop with a potential to extend arable marginal lands in stressful environments (Schahbazian et al., 2006).

2.5 Strategies to enhance the quality of complementary foods

2.5.1 Germination

Germination increases endogenous phytase activity in cereals through *de novo* synthesis, activation of intrinsic phytase, or both. Germination has a reducing effect of phytic acid on amaranth grain (Colmenares and Bressani, 1990; Egli et al., 2002) thus a mixture of cereal flours prepared from germinated and ungerminated cereals will reduce phytates when prepared as porridge for IYC feeding (Sharma and Kapoor, 1996). The rate of phytate hydrolysis varies with the species and variety as well as the stage of germination, pH, moisture content, temperature (optimum 45–57°C), solubility of phytate, and the presence of certain inhibitors (Egli et al., 2002). Lower inositol phosphates have less binding capacity and have little influence on mineral bioavailability in humans. This degradation of phytic acid increases absorption of both iron (Hurrell et al. 1992; Davidsson et al., 1994; Sandberg et al., 1999) and zinc (Egli et al., 2004).

Certain tannins and other polyphenols in legumes and red sorghum may also be reduced during germination due to formation of polyphenol complexes with proteins and the gradual degradation of oligosaccharides (Camacho et al., 1992). The alpha-amylase activity is also increased during germination of cereals, especially sorghum and millet resulting into Amylase Rich Flours (ARF). Alpha amylase hydrolyzes starch to dextrans and maltose reducing the viscosity of cereal porridge while simultaneously enhancing the energy and nutrient densities of the porridge (Besten et al., 1998) resulting in improved child growth (Haug et al., 2010). Several factors limit use of ARF complementary foods such as long

preparation time, cultural obstacles linked to alcohol processing and high risk of toxicity from hydrocyanide (in sorghum) and aflatoxins (Brown et al., 1998).

2.5.2 Soaking

Soaking has been an indigenous technique practiced in Kenya especially for cereal grains before cooking. It softens the grains making them cook faster among other advantages. Soaking cereals and legumes in water can result in passive diffusion of water-soluble phytates, which can then be removed by decanting the water (Hortz and Gibson, 2007). The extent of the phytate reduction depends on the species, pH, and length and conditions of soaking (Hortz and Gibson, 2007). Some polyphenols and oxalates that inhibit iron and calcium absorption, respectively, may also be lost by soaking (Erdman and Pneros-Schneier, 1994). Soaking under optimal conditions (about 12 hours at 30°C) also activates naturally occurring phytases in cereals and results in varying degrees of phytate hydrolysis, depending on the kind of cereals (Lindsay and Ahluwalia, 1997).

2.5.3 Fermentation

Fermentation is an age old practice in Kenya and elsewhere. Fermentation induces phytate hydrolysis via the action of microbial phytase enzymes, which hydrolyze phytate to lower inositol phosphates which do not inhibit nonheme iron absorption (Sandberg et al., 1999; Hurrell, 2004). Microbial phytases originate either from the microflora on the surface of cereals and legumes or from a starter culture inoculate (Sandberg and Svanberg, 1991). The extent of the reduction in higher inositol phosphate levels during fermentation varies; sometimes $\geq 90\%$ of phytate can be removed by fermentation of some cereals and legumes (Hurrell, 2007). In cereals with a high tannin content (e.g. red sorghum), phytase activity is inhibited, making fermentation a less-effective phytate-reducing method for these cereal varieties (Sandberg and Svanberg, 1991). Prolonged fermentation (48 hours at 23°C) of

whole meal wheat bread increases the absorption of iron seven-fold (Lindsay and Ahluwalia, 1997). Additionally, lactic acid fermentation inhibits gram negative pathogenic bacteria as well as gram positive bacteria (Svanberg et al., 1992) and subsequently less spoilage during storage. Fermentation also increases the amount of water soluble vitamins such as riboflavin, improves protein digestibility in high tannin cereals such as brown sorghum (Lorri, 1993) and improves zinc bioavailability (Sandberg, 1991).

2.5.4 Mechanical processing

Mechanical processing such as household pounding of cereal grains have been practiced in Kenya. It is used to remove the bran and/or germ from cereals, which in turn may also reduce their phytate content when it is localized in the outer aleurone layer (e.g. sorghum) or in the germ (e.g. maize) (Nout and Ngoddy, 1997). Hence, bioavailability of minerals may be enhanced, although the content of minerals and some vitamins of these pounded cereals are simultaneously reduced. Household mechanical processing may only be possible for large seeds such as maize but may be cumbersome for small seeds like amaranth grains or millet making it less viable in some settings.

Extrusion cooking has also been used in developing countries since 1950s to process cereal-soy blends such as Pronutro (South Africa), Faffa (Ethiopia), and Multipurpose Food (India) among others (WHO, 2002). During extrusion the uncooked food material is fed into the extruder and subjected to intense mechanical shear, worked into viscous, plastic like dough and cooked before being forced through a die at 150 - 180 °C and up to 25 MPa for 60 -120 seconds at a moisture content of about 20% (WHO, 2002). Beneficial effects of extrusion cooking include improvement in protein digestibility and the bioavailability of sulphur-amino acids (WHO, 2002), reductions in microbial count, improved palatability and texture and enhanced starch digestibility (Owino et al., 2007b). Extrusion cooking may reduce dietary bulk possibly due to thermal hydrolysis of starch and moisture content

reduction and might also be used to eliminate or reduce anti-nutrients that occur in foods (Alonso et al., 2000). It also reduces cooking time during food preparation (Malleshi et al., 1996) and may also result in lower levels of aflatoxins in complementary foods (Treche & Mbone, 1999; Moraru and Lokini, 2003).

2.5.5 Addition of animal source foods

Iron bioavailability is higher from ASFs than from plant foods (Lartey et al., 1999; Allen, 2003; Hurrell et al., 2006). The “meat factor” is thought to be within the protein fraction of muscle tissue with a possibility of involvement of other components such as cysteine-containing peptides (Hurrell et al., 2006). Myofibrillar proteins in meat are digested extensively by pepsin in the stomach and thus could bind iron and prevent its precipitation at the higher pH of the duodenum, supporting absorption of iron (Hurrell and Egli, 2010). Addition of even a small amount of ASFs is another strategy to improve the content and bioavailability of micronutrients in plant-based diets and fish has been used in development of complementary foods leading to enhanced iron, zinc and calcium content and bioavailability (Mosha and Bennink, 2005; Haug et al., 2010). Inclusion of red meat significantly increase non-heme iron bioavailability in plant based foods (Davidsson, 2003; Sorensen et al., 2007) with reported negative associations between the avoidance of ASFs and mineral deficiency in IYC from developed countries (Black et al., 2002; Dagnelie et al., 1994). According to Kinyuru et al., (2009) and Ayieko et al., (2010), it is possible to develop commercial products from edible insects in Kenya with high nutrient content and acceptability among consumers. The same has been reported in the Democratic Republic of Congo with caterpillars for complementary foods development (Bauserman et al., 2013).

Edible insects have been scientifically discussed since at least 1885 by V. M. Holt in his book "Why not eat insects"? (Morris, 2008). Entomophagy however picked up between 1970 and 1999 when DeFoliart and others (Meyer-Rochow, 1973; Posey, 1979; DeFoliart, 1975;

DeFoliart, 1989 and 1992; De Conconi et al. 1984; Ramos-Elorduy, 1997) aimed to popularise the eating of insects and to investigate the place of insects in human nutrition. In 1951, Bodenheimer detailed reasons why entomophagy is beneficial to the environment and human populations. Recently, the focus has shifted to the environmental benefits of entomophagy using various strategies (DeFoliart, 1997 and 1999; Ramos-Elorduy, 2006; Van Huis et al., 2013). De Conconi et al., (1984) analysed the protein content of 101 insect species and concluded that their amino acid profiles compared favourably with those recommended by the FAO (1981). Edible insect research is now well-established on all continents and particularly in countries such as India (Chakravorty et al., 2011), Japan (Nonaka, 2010), Thailand (Yhoung-Aree and Vitwatpanich, 2005), Australia (Yen, 2012), Nigeria (Banjo et al. 2006), South Africa (Teffo et al., 2007; Dzerefos et al., 2009), Kenya (Ayieko et al., 2010; Kinyuru et al., 2013) and the Netherlands (Vogel, 2010).

In countries where edible insects are traditionally consumed, focus is on documenting the indigenous knowledge around the insects, identifying the species used and developing databases (FAO, 2012). In more industrialised countries, the focus is on developing insect products to enhance the palatability of insect protein to encourage consumption (Van Huis et al., 2010 and 2013; Gracer, 2010) and use as a source of income (DeFoliart, 1999). All over the world, there is a lucrative trade in insects that are preserved or fresh and sold to the general public in supermarkets, open air markets and restaurants (Nonaka, 2010; Chen et al., 1998; van der Waal, 1999; Makhado et al., 2009; Ayieko et al., 2010).

Insects as food and feed are gaining attention due to the rising cost of animal protein, food and feed insecurity, environmental pressures, population growth and increasing demand for protein among the middle classes (Van Huis et al., 2013). Thus, alternative solutions to conventional livestock and feed sources urgently need to be found. Globally, the most commonly consumed insects are beetles (Coleoptera-31%) and caterpillars (Lepidoptera-

18%) with termites (Isoptera) constituting only 3% (Rumpold and Schlüter, 2013). Insect consumption is thus worldwide and is practised regularly by about 2 billion people (FAO, 2012; Van Huis et al., 2013) with more than 1,900 documented edible insect species mostly in tropical countries (Halloran et al., 2014).

Insects are a highly nutritious and healthy food source with high fat, protein, vitamin, fibre and mineral content comparing well with other forms of protein such as beef, fish and poultry (Dufour, 1987; Gullan and Cranston, 2000). The nutritional value of edible insects is however highly variable because of the wide range of species thus even within the same group of species, nutritional value may differ depending on the metamorphic stage of the insect, the habitat in which it lives, and its diet (Van Huis et al., 2013). For example, the composition of unsaturated omega-3 and six fatty acids in mealworms is comparable with that in fish (and higher than in cattle and pigs), and the protein and micronutrients content of mealworms is similar to that in fish and meat (Van Huis et al., 2013). Entomophagy can therefore be promoted for health as alternatives to mainstream staples such as chicken, beef and even fish. There is also a relatively small ecological footprint of farming insects compared with farming conventional livestock e.g. in greenhouse gas emissions (Ooninx et al., 2010; Ooninx and de Boer, 2012). For livelihoods, insect harvesting/rearing is a low-tech, low-capital investment option that offers entry even to the poor of society while as a minilivestock offer livelihood opportunities for both urban and rural people (Ooninx et al., 2010; Halloran et al., 2014).

Abila et al. (2003) report that *dagaa* has mainly been used for human consumption as a source of protein, especially to many low and medium income fish consumers in Kenya. With most of the Nile perch going for export, *dagaa* remains the "staple fish" to many households around Lake Victoria and for a long time, its price has been low (LVEMP, 2005). However, of late the price of dried *dagaa* going for human consumption has risen considerably as

are a result of increased pressure due to fishmeal factories buying this fish (Abila et al., 2003). Despite the use of *dagaa* in fishmeal industry leading to increases in its price, it has continued to have a strong demand in many communities around Lake Victoria with 89-95% of rural households being ready consumers (Abila and Jansen, 1997, Abila et al., 1998). Many relatively poor consumers buy *dagaa* for only Ksh 10-20 (\$ 0.1-0.3) at a time as a source of animal protein in their diet (Abila, 2003). Its high protein content and the mineral rich skeleton composition is an advantage to the consumers, especially to children threatened with malnutrition (Abila, 2003). Use of such nutritious indigenous ASFs as edible termites and *dagaa* is therefore an option that should be investigated as an effective means of delivering micronutrients and thus the justification for this study.

2.5.6 Combined strategies

The discussions above reveal that an integrated approach that combines a variety of the strategies is likely to be superior. For instance, traditional food-processing and preparation practices including the addition of even a small amount of ASFs, is probably the best strategy to improve the content and bioavailability of micronutrients (Gibson et al., 2003). This is more so in plant-based diets in resource-limited settings and depending on applicability of the strategies or a combination thereof. Use of such a combination of strategies can almost completely remove phytate. This is important because phytic acid is a potent inhibitor of iron absorption, even at low concentrations (Hurrell, 2004). Moreover, despite the promotion of household-level food processing and other food-based strategies to improve nutritional adequacy, there has been little effort to assess their impact in well-designed trials (Hotz and Gibson, 2007). Further studies of the efficacy of these strategies to determine their impact on nutritional status is needed thus further justifying the current study.

2.6 Food supplement intervention to manage MAM

The immediate expected benefit of an adequate food supplement in a child with MAM is rapid improvement in nutritional status, and prevention of further deterioration. Different types of foods may provide a different benefit based on the nutritional adequacy of the food, its level of acceptability for children and families, and other factors. A possible side effect of food supplements in children with MAM, especially lipid rich foods is rapid weight gain, recognised as a risk factor for adult adiposity, obesity, and metabolic syndrome (Uauy, 2002; Ekelund, 2006; Gordon-Larsen, 2012; Adair, 2013) while homemade foods because of hygiene, may lead to diarrhoea. At a population level, strategies with great investment in externally provided supplementary foods can create dependence on external donors thus unsustainable. Sustainability of local strategies will therefore be key and feasibility of different options, such as locally produced foods and the integration of food strategies into complex intervention packages as in the current study are likely to give better results.

2.6.1 The Role of Animal Source Foods (ASFs) in IYC Nutrition

Limited availability, access and intake of ASFs in resource limited settings are common with the intake of meat protein providing only 15% of dietary protein compared with 60% in developed countries (Higgs & Pratt, 1998). ASFs are nutrient dense foods that provide protein of high biological value, energy, fat and are likely to be the only unfortified foods that can provide enough calcium, iron, and zinc for IYC (WHO, 2004). They are more energy dense than plant foods, as well as a good source of fat soluble vitamins and essential fatty acids (WHO, 2008). Moreover, Vitamin B12 requirements must be met by ASFs because there is none in plants. The bioavailability of micronutrients is generally higher from ASFs than from plant foods (Bæch et al., 2003) with about 40% of the iron in meat being haem iron, of which 15-35% is absorbed, whereas the absorption of non-haem iron from plant foods is estimated at only 2-20% depending on the amount of enhancers and inhibitors in the

diet and individual's iron status (Dwyer, 1991). The low bioavailability of micronutrients from plant sources can be increased through small amounts of ASFs even in the presence of dietary inhibitors (Hallberg & Hulthén, 2000). Studies have shown negative associations between the avoidance of ASFs and the health of children in developed countries (Black et al., 2002; O'Connell et al., 1989) while some cross-sectional studies have observed improved nutritional status and growth among children consuming ASFs in developing countries (Neumann and Bwibo 1987; Allen et al., 1992; Calloway et al., 1992). Controlled supplementation studies with ASFs other than milk are scarce, especially of nutritious but neglected sources; a trial in which dry fish powder was added to fermented maize porridge, did not improve growth or micronutrient status of Ghanaian children (Lartey et al., 1999). According to Dror and Allen (2011), dietary diversification and modification strategies have attempted to increase consumption of ASFs.

Gibson et al., (2003) in a 12-month intervention designed to increase intake of ASFs (dried fish with bones) and reduce dietary phytate in Malawian children aged 2.5 to 7.5 years reported significantly improved z-scores for mid-upper-arm circumference and arm muscle area. There was no effect on weight or height gain compared with controls. The authors hypothesized that lack of ponderal or linear growth change in the intervention group may have been due to the insufficient duration of the intervention, the age range of the children, or intergenerational effects of malnutrition. However, the intervention reduced the prevalence of inadequate intakes of vitamin B12, calcium and bio-available zinc. Additionally, mean haemoglobin was significantly higher and the incidence of anaemia was lower in the intervention group than in the control group.

A community-based, randomized behavioural and dietary intervention trial conducted in Peruvian adolescent girls encouraged ASF intake with the goal of improving iron status. Heme iron and ascorbic intakes increased significantly in the intervention group. Although

the intervention was not sufficient to improve haemoglobin or iron status, it prevented the fall in these indicators observed in the control group (Creed-Kanashiro et al, 2000).

Given that edible insects have been shown to have high quality proteins, minerals and even polyunsaturated fatty acids among other nutrients (Van Huis et al., 2013; Rumpold and Schlüter, 2013; Kinyuru et al., 2013), the possibility of incorporating the same in complementary foods for IYC will be of interest. This is particularly due to the increasing interest on the insects as alternative ASFs and especially in settings with high levels of undernutrition. [Table 2-1](#) show the composition of 5 edible insect species. [Tables 2-2 and 2-3](#) show the proximate and mineral composition respectively of the edible winged termites in Western Kenya.

Table 2-1: Nutrient, fatty acid, mineral, and vitamin composition of five edible insect species (based on dry matter). Adapted from Rumpold and Schlüter, 2013

| (based on dry matter) | Coleoptera (beetles) Rhynchophorus phoenicis (larvae) ¹⁻⁷ | Lepidoptera (butterflies, moths) Bombyx mori (pupae) ⁸⁻¹¹ | Orthoptera (crickets, grasshoppers, locusts) Cirina forda Westwood (larvae) ^{1,12-15} | Acheta domesticus (adults) ¹⁶⁻¹⁸ | Ruspolia differens (brown; adult) ¹⁹ |
|-----------------------------|---|---|---|--|--|
| <i>Nutrient composition</i> | | | | | |
| Protein [%] | 10.33–41.69 | 48.70–58.00 | 20.20–74.35 | 64.38–70.75 | 44.30 |
| Fat [%] | 19.50–69.78 | 30.10–35.00 | 5.25–14.30 | 18.55–22.80 | 46.20 |
| Fibre [%] | 2.82–25.14 | 2.00 | 1.80–9.40 | | 4.90 |
| NFE [%] | 5.49–48.60 | 1.00 | 2.36–66.60 | 2.60 | |
| Ash [%] | 2.54–5.70 | 4.00–8.60 | 1.50–11.51 | 3.57–5.10 | 2.60 |
| Energy [kJ/kg] | 20,038–20,060.63 | 23,236.74 | 15,030.61 | 19,057.89 | |
| <i>% Fatty acids</i> | | | | | |
| Palmitic acid (C16:0) | 32.40–36.00 | 22.77–26.20 | 13.00 | | 32.10 |
| Stearic acid (C18:0) | 0.30–3.10 | 4.50–7.00 | 16.00 | | 5.90 |
| SFA total | 38.90–40.90 | 28.80–33.20 | 31.60 | | 39.10 |
| Palmitoleic acid (C16:1n7) | 3.30–36.00 | 0.60–1.70 | 0.20 | | 1.40 |
| Oleic acid (18:1n9) | 30.00–41.50 | 26.00–36.90 | 13.90 | | 24.90 |
| MUFA total | 43.40–66.60 | 26.61–36.90 | 14.90 | | 26.30 |
| Linoleic acid (18:2n6) | 13.00–26.00 | 4.20–7.30 | 8.10 | | 29.50 |
| Linoleic acid (18:3 n3/6) | 2.00–3.50 | 27.70–38.02 | 45.50 | | 4.20 |
| PUFA total | 17.70–28.00 | 29.90–43.92 | 53.80 | | 33.80 |
| SFA/(MUFA + PUFA) | 0.43–0.64 | 0.40–0.50 | 0.46 | 0.56 | 0.65 |
| <i>Minerals [mg/100 g]</i> | | | | | |
| Calcium | 54.10–208.00 | 158.00 | 7.00–37.20 | 132.14–210.00 | 24.50 |
| Potassium | 1,025.00–2,206.00 | | 47.60–2,130.00 | 1,126.62 | 259.70 |
| Magnesium | 33.60–131.80 | 207.00 | 1.87–69.89 | 80.00–109.42 | 33.10 |
| Phosphorous | 352.00–685.00 | 474.00 | 45.90–1,090.00 | 780.00–957.79 | |
| Sodium | 44.80–52.00 | | 44.40–210.00 | 435.06 | 121.00 |
| Iron | 14.70–30.80 | 26.00 | 1.30–64.00 | 6.27–11.23 | 229.70 |
| Zinc | 26.50–15.80 | 23.00 | 4.27–24.20 | 18.64–21.79 | 13.00 |
| Manganese | 0.80–3.50 | 0.71 | 7.00–10,163.10 | 2.97–3.73 | 12.40 |
| Copper | 1.60 | 0.15 | | 0.85–2.01 | 2.50 |
| Selenium | 1.60 | 0.15 | | 0.60 | 0.50 |
| <i>Vitamins</i> | | | | | |
| Retinol [µg/100 g] | 11.25 | | 2.99 | 24.33 | 2.80 |
| α-Tocopherol [IU/kg] | | | | 63.96–81.00 | 22.64 |
| Ascorbic acid [mg/100 g] | 4.25 | | 1.95 | 9.74 | 0.1 |
| Thiamin [mg/100 g] | 3.38 | | | 0.13 | n.d. |
| Riboflavin [mg/100 g] | 2.21–2.51 | | 2.21 | 11.07 | 1.4 |
| Niacin [mg/100 g] | 3.36 | 0.95 | | 12.59 | 2.4 |
| Pantothenic acid [mg/100 g] | | | | 7.47 | |
| Biotin [µg/100 g] | | | | 55.19 | |
| Folic acid [mg/100 g] | | | | 0.49 | 0.9 |

NFE – nitrogen-free extract (i.e. carbohydrates).

SFA – saturated fatty acids.

MUFA – monounsaturated fatty acids.

PUFA – polyunsaturated fatty acids.

Sources:

1 - Banjo, Lawal, and Songonuga (2006), 2 - Bukkens (1997), 3 - Ekpo, Onigbinde, and Asia (2009).

4 - Elemo, Elemo, Makinde, and Erukainure (2011), 5 - Omotoso and Adedire (2007), 6 - Onyeike, Ayalogu, and Okaraonye (2005),

7 - Opara et al. (2012), 8 - Pan, Liao, Zhang, Dong, and Wei (2012), 9 - Ramos-Elorduy et al. (1997), 10 - Rao (1994),

11 - Tomotake, Katagiri and Yamato (2010), 12 - Agbidiye et al. (2009), 13 - Akinnawo and Ketiku (2000),

14 - Omotoso (2006), 15 - Osasona and Oloafe (2010), 16 - Barker et al. (1998), 17 - Finke (2002), 18 - Finke (2007),

19 - Kinyuru, Kenji, Muhoho, and Ayieko (2010)

Table 2-2: Proximate composition of the edible winged termites (g/100 g). Adapted from Kinyuru et al., 2013

| Termite | Moisture | Protein ¹ | Fat ¹ | Total Ash ¹ | Dietary fibre ¹ | Available Carbohydrate ¹ |
|--|-------------|----------------------|------------------|------------------------|----------------------------|-------------------------------------|
| <i>Macrotermes subyланus</i> ; dewinged | 6.50 ± 0.02 | 39.34 ± 0.12 | 44.82 ± 2.89 | 7.58 ± 0.05 | 6.37 ± 1.18 | 1.89 ± 0.76 |
| <i>Pseudocanthotermes militaris</i> ; dewinged | 5.04 ± 0.15 | 33.51 ± 0.85 | 46.59 ± 2.13 | 4.58 ± 0.06 | 6.59 ± 0.07 | 8.73 ± 1.87 |
| <i>Macrotermes bellicosus</i> ; dewinged | 5.13 ± 0.18 | 39.74 ± 0.61 | 47.03 ± 1.04 | 4.65 ± 0.09 | 6.21 ± 2.04 | 2.37 ± 0.98 |
| <i>Pseudocanthotermes spiniger</i> ; dewinged | 8.76 ± 1.61 | 37.54 ± 0.12 | 47.31 ± 0.13 | 7.22 ± 0.38 | 7.21 ± 0.44 | 0.72 ± 0.01 |

Values are mean ± SD; n=6

¹Values are on dry weight basis

Table 2-3: Mineral composition of the edible winged termites (mg/100 g). Adapted from Kinyuru et al., 2013

| Termite | Calcium | Iron | Zinc |
|--|--------------|---------------|--------------|
| <i>Macrotermes subyланus</i> ; dewinged | 58.72 ± 1.29 | 53.33 ± 1.46 | 8.10 ± 2.80 |
| <i>Pseudocanthotermes militaris</i> ; dewinged | 48.31 ± 7.09 | 60.29 ± 1.11 | 12.86 ± 0.92 |
| <i>Macrotermes bellicosus</i> ; dewinged | 63.60 ± 6.53 | 115.97 ± 3.46 | 10.76 ± 1.93 |
| <i>Pseudocanthotermes spiniger</i> ; dewinged | 42.89 ± 1.75 | 64.77 ± 2.66 | 7.10 ± 1.82 |

Values are mean ± SD on dry weight basis; n = 6

2.6.2 Food fortification and IYC Nutrition

Although some of the strategies like extrusion cooking are proposed to enhance quality of complementary foods, some workers have reported a decreased apparent absorption of Zinc, Magnesium and Phosphorus (Kivitso et al., 1986) and an insignificant decrease in iron absorption (Hurrell et al., 2002). This may be due to extrusion cooking causing deactivation of phytase resulting in only about 25% digestion of phytate or due to the formation of indigestible phytate complexes (Sandberg et al., 1987). The decrease in micronutrient absorption by extrusion cooking and thermal treatment of food may be mitigated by the multi-micronutrient fortification of complementary foods.

Food fortification can be mandatory by law or voluntary at the discretion of the food manufacturer. Many countries currently require mandatory fortification of some foods with micronutrients, including vitamin A and iron, and several other countries practice voluntary food fortification (ILSI, 2003; van Ameringen, 2005). [Table 2-4](#) shows food fortification

programs in some African countries. The adoption rate for fortification is not uniform in all countries due to either lack of political will or variation among countries in the recognition of the importance of micronutrients in child survival. This has been taken up by the Scaling Up Nutrition (SUN) movement for some years to place the nutrition agenda up in the priorities of the countries in the developing world. By mid September 2014, 54 countries have joined the movement with an aim to progress towards the 2025 World Health Assembly Targets as part of a global movement led by the countries themselves.

Table 2-4: Some Food fortification programs in some of the African countries*

| Country | Program** | Vitamins and minerals added |
|----------------|---|---|
| Burkina Faso | Cotton seed oil ¹ | vitamin A |
| Cote d'Ivoire | Wheat ¹ | Folic acid, iron |
| Ghana | Edible oils ¹ | vitamin A |
| | Wheat ² | vitamins A, B1, B2, B6, B12, folic acid, niacin; iron, zinc |
| Kenya | Edible oils ² | vitamin A |
| | Maize ¹ | vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc |
| Lesotho | Edible oils ¹ | vitamin A |
| | Wheat ² | vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc |
| Malawi | Maize ² | vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc |
| | Maize ² | vitamins A, B1, B2, B6, B12, folic acid, niacin; iron, zinc |
| Namibia | Sugar ² | vitamin A |
| | Maize ² | vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc |
| Nigeria | Wheat ³ | vitamins A, B1, B2, niacin; iron |
| | Maize ³ & Edible oils ³ | vitamin A |
| | Sugar ³ | vitamin A |
| South Africa | Wheat ³ & Maize ³ | vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc |
| Uganda | Wheat ¹ & Maize ² | vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc |
| | Edible oils ¹ | vitamin A |
| Zambia | Sugar ¹ | vitamin A |
| | Wheat ² | vitamins B1, B2, niacin |
| | Maize ² | vitamins A, B1, B2, B6, folic acid, niacin; iron, zinc |
| | Sugar ³ | vitamin A |

* adapted from van Ameringen, 2005. ** 1 and 2 = voluntary; 3 = mandatory (NB 1 was pending as at 2005)

Food fortification has been used to control micronutrient deficiencies in the industrialised world (Chen and Oldewage-Theron, 2002; ILSI, 2003) as well as in the developing world (ILSI, 2003; van Ameringen, 2005). The effectiveness of a food-fortification programme however depends on whether or not the fortified food is accepted, purchased, and consumed by the targeted population. The quality, taste, and the price of the fortified product play

important roles in determining the effectiveness of the fortification programme (Wirakartakusumah and Hariyadi, 1998). For a successful food fortification program; 1) the food fortified should be consumed in sufficient quantities to make a significant contribution in the diet of the target population; 2) the addition of nutrients should not create an imbalance of essential nutrients due to interaction effect (among the added and the nutrients that are naturally present in the food carrier); 3) the added nutrient should be stable under normal conditions of storage and use; 4) the fortified food should be affordable for the targeted population; 5) the fortification programme should be centralised and involve mass production for easy quality assurance and control of fortified food; 6) the food should be distributed to the target population as widely as possible (Wirakartakusumah and Hariyadi, 1998; FAO, 1995).

There are many studies on the nutritional and health effects of fortified complementary foods and very few on improvements based on blending locally available foods especially indigenous ASFs for children in resource limited settings. For instance, Ruel et al, (2004) in the Central Plateau of Haiti assessed the benefits of fortified cereal blends CSB or wheat-soy blend (WSB) in improving the quality of the diet of IYC 6-23 months of age. Participatory recipe trials were conducted to assess current complementary feeding practices and to develop new, improved recipes by using a combination of locally available ingredients and foods and donated fortified cereal blends. The results showed that only preparations using CSB could achieve the recommended concentrations of iron and zinc in complementary foods for young children 12-23 months old. In contrast, the iron and zinc needs of infants, especially those between 6 and 8 months of age, could not be met, even with a combination of fortified CSB and other locally available, acceptable, and affordable foods. They recommended higher fortification levels if iron and zinc needs of infants are to be met. The study did not include determination of micronutrient status.

Table 2-5 summarizes some studies that have assessed the effect of fortified complementary foods on growth and micronutrient status of IYC whose results have been mixed. The need to conduct well-designed studies to demonstrate the effectiveness of fortification has been highlighted (Townbridge and Martorell, 2002). The studies reviewed in **Table 2-5** show that fortification generally improves haematological measurements in pre-school and primary school children. However, the results on growth and serum retinol are inconsistent and the differences can be explained by the coexistence of micronutrient deficiencies and micronutrient interactions. The amounts and the form of the fortificant in a fortified complementary food also affect the outcomes. Among the studies reviewed however, there is none incorporating edible insects/termites in the complementary foods and thus the current study will provide data on the same. This is in line with the current FAO strategy (FAO, 2012) to promote insects as food and especially in localities where they are already acceptable as food.

Table 2-5: Effects of ASFs and multi-micronutrient fortified complementary foods on growth and micronutrient status of IYC

| Reference | Country | Age (mo) | Duration | N | Design | Setting | Results |
|-----------------------|--------------|----------|-------------------|-----|--|--|---|
| Lartey A. et al, 1999 | Ghana | 6 | 6 months | 208 | Randomized community-based trial (4 centrally processed complementary foods namely, 1) weanimix with vitamins and minerals, 2) Weanimix only, 3) Weanimix with fish powder, and 4) Koko with fish powder; Cross sectional data before and after on 464 infants not included in intervention | Rural breastfed infants | No differences among intervention groups in growth and micronutrient status. Significantly higher WAZ and LAZ in combined intervention from 9-12 mo. Significantly greater change in plasma retinol in fortified group & lower proportion of infants with low ferritin values in Weanimix than other diets |
| Torrejón et al, 2004 | Chile | 12 | at least 6 months | 42 | Self-controlled trial (cow's milk fortified with iron (10 mg/L, zinc (5 mg/L and copper (0.5 mg/L)) | Healthy male children, normal growth; lower socioeconomic groups | Favourable effect on iron status, but no effect on zinc status |
| Oelofse et al, 2003 | South Africa | 6 | 6 months | 60 | Randomized controlled trial (fortified complementary food versus controls on traditional diet. | Rural and Urban disadvantaged black communities in KwaZulu Natal and Western Cape respectively. | Significantly higher serum retinol at 12 months. Less pronounced decline in serum iron. No effects on Hb conc., weight or length gain. |
| Nesamvuni et al, 2005 | South Africa | 12-36 | 12 months | 44 | Parallel, single-blind RCT (Maize meal fortified with vitamin A, thiamine, riboflavin and pyridoxine versus unfortified maize meal). Children with HAZ or WAZ below the 5 th percentile of the NCHS criteria | A small town and attending local crèche or clinic | Significant weight gain in experimental group. No significant differences in haemoglobin concentration and serum retinol. Significant decrease in retinol binding protein in control group. |
| Faber M. et al 2005 | South Africa | 6-12 | 6 months | 361 | RCT with infants assigned to receive either the fortified or unfortified porridge. Primary outcomes were Hb and serum retinol, Zn, and ferritin conc. and motor development. Growth assessed as secondary outcome. Primary & secondary outcomes assessed at baseline and 6 mo. | Rural area low socioeconomic status & pop. density at the Valley of 1000 Hills in KwaZulu-Natal province | An intervention effect of 9.4 microg/L for serum ferritin and 9 g/L for Hb. Anaemia decreased from 45% to 17%, remained >40% in the control group. On average 15.5/25 motor development score items, control group achieved 14.4 items. Serum retinol concentration inconsistent effect and no intervention effect for serum Zn concentrations. |
| Owino VO et al. 2007 | Zambia | 6 | 3 months | 81 | Infants were randomly assigned to receive a fortified blend of maize, beans, bambaranuts, and groundnuts [Chilenje Baby Mix (CBM); energy density: 68 kcal/100 g; n=37] or a similar blend with alpha-amylase (CBMA; energy density: 106 kcal/100 g; n=44). Outcomes growth, Hb conc, & breast milk intake. Cross-sectional data got at 9 mo for a control group (n=69) not given the diets. | Peri urban set up | No differences in weight or length z scores, all were within normal ranges at 9 mo. Percentage fat mass was significantly greater in the infants in both the CBM and CBMA groups than in the control group Hb concentrations were significantly greater in both intervention groups than in the control group. Breast milk intake was not significantly different between groups. |

Table 2-5 continued

| Reference | Country | Age (mo) | Duration | N | Design | Setting | Results |
|-----------------------------|---------|----------------|--|-----|--|-----------------------------|--|
| Grillenberger M, et al.2006 | Kenya | Median 7 years | 2 years | 544 | The study investigated which specific nutrients from the diet of Kenyan school children predicted their growth. | Rural resource poor setting | Height gain positively predicted by average daily intakes of energy from ASFs, haem Fe, preformed vit A, Ca and vit B12. Weight gain positively predicted by average daily intakes of energy from ASF, haem Fe, preformed vit A, Ca and vit B12. Gain in mid-upper-arm muscle area positively predicted by average daily intakes of energy from ASF and vit B12. Gain in mid-upper-arm fat area positively predicted by average daily intakes of energy from ASF. Negative predictors of growth were total energy and nutrients contained in high amounts in plant foods. growth positively predicted by energy and nutrients provided in high amounts and in a bioavailable form in meat and milk |
| Neumann CG, et al.2003 | Kenya | Median 7 years | For 7terms on sch. days during 21mo measuring a child repeated at intervals over 2 y | 544 | A RC school feeding study designed to test if ASFs would improve micronutrient status, growth and cognitive function in to four feeding interventions using a local vegetable stew. Groups designated as Meat, Milk, Energy and Control with no feedings. Preintervention baseline measures included nutritional status, home food intake, anthropometry, biochemical measures of micronutrient status, malaria, intestinal parasites, health status, cognitive & behavioral measures. | Rural setting | Stunting and underweight in approximately 30% of children and widespread inadequate intakes and/or biochemical evidence of micronutrient deficiencies, Malaria was present in 31% of children, and hookworm, amebiasis and giardia were widely prevalent. Milk and Meat groups had significant higher levels of micronutrients and cognitive and behavioral outcomes |
| Adu-Afarwuah S, et al.2008 | Ghana | 6 months | 6 months | 313 | Randomly assigned to Sprinkles (SP), crushable Nutritabs (NT), and fat-based Nutributter (NB; 108 kcal/d), which provided 6, 16, and 19 vit& minerals, respectively. SP &NT each (n=105), or NB (n=103) daily and assessed dietary intake, morbidity & compliance weekly. Hb and plasma ferritin, TfR, CRP & Zn measured at 6 & 12 mo. Random control group no intervention (NI;n=96) assessed at 12 mo. | | All 3 intervention groups had significantly higher ferritin and lower TfR conc. than did the NI control group. Mean Hb significantly higher in NT and NB but not in SP infants than in NI infants. The prevalence of iron deficiency anaemia was 31% in the NI control group compared with 10% in the intervention groups combined. |

Table 2-5 continued

| Reference | Country | Age (mo) | Duration | N | Design | Setting | Results |
|----------------------------------|--------------|-------------|-----------------------------|-----|--|---|---|
| Nesamvuni AE, et al.2005 | South Africa | 1-3 years | 12 months | 54 | A parallel RCT: 21 experimental children & their families received maize meal fortified with vit. A, thiamine, riboflavin & pyridoxine, while 23 control children & their families received unfortified maize meal. Baseline measurements were demographic, socio-economic and dietary data, as well as height, weight, Hb, haematocrit, serum retinol & retinol-binding protein (RBP). | Rural setting | The children in the experimental group had a significantly higher increase in body weight than control (4.6 kg vs. 2.0 kg) and both groups had significant but similar increases in height. Study showed positive effects of a vitamin-fortified maize meal on weight gain and some variables of vit A status confirming the relationship between vit A & Fe status. |
| van Stuijvenberg ME, et al. 1999 | | 6-11 years | for 43 wk over 12-mo period | 115 | Micronutrient status was assessed in an RCT with biscuits (fortified with iron, iodine, and beta-carotene) compared with that in a control group (n = 113) who consumed nonfortified biscuits. Cognitive function, growth, and morbidity assessed as secondary outcomes. | primary school children from a poor rural community | A significant between-group treatment effect on serum retinol, serum ferritin, serum Fe, transferrin saturation, and urinary iodine and in Hb and hematocrit. Significant improvement in micronutrient status & favourable effect on morbidity & cognitive function. |
| Gibson RS, et al.2011 | Zambia | 6 months | 12 months | 373 | In a double-blind RCT compared the efficacy of a locally produced porridge based on maize, beans, bambaranuts, and groundnuts fortified with 19 (rich) or 9 (basal) micronutrients. Randomized to the richly fortified (n = 373) or basal (n = 370) porridge daily for along with routine vit A supplements. Baseline and final micronutrient status and inflammation (α -1-glycoprotein) assessed using nonfasting blood samples. | Urban setting, Lusaka | Baseline prevalence of anaemia (39%) and Zn deficiency (51%). Overall treatment effects on Hb, serum TfR, serum ferritin and serum Se .biomarker responses for Fe & Zn were modified by baseline conc. & for Hb and Fe by socioeconomic status. No overall treatment effect on serum Zn but improved serum Zn in children with lower Hb conc. at baseline. Reduced anaemia and improved Fe & Se status but not biochemical Zn status. |
| Liu DS, et al.1993 | China | 6-13 months | 3 months | 226 | Randomly assigned A micronutrient-fortified rusk for weanling children full-term, healthy infants to either a micronutrient-fortified or an unfortified rusk. The fortified rusk contained extra Zn; Fe; Ca; vit A, D, and B-12; thiamin, riboflavin, niacin, and folic acid. 15% of all infants initially anemic, not severely. | Mi-yun rural area near Beijing | Mean Hb levels decreased in the infants in the unfortified rusk but not for fortified rusk group. The fortified rusk group had a significant greater fall in vit. E levels. Improvements in erythrocyte porphyrin, plasma vit. A, & riboflavin status, in both groups. Micronutrient fortification beneficial for Fe status. |

2.7 Complementary foods intake measurement techniques

It is important to accurately determine the intake of complementary foods for designing the nutrient composition of a fortified processed food and as the basis for the recommendation of daily rations (Lutter, 2003; Owino et al., 2008). This may also be useful in educating mothers and caregivers on sound weaning practices that can help prevent early growth faltering. Dietary intake assessment techniques include self-reported data from questionnaires and interviews (Gibson and Ferguson, 1999 and 2008), biological markers such as urinary nitrogen, circulating nutrients in blood such as ascorbic acid and double labelled water for total energy expenditure (McKeown et al., 2001). However, since biomarkers are influenced by intervening factors such as smoking status and use of supplements, they do not reflect absolute dietary intakes and can only be used to validate other dietary assessment methods (McKeown et al., 2001).

Dietary assessment methods based on self-reported data include observer-recorded food records, food frequency questionnaires (FFQs) and 24-hour recalls (Gibson and Ferguson, 1999). Although techniques based on self-reported data have been associated with underestimation of nutrient intake (Haisma et al, 2005), they are used widely since they are relatively cheap and are easy to use (McKeown et al, 2001). Food records have been the most commonly used method to quantify dietary intake in developing countries (Gibson and Ferguson, 1999). However, food records are expensive, time consuming and invasive and might result in decreased response rates, bias and loss of statistical power and a possibility of change in dietary intake during the record period (Gibson and Ferguson, 1999).

24-h recall is used to quantitatively assess current nutrient intake and is brief with less respondent burden (Gibson and Ferguson, 1999). Repeated 24-h recalls may be used to assess actual food intakes for both individuals and groups while single 24-h recalls are applicable for the determination of group food intakes. 24h-recall may also be used to measure dietary

enhancers or inhibitors that influence nutrient absorption (Gibson and Ferguson, 1999). Gibson and Ferguson, 1999 report that FFQs can be used to estimate dietary intakes for groups over time and to place individuals into broad categories based on a distribution of nutrient intake. The FFQs are cheap and able to assess usual and longer term intake but demands increased respondent literacy, respondent burden and structure and lacks detail regarding specific foods (Gibson and Ferguson, 1999).

2.8 Iron deficiency and interventions to improve iron status

Iron is essential for biological systems such as haematopoiesis, immune function, oxygen delivery, neurological function, physical development and in micro-organisms physiology (Drakesmith & Prentice, 2008). The body has a regulated system for iron transport and storage (Dunn et al., 2007) and benefits of iron interventions have been demonstrated (Menendez et al., 2004; Richard et al., 2006). Erythrocytes carry most of the body's iron and an adult human produces about 200 billion erythrocytes daily to replace an equal number of cells that reach the end of their life span. The body maintains the concentration of iron at about 40 mg iron/kg in women and about 50 mg iron/kg in men (Brittenham, 2000) and humans are incapable of excreting excess iron thus they regulate the total iron in their body by controlling iron absorption in the gastrointestinal tract. If iron stores increase, absorption decreases and vice versa (Bothwell et al., 1979).

Iron deficiency is an imbalance in total body iron that results when the supply of iron is less than the body requirements and losses (Brittenham, 2000). There are three stages of iron deficiency: storage iron depletion, early functional iron deficiency, and established functional iron deficiency. Iron deficiency may cause anaemia, impaired behavioural development, decreased work capacity and may lead to clinically significant immune deficiency and infections in children (Prentice et al., 2007). If severe, iron deficiency causes increased mortality during pregnancy and infancy.

2.8.1 Iron metabolism

Human iron metabolism is the set of chemical reactions maintaining human homeostasis of iron. Ferritin and haemosiderin found primarily in the liver, spleen, reticuloendothelial cells and bone marrow, are the major iron-storage compounds in the body (Ramakrishnan & Semba., 2008). Intracellular iron is spread over three different pools: 1) the functional pool, where it is bound mostly to haemoglobin; 2) the storage pool, where it is bound to ferritin; and 3) the regulatory pool, where iron regulating proteins iron regulatory protein (IRP) 1 and IRP2 regulate transcription and translation of iron binding proteins. Serum ferritin is a good indicator of iron status, although its expression is up-regulated by inflammatory cytokines during periods of infection. Small amounts of iron are also found in plasma (Kumar & Clark., 1998). Iron is transported in the plasma bound to transferrin, a β -globulin that is synthesized in the liver with about two-thirds of the total body iron in the circulation as haemoglobin (Kraemer & Zimmermann, 2007).

2.8.2 Absorption of iron from the diet

Iron is absorbed in the duodenum by enterocytes of the duodenal lining and the jejunum. It must be in its ferrous (Fe^{2+}) form in order to be absorbed. Heme iron transporter (HCP 1) transports heme iron, which undergoes endocytosis after which Fe^{2+} is liberated within the endosome or lysosome. Non-heme iron includes ferrous and ferric iron (Fe^{3+}) salts. Fe^{3+} is reduced to Fe^{2+} by ascorbic acid in the lumen or by membrane ferrireductases that include duodenal cytochrome B (DCYTB) (McKie et al., 2001). Transport of non-heme Fe^{2+} from the intestinal lumen into the enterocytes is facilitated by the divalent metal ion transporter 1 (DMT 1) mainly because the acid microclimate at the apical membrane provides an H^+ electrochemical gradient that drives Fe^{2+} transport into the enterocytes (Gunshin et al., 1997). Once inside the enterocyte, iron that is not directly transferred to the circulation is stored as ferritin and is eventually lost when the cell is exfoliated at the villus tip. At the basolateral

membrane, iron transport to transferrin in the circulation is mediated by ferroportin 1, in association with hephaestin. Hepcidin, which is produced by the liver, binds to ferroportin 1 causing its internalization and degradation and decreasing iron transfer into the blood (Nemeth et al., 2004; Nemeth & Ganz, 2006). It is regulated by iron levels and erythropoiesis. In turn, it regulates iron uptake by enterocytes and release of iron stores from macrophages and hepatocytes. Ferroportin 1 also mediates export of iron from other cells, including macrophages (Donovan et al., 2005).

The regulation of ferroportin is the main way of regulating the amount of iron circulating in the body. This is because though DCYTB and DMT1 are unique to iron transport across the duodenum, ferroportin is distributed throughout the body on all cells that store iron. Iron deficiency and hypoxia stimulate duodenal expression of DMT1, DCYTB and ferroportin and thereby increase iron absorption (McKie et al., 2001; Collins et al., 2005). Senescent red blood cells are broken down by macrophages in the spleen, bone marrow, and liver. The iron extracted is returned to the circulation where it binds to transferrin as detailed in [Figure 2-1](#)

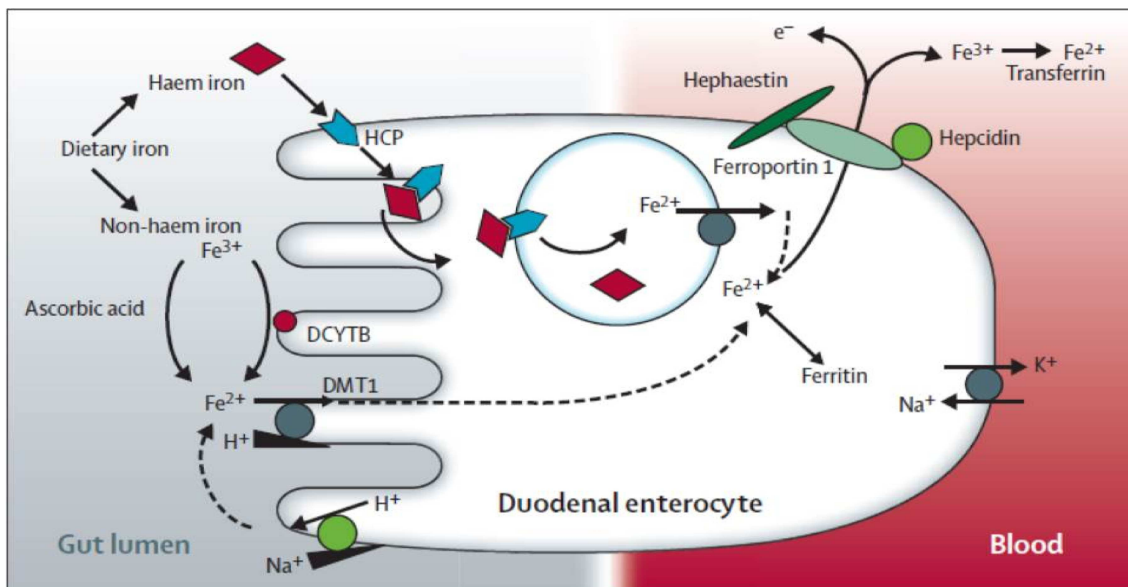


Figure 2-1: Regulation of intestinal iron uptake (Source: Zimmermann and Hurrell, 2007)

2.8.3 Reasons for iron deficiency

High demand for iron beyond what the diet can supply, increased iron loss usually through loss of blood, nutritional deficiency, inability to absorb iron because of damage to the intestinal lining e.g. in case of celiac sprue which severely reduces absorption surface area and inflammation leading to hepcidin-induced restriction on iron release from enterocytes are some of the reasons for iron deficiency.

2.8.4 Regulation of intracellular iron homeostasis

Hepcidin, 25-amino-acid peptide hormone produced by the liver is the central regulator of iron homeostasis. It regulates the export of iron from cells into plasma by controlling absorption from the intestine, export from macrophages, and release from body stores by controlling the entry of iron into plasma (Nemeth & Ganz, 2006; Ganz, 2007). An increase in hepcidin synthesis causes a subsequent decrease in plasma iron and intestinal iron absorption. Hepcidin synthesis is increased by iron loading, inflammation, and infection and decreased by iron deficiency, and ineffective erythropoiesis. Iron in circulation is tightly bound to transferrin. The level of serum ferritin in the body is directly proportional to the amount of stored iron in the body. Body cells have receptors for transferrin– iron complexes on their surfaces that engulf and internalize both the protein and the iron attached to it. Once inside, the cell transfers the iron to ferritin, the internal iron storage molecule. Iron absorption is influenced by dietary iron content, bioavailability of dietary iron, the amount of storage iron and the rate of erythrocyte production with only 5% of dietary iron normally absorbed from the average daily diet in developing countries (Kraemer & Zimmermann, 2007). Most of the iron in the body is obtained by recycling aged red blood cells in the reticuloendothelial system. Iron is lost through menstruation, sweat, urine, breast milk, shedding of skin cells and the mucosal lining of the gastrointestinal tract (Kumar and Clark., 1998). Thus people must continuously absorb iron. When iron loss exceeds iron absorption, the iron stores become

depleted and the transferrin saturation in the blood then falls. If this drops to below 10%, then abnormal iron deficient erythropoiesis occur leading to microcytic anaemia.

2.8.5 Iron and immunity

Iron is intricately involved in both innate and adaptive immune responses to infection (Weiss, 2002). Since almost all pathogenic microorganisms require iron for growth, the immediate response to infection is usually to withhold iron to invading pathogens. Increased hepcidin synthesis restricts delivery of iron to the plasma from macrophages, from intestinal absorption, and from hepatocyte stores (Ward et al., 2011). Many of the genes and proteins involved in iron homeostasis play a vital role in controlling iron fluxes such that bacteria are prevented from utilising iron for growth (Ward et al., 2011). Cells of the innate immune system, monocytes, macrophages, microglia and lymphocytes, are able to combat bacterial attacks by carefully controlling their iron fluxes, which are mediated by hepcidin and ferroportin. A variety of effector molecules, e.g. tolllike receptors, NF- κ B, hypoxia factor-1, haem oxygenase, orchestrate the inflammatory response by mobilising a variety of cytokines, neurotrophic factors, chemokines, and reactive oxygen and nitrogen species (Ward et al., 2011). Imbalances in the host iron availability impair the host immune system (Weiss, 2002). The virulence of many bacteria is enhanced through exposure to iron (Ratledge & Dover, 2000). Some bacteria acquire iron by secreting organic iron chelators called siderophores, by expressing surface receptors that interact with host iron-containing proteins, or both.

2.8.6 Anaemia

Anaemia is defined as a haemoglobin concentration below -2 standard deviations of the age- and sex-specific reference mean (Ramakrishnan & Semba., 2008). The cut-off values most commonly used to define anaemia are haemoglobin concentrations below 110 g/L for children under 5 years old and pregnant women, below 120 g/L for non-pregnant adult

women, and below 130 g/L for adult men. According to WHO (2001), the main causes of anaemia are: dietary iron deficiency; infectious diseases such as malaria, hookworm infections, or schistosomiasis infections; deficiencies of key micronutrients such as folate, vitamin B12, or vitamin A; and inherited conditions that affect cell stability such as thalassaemia and sickle cell anaemia. The three main exogenous causes of anaemia, namely disease, blood loss and diet (Thurnham & Northrop-Clewes, 2007), are shown in [Figure 2-2](#)

Although there is great variation by region, young children and women of reproductive age are at greatest risk of anaemia, followed by the elderly and men. Anaemia may lead to: fatigue, headaches, faintness, breathlessness, angina of effort, intermittent limping due to weakness (claudication), palpitations, pallor (extreme paleness), tachycardia, systolic flow murmur, cardiac failure and rarely, papillo-edema and retinal haemorrhages after an acute bleed (Thurnham & Northrop-Clewes, 2007). Very severe anaemia (haemoglobin < 50 g/L) is associated with increased childhood and maternal mortality (Allen, 1997). In areas where severe anaemia (haemoglobin < 80 g/L) is common, iron deficiency is usually one of multiple causes of anaemia (Brooker et al., 1999).

2.8.6.1 Iron deficiency anaemia (IDA)

Anaemia is the primary sign of iron deficiency (Kraemer & Zimmermann, 2007). Stored iron which is physiologically bound by ferritin molecules is usually almost entirely depleted before the development of IDA (Ramakrishnan & Semba, 2008). The causes of IDA include: blood loss, increased demands such as growth and pregnancy, decreased absorption (e.g. postgastrectomy) and poor intake. IDA develops when there is inadequate iron for haemoglobin synthesis (Zimmermann & Hurrell, 2007). Normal levels of haemoglobin are maintained for as long as possible after the iron stores are depleted; latent iron deficiency is said to be present during this period.

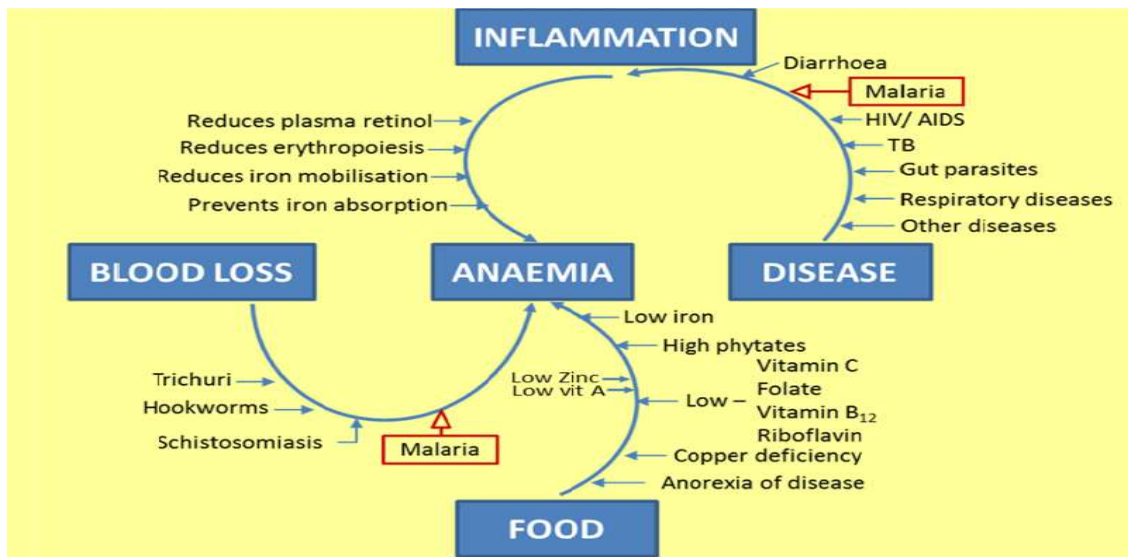


Figure 2-2: Exogenous factors contributing to anaemia. Adapted from: Thurnham and Clewes 2007 in Nutritional Anaemia (Kraemer & Zimmermann, 2007)

The highest risk groups for Iron deficiency are the pre-term and low birth weight infants, infants and children during the rapid growth period and IYC consuming milk and who have sensitivity to cow's milk, premenopausal women, pregnant women, and individuals with nematode infections in the gastrointestinal tract (Ramakrishnan & Semba, 2008). Low consumption of iron containing foods and consumption of foods that interfere with iron absorption, such as phytates, also increases the risk of iron deficiency.

The correct management of iron deficiency anaemia is to find and treat the underlying cause. Iron deficiency anaemia can also be corrected with oral iron supplements. The preparation most commonly used is ferrous sulphate, from which iron is best absorbed when the patient is fasting. Iron stores are replaced much faster with parenteral iron than with oral iron, but the haematologic response is not quicker. Oral iron should be given for long enough to correct the haemoglobin level and to replenish the iron stores. This can take six months. Failure of response to oral iron may be due to lack of compliance, continuing haemorrhage, severe malabsorption or another cause of the anaemia e.g. malaria infection. Although not all anaemias are caused by iron deficiency, in areas where the prevalence of anaemia exceeds

30–40%, most anaemia is caused largely by iron deficiency. This assumption may not hold in regions such as sub-Saharan Africa, where conditions such as thalassemia and infections such as malaria are endemic.

2.8.6.2 Anaemia caused by infections and inflammation

The infectious diseases that significantly cause anaemia are malaria, tuberculosis (TB) and HIV/AIDS. They act either individually or in combination and are most serious in developing countries. Malaria frequently causes acquired haemolytic anaemia. The anaemia of malaria has several causes namely: rupture of parasitized red blood cells in tissue venules, destruction of parasitized and unparasitized red blood cells in the reticulo-endothelial system (especially the spleen), haemolysis due to the presence of malaria antigen, antibodies and marrow suppression (Shankar, 2008; Graves & Gelband, 2006). In absence of treatment, this cycle of invasion and destruction of red blood cells is continuous thus making the person more anaemic. Blood transfusion is indicated when there is acute intravascular haemolysis and when the haemoglobin concentration falls below critical values. It is effective in severely ill patients especially when more than 20% of red blood cells are parasitized (Moxham, 1994). Malaria not only causes blood loss leading to haemolysis but also causes inflammation leading to reduced iron absorption and mobilization in the gut (Kanjaksha & Kinjalka, 2007). The most common cause of iron deficiency anaemia worldwide is blood loss from the gastrointestinal tract resulting from hook worm infestation (Ong'echa et al., 2006). In such settings, the potential impact of deworming can be justified as part of the anaemia control program.

Promoting iron-rich ASFs and undertaking other food-based strategies may also increase iron intakes and contribute to anaemia reduction but research is still needed to document their efficacy and effectiveness (Ruel and Levin, 2000). The use of local iron rich but neglected foods, such as edible termites, *dagaa* small fish, and germinated grain amaranth to improve

the quality of complementary foods needs to be explored. Higher iron status has associated with the consumption of an omnivorous diet mainly due to the intake of heme iron than to the enhancing effect on nonheme iron absorption (Reddy et al., 2006) while vitamin C enhances iron absorption (Cook and Reddy, 2001).

CHAPTER THREE

3. ACCEPTABILITY OF AMARANTH GRAIN-BASED NUTRITIOUS COMPLEMENTARY FOODS WITH DAGAA FISH (*RASTRINEOBOLA ARGENTEA*) AND EDIBLE TERMITES (*MACROTERMES SUBHYLANUS*) COMPARED TO CORN SOY BLEND PLUS AMONG YOUNG CHILDREN/MOTHERS DYADS IN WESTERN KENYA

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Abstract

We assessed acceptability of two flours and porridges of complementary foods based on germinated grain amaranth and maize with or without edible termites and *dagaa* small fish named “WINFOOD Classic” (WFC) and “WINFOOD Lite” (WFL), respectively, compared to Corn Soy Blend Plus (CSB+) among mothers and young children. 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. All foods were acceptable and can be further developed and be tested for efficacy.

Keywords: complementary food, amaranth grain, *Dagaa* fish, termites, acceptability, CSB+

Key messages

- (1). Complementary foods developed from locally available food materials are possible and when well formulated is appropriate for resource poor settings.
- (2). Affordable complementary foods formulations are needed, but such new formulations must be acceptable and not associated with any adverse health effects among target children

3.1 Introduction

Accessibility to affordable, healthy food is essential for good health as poor nutrition is one of the major determinants of impaired growth and development, acquisition of certain diseases and later chronic diseases (Allen, 2003). Children are most vulnerable to poor nutritional status during the complementary feeding periods when both macro and

micronutrients are insufficient to maintain growth and development, leading to malnutrition (Dewey & Brown, 2003). One way of sustainably reducing malnutrition is by using available traditional and indigenous plant and animal foods to prepare complementary foods that are both hygienically and nutritionally adequate to meet the needs of fast-growing infants and young children (WHO, 2008).

In some communities in Kenya, entomophagy (the practice of eating insects) has been common and widespread depending on the availability of the insects and the ability to trap them for use (Ayieko, 2007). Although small fish such as *dagaa* (*Rastrineobola argentea*) have been used in the preparation of complementary foods in some settings, there is scarcity of data on acceptability and tolerance of such foods among infants and young children, as well as their caretakers. The objective of this study was to determine acceptability of nutritionally dense complementary foods developed from traditional ingredients in comparison to Corn Soy Blend plus (CSB+), a standard humanitarian food ration, among local mothers and young children. This study was a prelude to an intervention study aimed at the evaluation of the effects of the amaranth grain-based complementary foods processed using *dagaa* fish and edible termites on growth and micronutrient status of infants in western Kenya.

3.2 Material and Methods

3.2.1 Foods Description

The two complementary foods named as “WINFOODs” were processed using germinated grain amaranth, maize soy oil and sugar but differed in the content of *dagaa* and edible termites. WINFOOD Classic (WFC) had 3% *dagaa* and 10% edible termites added, while WINFOOD Lite (WFL) had neither *dagaa* nor termites added. The ingredients were chosen after focus group discussions and interviews with mothers and health workers showed (results not shown) that some of the ingredients were available within the study area and were

commonly used for the preparation of complementary foods (Kinyuru et al., 2010). “WINFOODs” acceptability was compared to that of Corn-Soy Blend (CSB+) processed to World Food Programme specifications of 2010 (World Food Program, 2010) and purchased from Wanjis Foods Industries Ltd, Nairobi, Kenya. All the ingredients were locally identified and sourced from within the study region. WFC and WFL were pre-cooked based on heat extrusion. The final microbial analysis was carried out and safety ascertained by the Kenya Bureau of Standards and found to comply with limits set for CSB+ meant for similar target groups (World Food Program, 2010). CSB+ was also extruded and availed in flour form.

Estimated nutrient values of the developed complementary foods and the CSB+ are as shown in **Table 3-1**. The values are as obtained from estimated values of the nutrients composition of the ingredients on dry weight basis. They acted as a guide during the nutrient optimization process in order to achieve nutrient dense blends. On cost analysis based on costing the ingredients and the unit operations involved to produce the products, WFL had the lowest cost (USD 1.82/ kg) due to the fact that it did not have the termites while WFC cost (USD 3.76/kg). The cost of the foods was lower than an average commercially processed complementary food which retailed at about USD 7.0/ kg in Kenya (as per market survey of November, 2011). One way to reduce the cost is by encouraging more termite harvesters to harvest thereby increasing supply and consequently lowering the price. Currently, the termite is a delicacy in Western Kenya where it retails at USD 12.0 in the markets when they are in season. The prices go even higher to USD 18.0 when they are off season. However, the developed complementary foods may be affordable by the target population in western Kenya where the ingredients are sourced from and thus the middlemen effect which increases the prices drastically is eliminated when sourcing the termites (Kinyuru et al., 2010).

Table 3-1: Estimated nutrient values of the study foods on dry weight basis (WFC,WFL& CSB+)

| | Moisture g/100g | Energy Kcal | Protein g/100g | Fat g/100g | Fe mg/100g | Zn mg/100g | Ca mg/100g |
|------|----------------------------|------------------------|---------------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| WFC | 8.7 | 423.6 | 19.1 | 12.3 | 12.2 | 6.3 | 48.2 |
| WFL | 9.4 | 407.2 | 14.6 | 9.0 | 12.5 | 5.5 | 139.4 |
| CSB+ | 9.7 | 391.7 | 15.1 | 5.8 | 7.7 | 5.1 | 141.9 |

3.2.2 Study Area

The study was carried out at Makunga Sub-District Hospital, Mumias District of Kakamega County, Western Kenya in March 2011. The site was chosen to reflect the resource poor settings targeted in the subsequent efficacy study and in the region where the foods used to process the products are already accepted. Most of the inhabitants of the area have turned their farms into small plantations of sugar cane with the average size of plot holdings of 4 acres in an effort to earn cash from the cash crop. The sugar cane is harvested after 18-24 months. In the meantime, farmers are left without a viable source of income till the next harvesting season which 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.

3.2.3 Study Design and Data Collection Methods

The study was done in three phases where phase 1 involved hedonic ranking by mothers of three flours and, subsequently, porridges as detailed by Meilgaard et al. 2007. The porridges had been prepared by the study team prior to the ranking. Mothers evaluated colour, taste, texture and smell of flours and prepared porridges based on a 5-point hedonic scale (from “5” – like very much, to “1” - dislike very much) (Meilgaard et al., 2007). Overall comments about the products and how to improve them for feeding infants and young children were also captured.

Phase 2 involved having the mothers centrally prepare the porridges and feed the infants. In a cross-over design children were initially randomized to receive one of the three study

foods (WFL [n=15], WFC [n=17], CSB+ [n=15]) on the first day followed by a one-day washout after which they switched to the second porridge on the 3rd day followed by another one-day washout period before completing with the third porridge on the 5th day. On each feeding day the study team observed how the child reacted during feeding and scored response based on a 5-point hedonic scale (Meilgaard et al., 2007). About 150 ml of porridge was offered to the infant in a graduated feeding cup. The amount of porridge consumed was calculated by subtracting amount left and spilt from 150 ml. The mother/infant pairs were divided into three groups and each infant was scheduled to eat one product one day over a 5-day period with a wash-out day in separating the porridges.

Phase 3 involved the mothers taking home 100g of a flour on each of the three visits at the health facility, preparing the food at home in their usual way and feeding the enrolled child as well as any other willing person at home. On subsequent visits mothers were asked how well the child and any other person in the household liked the porridge they prepared at home and responses recorded on a 5-point scale.

Morbidity data for children including diarrhoea, stomach-ache, vomiting, skin rashes and difficulty in breathing, were collected by a two-day recall at recruitment and at every subsequent visit along with anthropometric measurements once for weight, length and mid upper arm circumference. For infants, each food was deemed acceptable if the child consumed at least 75% of the serving and if less than 10% of adverse effects cases were reported for that particular test food based on similar work (Valid International, 2006). Mothers attending the Makunga Sub-District Health Centre with infants 6-24 months of age were assessed for eligibility to participate in the acceptability study. To be eligible, the infant had to be 6-24 months old, the caregiver had to be willing to prepare and take/serve the porridge as advised. Moreover, the infant had to be free of any evidence of chronic disease and weighed at least 2500g at birth. Those with chronic illness requiring medication, genetic disorders interfering with normal growth were excluded and referred for treatment at the

partnering Makunga health facility after assessment by qualified medical personnel. The mothers were not forced to take part in the study and the purpose of the study was explained to obtain informed consent and further permission obtained from the Mumias District Health Management Team, the Chief and other local administrators. All caregivers/mothers gave written informed consent.

3.2.4 Data Analysis

All data collection forms were checked by the supervisors in the field to allow immediate re-visits for gross errors or missing data. Data was analysed using SPSS (Version 17) (SPSS, 2010) and anthropometric data was analysed using WHO Anthro (WHO Anthro 3.2.2). The anthropometric data was to confirm that only normal and moderate malnutrition cases were included in the study. Means and standard deviations were calculated for acceptability of the sensory attributes of the complementary foods. A two-way analysis of variance (ANOVA) was conducted to test for significant differences ($p \geq 0.05$) in the sensory attributes (appearance, smell, taste, and texture) among the three foods. Mothers' comments were also recorded verbatim and organised into themes.

3.3 Results

3.3.1 Demographic and Anthropometric Information

Fifty seven child/mother pairs were recruited into the study with 31 of the infants/children being boys and 26 girls. Descriptive statistics for the anthropometric information before the acceptability study are presented in [Table 3-2](#). Age of the participating infants ranged from 6 months to 24 months. Boys had a mean age of 12.3 months while the girls had a mean age of 11.3 months. Girls had a slightly lower LAZ than the boys (-0.2 ± 1.3 and 0.3 ± 1.5 , $p=0.001$), respectively, though none of them were stunted ($LAZ < -2$).

None of the participating infants were wasted ($WLZ < -2$) and the average WLZ was not significantly different ($p \leq 0.05$) between boys and girls. All the infants participating in the

study were well nourished (MUAC>12.5) and were already consuming complementary foods back home while among them, 80.7% were still breastfeeding.

Table 3-2: Background demographic and anthropometric information

| | Boys (n=31) | Girls(n=26) |
|-----------------------------------|-------------|-------------|
| Age (months),(Mean±SD) | 12.3 ± 5.6 | 11.3 ± 6.9 |
| Length (cm) | 75.1± 7.6 | 74.6 ± 5.8 |
| Weight (kg) | 9.5 ± 2.2 | 8.9 ± 1.2 |
| MUAC(cm) | 14.7 ± 1.4 | 14.3 ± 1.1 |
| WAZ | -0.1 ± 1.2 | -0.5 ± 1.3 |
| LAZ | 0.3 ± 1.5 | -0.2 ± 1.3 |
| WLZ | -0.2 ± 1.1 | -0.5 ± 1.6 |
| Consuming complementary foods (%) | 100.0 | 100.0 |
| Still breastfeeding (%) | 80.7 | 80.7 |

MUAC-Mid Upper Arm Circumference; LAZ-Height/Length for Age Z-score; WAZ-Weight for Age Z-score; WLZ-Weight for Height/Length Z-score.

3.3.2 Maternal Food Acceptability

Data on the sensory evaluation and acceptability of the flours by mothers are presented in **Table 3-3**. The consumers ranked WFL flour highest in terms of colour, smell and texture. The colour and smell of WFL were however not significantly different ($p > 0.05$) from CSB+ which was being used as a positive control. The colour and smell of WFC flour were significantly lower than in both WFL and CSB+. This could have been due to the addition of *dagaa* fish and termites added to the composite products that slightly changed the attributes. CSB+'s texture was rated lowest.

Table 3-3: Sensory evaluation of the products' flours by mothers

| Product | Attributes | | |
|---------|-------------------------|-------------------------|-------------------------|
| | Colour | Smell | Texture |
| WFL | 3.9 ± 0.14 ^a | 3.9 ± 0.14 ^a | 4.6 ± 0.07 ^a |
| WFC | 3.3 ± 0.18 ^b | 3.0 ± 0.18 ^b | 4.4 ± 0.12 ^a |
| CSB+ | 3.8 ± 0.16 ^a | 3.8 ± 0.16 ^a | 2.4 ± 0.12 ^b |

Mean ± SD; N=57; Values on the same column with different superscripts are significantly different ($p \leq 0.05$).

Similar trends as shown in the flour were reported by the mothers rating of the porridge

(Table 3-4). Mean scores for WFL on the five point hedonic scale were 4.0 ± 0.1 indicating that the color, smell, taste and texture were liked. There were no significant differences between the mothers' scores for sensory attributes of colour, smell and taste of the WFL and CSB+. WFC's smell and taste were however rated at 2.0 meaning they were slightly disliked even though the texture was significantly liked ($p < 0.05$).

Table 3-4: Sensory evaluation of the products' porridge by mothers

| Product | Attributes | | | |
|---------|-----------------|-----------------|-----------------|-----------------|
| | Colour | Smell | Taste | Texture |
| WFL | 4.0 ± 0.1^a | 4.0 ± 0.1^a | 4.0 ± 0.1^a | 4.0 ± 0.1^a |
| WFC | 3.0 ± 0.2^b | 2.0 ± 0.1^b | 2.0 ± 0.2^b | 4.0 ± 0.2^a |
| CSB+ | 4.0 ± 0.2^a | 4.0 ± 0.1^a | 4.0 ± 0.2^a | 3.0 ± 0.2^b |

Mean \pm SD; N=57; Values on the same column with different superscripts are significantly different ($p \leq 0.05$).

WFL was the most preferred flour and porridge (63.2% and 70.2% respectively) by the mothers thus WFL was the most preferred product of the three foods presented. More mothers preferred WFC flour (24.6%) than CSB+ flour (12.3%) while the porridges were preferred at 10.5% and 19.3% for WFC and CSB+ respectively by the mothers. All the mothers indicated that they preferred their product of choice due to the inherent properties of color, smell, taste and texture and none indicated that their preferences was based on extrinsic factors.

3.3.3 Infant Food Acceptability

Maternal interpretation of their infant's responses was evaluated at three levels of feeding starting at when the infant sees the food, at the initial food offer and subsequent offers at the health centre. The interpretation indicated that the infants preferred WFL more, in comparison to the other products (Table 3-5). Infants preferred WFC porridge more than CSB+ based on the level of liking with the exception of "subsequent offers". At subsequent feeding offers, there was no significant difference between WFC and CSB+.

Table 3-5: Sensory evaluation of the porridge by enrolled infants

| Product | Sees food first | Initial offer | Subsequent offers |
|---------|------------------------|------------------------|------------------------|
| WFL | 4.2 ± 0.1 ^a | 4.2 ± 0.1 ^a | 4.2 ± 0.1 ^a |
| WFC | 3.6 ± 0.2 ^b | 3.6 ± 0.2 ^b | 3.4 ± 0.2 ^b |
| CSB+ | 3.3 ± 0.2 ^c | 3.3 ± 0.2 ^c | 3.3 ± 0.2 ^b |

Mean ± SD; N=54 (3 were lost to follow up); Values on the same column with different superscripts are significantly different (p≤0.05).

3.3.4 Amount of Food Consumed

On average, 73.0% of WFL porridge was consumed while 48.5% and 48.0% of CSB+ and WFC were eaten respectively by the enrolled infant during central feeding at the health centre (Table 3-6). However, the amount consumed significantly increased during home feeding with over 75% of CSB+ and WFL consumed and WFC reporting 68.9% consumption.

Table 3-6: Amount of porridge consumed (%) by infants

| | Central feeding | Home feeding |
|------|--------------------------|--------------------------|
| WFL | 73.0 ± 30.4 ^b | 91.6 ± 39.5 ^a |
| WFC | 48.0 ± 34.3 ^b | 68.9 ± 32.3 ^a |
| CSB+ | 48.5 ± 33.5 ^b | 75.0 ± 39.5 ^a |

Mean ± SD; Values on the same row with different superscripts are significantly different (p≤0.05).

Upon evaluating different proportions eaten, Figure 3-1 shows that 43% of the infants consumed over 75% of the served WFL porridge. Twenty one (21%) of the infants consumed over 75% CSB+ served while 19.6% of the infants consumed over 75% of the WFC served. Similar trend was observed for CSB+ and WFC with over 50% of the respondents consuming less than 50% of the food served while only 27.8% of the respondents consumed less than 50% of the WFL served.

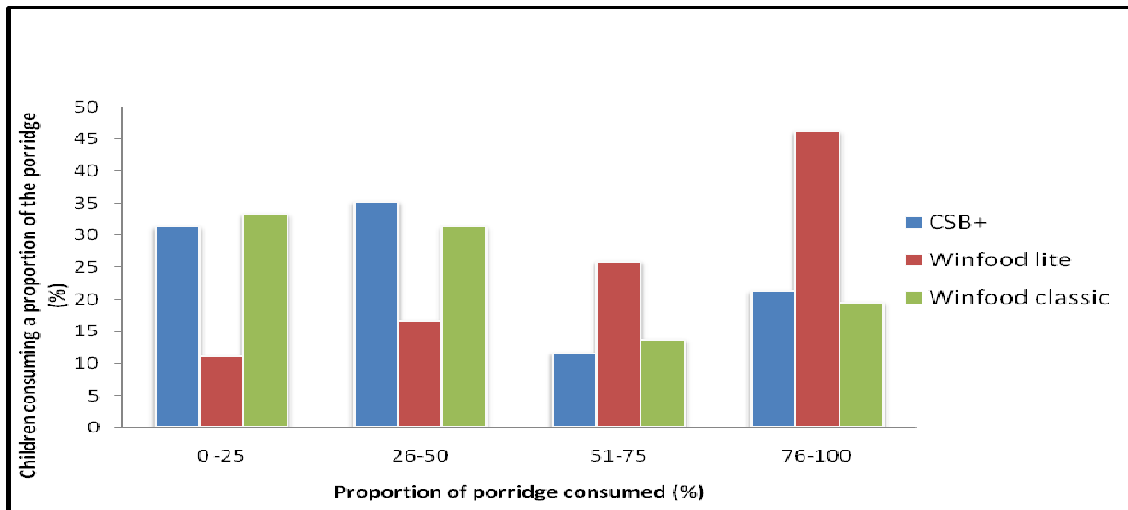


Figure 3-1: Proportion of porridge consumed by the enrolled young children at the health centre

3.3.5 Food Tolerance

Diagnosis of infant and young child food allergy and intolerance is a challenge due to lack of appropriate communication. In this study, infant tolerance of the foods was evaluated on consuming each of the foods. Adverse health effects on consumption of the foods were evaluated and presented as shown in [Figure 3-2](#).

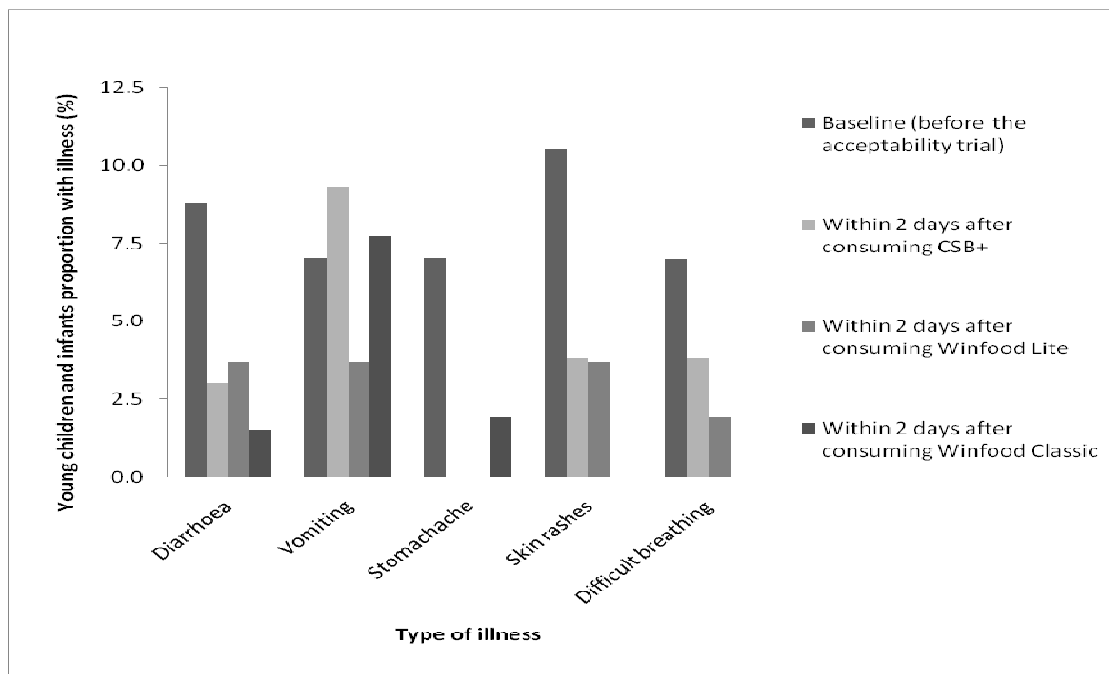


Figure 3-2: Infant/young child illness just before, during and shortly acceptability study

In all illnesses evaluated except vomiting, the levels were higher before the acceptability study than after feeding the specific foods. This shows that the level of morbidity was high among the participants with 10.5% of participants showing signs of skin rashes before the acceptability study. Others illnesses evaluated according to the mothers knowledge were whether the infants had shown signs of fever and cough. Cases of vomiting were reported (9.3%) after consumption of CSB+ which was slightly higher than the value before the acceptability study (7%). Cases of stomach-ache were not reported among the infants who ate WFL and CSB+. However, some symptoms can be difficult to interpret as life-threatening reactions such as the crying, fussing, irritability, inappropriate drowsiness or fright, spitting up, vomiting, loose stool/diarrhoea or abdominal pain/ stomach-ache. Within a population, the number of allergic/intolerance cases towards a certain food should not exceed 10% (Bovell-Benjamin et al., 1999). In this study, all the cases reported were below 10% threshold required to declare a product to have adverse effects thus even the 9.3% observed for vomiting was still acceptable.

3.3.6 Post-tasting Comments by Mothers

Mothers were requested to comment on the product. The comments were captured and are represented in **Figure 3-3**. The main comments relating to how to further improve the foods were identified as thickness (consistency) and taste (sweetness). The highest percentage of the mothers (39.4%) suggested they would prefer WFC with sugar while 23.5% of the mothers indicated they would prefer a WFC if it had a thicker consistency. Other comments included 'baby likes' and 'good for babies'.

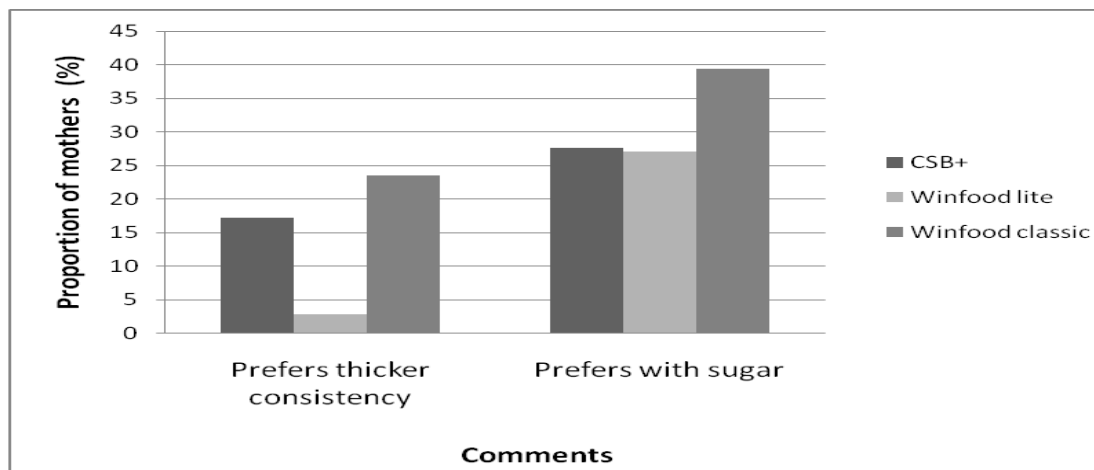


Figure 3-3: Some post taste comments by mothers

3.4 Discussion

The aim of this study was to assess the acceptability of the three improved complementary foods. In infants, means for the three porridges were >3.0 thus a general liking of all the products with no evidence of allergic reaction from the foods consumed. The observation that both WFL and WFC porridges were generally accepted agrees with previous observations that mothers often find complementary foods processed from locally available staples attractive (Mensa-Wilmot et al., 2001; Owino et al., 2007). It appears that, in addition to a sufficient energy density, sensory qualities of complementary food formulations corresponding to food preferences of mothers and their infants are of importance. It has been noted that dietary quality rather than quantity is a key aspect of complementary food development that needed improvement (Lutter & Rivera, 2003). Infant age has been found to affect food preferences for consistency of complementary foods (Parker et al., 1998), with infants not showing dislike for some foods that mothers reportedly disliked. However, it is necessary to ensure that there is maternal preference of complementary food if a food is to be widely accepted (Muroki et al., 1997). Involving mothers in nutrition education activities is recommended for improved nutritional status of young infants (Muroki et al., 1997; Abebe et al., 2006), and the mothers in our study were enthusiastic about preparing the WINFOOD complementary foods.

Consistency has been found to affect acceptability of complementary porridge (Owino et al., 2007). The Codex Standard (1981) for processed cereal-based foods for infants and children states that reconstituted dry cereal should be suitable for spoon feeding of infants and children. Previous studies have reported that mothers preferred semi-liquid porridges which are easy to spoon feed (Muroki et al., 1997; Mosha & Svanberg, 1993). If the porridge has a very low or very high consistency, its acceptability may be low. Some previous studies have found that complementary food formulations with addition of sugar were found to be more tasty and appealing than those without sugar, indicating that inclusion of sugar not only increased the energy density of the porridge but enhanced the taste and characteristic improved flavour (Martin et al., 2010; Muhimbula et al., 2011). Owino et al. (2007) found that sweetness improved the acceptability of α -amylase-treated maize-beans-groundnuts-bambaranuts complementary blends. This shows that inclusion of sugar or sweetener is important for the acceptability of product by target groups.

There is evidence that olfactory preferences in infants and young infants are learned, not innate and that they develop slowly (Bovell-Benjamin et al., 1999; Pangborn et al., 1988; Schaal, 1988), even though the process may start as early as in the womb (Marlier et al., 1998; Sonssignan et al., 1997). Although infants and toddlers may detect 'unpleasant' smells, they may not judge them as 'unpleasant' until they are about 5 years of age (Mennella & Bauchamp, 1997). In general, exposure drives preference, except in those cases when negative reactions from peers or parents teach young children that a given olfactory stimulus is unpleasant (Birch, 1992). In this study however, young children as old as 24 months did not dislike any of the foods and this may mean that all the foods were acceptable among the young ones.

Most of the theoretical base for conducting sensory evaluation relates to normal circumstances where food resources are adequate. Infants who lack adequate food may eat foods eagerly due to hunger, which might not represent an accurate assessment of the

acceptability of tested products using standard sensory evaluation procedures. In this study evaluation was done both at a central place and at home to avoid bias due to hunger as well influence of changing the feeding environments. In this study we observed that infants tended to eat more of the served porridge at home compared to the health centre. Apart for the fact that at home there is little observer influence, it is also likely that mothers/caretakers added more sugar and other ingredients to the porridge thereby making it more palatable. This observation may also have been as a result of bias from mother/caregiver self-reported data as opposed to the observed measurements at the health facility. Also, low-income caregivers in such situations may be inclined to give higher scores (Ashbrook & Doyle, 1985). This suggests the need for applicable methods for conducting sensory evaluation in low-income households with illiterate and semi illiterate participants.

Food fortification may have adverse effects on the sensory qualities of foods especially if fortifying with some foods such as fish and insects due to the high rate of rancidity development. Rancidity is influenced by the type of fatty acid composition of the high oil content foods. However, infants may not always detect the rancid smell in the foods or may not view it as unpleasant or do not care. The logical explanation for this is that their olfactory preferences are not yet well developed (Schaal, 1988; Marlier et al., 1998; Sonssignan et al., 1997). In our study, the infants' non-verbal cues were mostly positive, intake was good and it is difficult to imagine how mothers could have influenced such responses. The degree of liking of the porridges was read by the primary caregiver from non-verbal cues exhibited by the toddler during feeding. The indirect approach of using non-verbal cues as indicators of acceptance by infants and young children has been used before by researchers (Mennella & Bauchamp, 1997; Birch, 1992; Ashbrook & Doyle, 1985 and Melcer 1997), and it is the practice of choice in the baby-food industry (Kevin, 1995; Neumann et al., 2002). Many variables (other than sensory variables) affect consumer behaviour. It is speculated, however, that the respective weights of these non-sensory (mostly cognitive or psychological) variables

in decisions to consume must be much lower for infants and young children than they are for adults, simply because the former are not aware of most of them (Bovell-Benjamin et al., 1999). The influence of food security on acceptability assessment was equally distributed across the groups by randomization at recruitment.

3.5 Conclusion

All three foods are acceptable to the target population with no adverse effects and may be developed further and be used for efficacy trial. Despite the differences, it is important to notice all the foods score reasonably high in the parameters tested.

3.6 Acknowledgements

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3.7 CONFLICT OF INTEREST

None of the authors had a conflict of interest to disclose.

3.8 FUNDING

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CHAPTER FOUR

4. PREVALENCE AND SEVERITY OF MALNUTRITION AMONG INFANTS AND YOUNG CHILDREN IN AN HIV AND MALARIA PRONE RURAL SETTING, WESTERN KENYA

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ABSTRACT

Objective: To determine the nutritional status of infants and young children 6-24 months of age in an area in rural Western Kenya.

Design: Cross sectional survey.

Setting: A rural community in Western Kenya, approximately 425 km North-West of Nairobi with high malaria prevalence ($\geq 40\%$), a high prevalence of human immunodeficiency virus (7.5%), and high infant and under-five mortality (176/1,000 and 259/1,000) respectively.

Methods: A random sample of 618 children-caregivers pairs selected from the catchment areas of Lusheya, Khaunga and Makunga health facilities in Mumias Sub-county of Kakamega County. Anthropometric, current food and nutrient intake and morbidity data were collected using standard procedures.

Results: Anthropometric indices are presented for 614 children collected during the survey. The prevalence of stunting (Z-scores for height-for-age [HAZ] < -2), wasting (Z-scores for weight-for-height [WHZ] < -2) and underweight (Z-scores for weight-for-age [WAZ] < -2) was 35.7%, 4.1%, and 13.4%, respectively. About 83% were breastfeeding while complementary feeding was introduced at mean age of 3.8(SD0.3) months. Solid complementary foods used mirrored the family diet at the time. Fruits were rarely consumed. The energy intake from the traditional complementary foods were 1.8 ± 0.5 , 2.2 ± 0.4 and 2.6 ± 0.8 MJ/day for age ranges 6-8, 9-11 and 12-18 months respectively. The daily iron, calcium, zinc and Vitamin A intake from the complementary foods was 0.4 ± 0.2 mg, 130.0 ± 90.0 mg, 2.0 ± 0.6 mg and 205.1 ± 100.0 μ g respectively. About 48% of the children had experienced moderate illness that required treatment while about 30% had experienced watery loose diarrhoea with 53% experiencing both a cough and a fever within the same period. WAZ was significantly predicted by the age, marital status and education level of the caregivers (P= 0.001, 0.003 and 0.003 respectively) while HAZ was significantly predicted by education level of the caregiver (P=0.005). HAZ and WAZ deficits increased rapidly in children already at 6 to 18 months old and were greatest in children 18–23 months old. Mean HAZ and WAZ stabilized towards 24 months of age but remained substantially below the reference median with no evidence of catch-up growth.

Conclusions: Malnutrition is highly prevalent. Complementary feeding is sub-optimal. Marital status, education level and age of the caregiver significantly predict the nutritional status of the children in the study population. Given both the acute and long-term consequences of malnutrition in this vulnerable age group, community-based interventions aimed at reducing child malnutrition in such populations should focus on all children less than two years of age and especially from households whose caregivers are young, have low education attainment and are single. Nutrition education and food intervention strategies should be put in place to address the high prevalent malnutrition.

Keywords: Malnutrition, Stunting, Resource limited setting, Western Kenya

4.1 INTRODUCTION

Despite the envisaged decline in global trends of acute malnutrition, the figures remain alarmingly high with about 25% of children under five years still stunted in 2012 (WHO, 2012). A decade ago, the United Nations Administrative Committee on Coordination/Standing Committee on Nutrition (ACC/SCN) observed that malnutrition was still unacceptably high and progress to reduce it in most regions of the world was slow with approximately 27% of children estimated to be underweight (ACC/SCN, 2004). While the overall trend in nutritional status in developing countries over the last 30 years has improved and is expected to continue, Eastern Africa (Kenya included) is one of the regions where the trend has been in the opposite direction (Kwena et al., 2003; KDHS, 2008-09).

In Kenya, the prevalence of stunted and underweight children remained stable throughout the 1990s, as did the gross national product per capita, while the under fives five mortality rate increased slightly from 105/1,000 to 112/1,000 (ACC/SCN, 2004). According to the Kenya Demographic Health Survey (KDHS) 2008-09, nationally, 35% of children under five are stunted, 16% are underweight and 7% are wasted. Among the stunted, 14% are severely stunted with stunting highest (46%) among children aged 18-23 months and lowest (11%) in children age less than 6 months. Severe stunting shows a similar trend, where children aged 18-23 months have the highest proportion of severe stunting (22%) and those less than 6 months have the lowest proportion (4%). A higher proportion (37%) of male children under five years are stunted compared with 33% of female children with an inverse relationship between the length of the preceding birth interval and the proportion of children who are stunted. The KDHS further estimates the prevalence of malnutrition in children less than five years of age showing a large variation by province, reflecting the considerable variability in environmental and socioeconomic risk factors. These estimates were approximately 28% (stunting), 15% (underweight), and 3% (wasting) for the former Western Province in Kenya.

It is unknown whether these statistics apply currently to the study area in Mumias Sub-county. This poor rural area located in Kakamega County in Western Kenya has intense malaria transmission estimated at $\geq 40\%$ (Noor et al., 2009) and a human immunodeficiency virus (HIV) prevalence of 7.5% (Kenya Aids Indicator Survey (KAIS), 2007). Both malaria and HIV are perceived as the main contributors to the very high infant and less than five mortality (176/1,000 and 259/1,000) respectively, which is considerably higher than in other parts of Kenya (KAIS, 2007; KDHS, 2008-09; Noor et al., 2009).

Public perception assumes that children in this area are prone to malnutrition but no area specific data is available to support this or to make comparisons with other populations. The cross sectional survey aimed to establish the prevailing nutritional status (weight, height and MUAC), morbidity and food intake of infants and young children 6-24 months of age in the area. Currently, DHS and the UNICEF-supported Multiple Indicator Cluster Surveys (MICS) programmes include around 15,000 and 10,000 households respectively (Hancioglu and Arnold, 2013). This sample size is sufficient to produce statistically reliable estimates of most indicators at the national, urban-rural, and regional levels, but not at lower administrative levels, such as districts, slums, and small population groups as is the case in the study area (Hancioglu and Arnold, 2013). The study area being a sugarcane farming zone tends to have a small population group whose characteristics cannot be lumped with the general cluster characteristics. Moreover, the recent crop failure following the recent maize streak virus attack of 2012 (Ministry of Agriculture, 2013), improvement in PMTCT and other health systems management structures (KAIS, 2007) among other changes are likely to have altered the figures as reported in KDHS 2008-09 since it has been four years since the last KDHS data were collected and the DHS does not give trends but rather point in time data thus justifying this specific study.

4.2 METHODS

4.2.1 Study site and participants

Details of the study site and methods have been described in details by Konyole et al., (2012). Briefly, the study site was in Mumias Sub-county of Kakamega County in Western Kenya in three health facilities namely Makunga, Lusheya and Khaunga. The population is ethnically homogeneous except in Mumias town where because of the sugar milling factory and other socio-economic activities other ethnic groups have been attracted as traders and labourers. More than 90% of the population are members of the Wanga sub-tribe of the larger Luhya tribe. Introduction of solid and liquid foods other than breastmilk occurs in about 88% of the children by 4 months (Mbagaya, 2009). Malaria transmission is intense and occurs throughout the year at $\geq 40\%$ (Noor et al., 2009; KDHS, 2008-09) with the prevalence of HIV among pregnant women in this community being 7.5% (NAS COP/MoH, 2006; NACC, 2009).

4.2.2 Participants recruitment and measurements

A cross sectional survey was conducted between November and December 2011 in the catchment areas of Khaunga, Lusheya and Makunga health facilities. All infants from the age of 6-24 months of age were targeted. Mothers-infants (at 6 months) pairs were recruited in the study as they came to the child welfare clinics for diphtheria, pertussis (whooping cough) and tetanus (DPT; also DTP and DTWP) or oral polio and other vaccinations from 6 months of age onwards. The main study outcomes were: weight, length, mid upper arm circumference (MUAC), Skinfolds measurements (including triceps skinfold, subscapular skinfold, suprailiac skinfold, biceps skinfold,) morbidity prevalence and 24 hour dietary maternal recall.

4.2.2.1 Inclusion criteria

The infants had to be free of any evidence of chronic disease and must have weighed at least 2500g at birth. Children had to possess: WHZ > -3, a MUAC >115 mm, no bilateral pitting oedema, anaemia (Hb > 80 g/L) nor clinical signs of vitamin A deficiency (xerosis or Bitot spots)

4.2.2.2 Exclusion criteria

Children with WHZ < -3, a MUAC <115 mm, and/or bilateral pitting oedema, or with anaemia (Hb < 80 g/L) or clinical signs of vitamin A deficiency (xerosis or Bitot spots) were excluded and referred for treatment at the partnering health facilities. Those with chronic illness requiring medication, genetic disorders interfering with normal growth and twins were also excluded, and referred for care by qualified medical personnel.

Anthropometric measurements were taken by 2 trained assistants whose techniques were standardized according to World Health Organization (WHO) procedures on a purposive sample of 600 infants (UN, 1986). Briefly, nude weight was measured to the nearest 100 grams using a 25kg±100g hanging weighing scale (CMS Weighing Equipment, London, United Kingdom) which was calibrated daily. Recumbent length (children less than two years old) was measured to the nearest 0.1 cm using wooden measuring boards. Triceps and subscapular skinfold-thickness measurements were taken on the left side to the nearest 0.1 mm by using Holtain skinfold calipers (Crymych, United Kingdom), and midupper arm and head circumferences were measured to the nearest 0.1 cm by using an insertion tape. Current food and nutrient intake data were collected based on 24 hour recall technique as detailed in Gibson and Ferguson (1999 and 2008) while morbidity was collected by maternal recall in the past week before the study as detailed in Lartey et al. (1999). Measurements were completed at Maternal and Child Health centers when mothers reported with their infants for the regular monthly growth monitoring.

4.2.3 Data management

Prior to the fieldwork, all field assistants were trained on the standard operating procedures (SOP) such as anthropometric measurements among others until they were able to correctly carry them out. The study questionnaires were also pretested to ensure quality. Case record forms were checked daily and data entered into a computer within 2 weeks. Quantitative data were double entered using WHO Anthro v3.2.2 (WHO, 2009).

4.2.4 Data analysis

The Z-scores for length/height-for-age (L/HAZ), weight-for-age (WAZ), and weight-for-height/length (WH/LZ) were calculated using reference data from the WHO (2006) in WHO Anthro v3.2.2. Frequencies, means and median values were calculated using SPSS™ software version 19 (Chicago, Illinois, USA). Stunting, underweight and wasting were defined as LAZ, WAZ and WLZ respectively, < -2 standard deviations of the WHO reference standards (WHO, 2008). Moderate-to-severe and severe malnutrition in infants were defined as MUAC < 12.5 cm and MUAC < 115 mm, respectively (Cogill, 2003). 24 hr recall data were analysed to generate the most commonly consumed foods by the children where possible. Dietary intake and morbidity data were analysed as detailed in Lartey et al. (1999). Briefly, the dietary data were used to calculate the daily intake of energy, food solids, iron, zinc, calcium and Vitamin A of traditionally used foods based on a Kenyan and regional food composition tables (Sehmi, 1993; Calloway et al. 1994; Lukmanji et al., 2008) using Food Processor Plus™ software (ESHA Research, Salem, OR, USA) as detailed in Lartey et al. (1999). The midupper arm fat area (MAFA) and midupper arm muscle area (MAMA) were calculated by using the following equations 4-1 and 4-2 adapted from Lartey et al. (1999).

$$\text{MAFA} = (\text{TSF} \times \text{MUAC})/2 - [\pi \times (\text{TSF})^2]/4 \dots\dots\dots(\text{Equation 4-1})$$

$$\text{MAMA} = [\text{MUAC} - (\pi \times \text{TSF})]^2/4\pi \dots\dots\dots(\text{Equation 4-2})$$

Where TSF is triceps skinfold thickness (mm) and $\pi = 3.14$

4.2.5 Permission and consent

The study was reviewed and approved by the Kenyatta National Hospital-University of Nairobi ethics review committee (KNH-UON ERC-P436/12/2010). Permission to conduct the study was obtained from the Mumias district health management board and team. The local administration also granted permission to carry out the study. Written informed consent was obtained from caregivers for each individual participant. The mothers were free to withdraw from the study anytime they wished.

4.3 RESULTS

A total of 618 infants and children 6-24months old were included in the cross sectional survey. Current food and nutrient intake data were collected based on 24 hour maternal recall technique in a random sub-sample of 84 (27 each in Lusheya and Khaunga; 30 in Makunga) IYC with emphasis on micronutrient-rich foodstuffs such as meat, liver, eggs, etc. A further random sub-sample of 160 participants was taken for skinfold measurement (59 Lusheya, 48 Khaunga and 53 Makunga). Of the 618 children, two were excluded because they were over the maximum age limit. Anthropometric data were missing for two other children. The characteristics of the remaining 614 children (with at least one anthropometric observation) are shown in **Table 4-1**. The children were also examined for thin flaky skin texture, visible severe wasting, light thin hair colour and bipedal oedema.

4.3.1 Socio-demographic data

A majority (96.3%) of the caregivers were mothers to the IYC. Christianity was the main faith (about 83%) practiced by the study population and the main source of income was farming at 52.4%. The mean age for the caregivers was 26 ± 7 years while the mean age for IYC was about 13.5 ± 5.3 months old. There were 52% male infants in the study while the presence of the clinic card was taken as a proxy indicator of immunization coverage which was at 90.9%. A majority of the caregivers had only completed primary level of education

(54.5%) while another 19.4% were neither able to read nor write (illiterate). Most households (83%) obtained water for domestic use from untreated sources (Table 4-1).

Table 4-1: Socio demographics characteristics of the study participants

| Characteristic | Mean (SD)/Percent |
|--|-------------------|
| Age of caregivers (Mean, SD) years | 26.5±7.3 |
| Number of people in the households (Mean, SD) | 5.5±2.0 |
| Sex of Main caregiver, Female | 98.9% |
| Marital status of main caregiver | |
| Married | 90.2% |
| Widowed | 2.1% |
| Single | 6.0% |
| Separated | 1.6% |
| Relation of main caregiver to infant | |
| Mother | 96.3% |
| Grand mother | 2.9% |
| Religion | |
| Christian | 82.8% |
| Indigenous churches | 1.6% |
| Muslim | 9.8% |
| Others | 5.9% |
| Main source of income | |
| Farming | 52.4% |
| Self employed | 29.0% |
| Salaried employment | 8.8% |
| Remittance | 4.6% |
| Others | 5.2% |
| Presence of clinic card(growth monitoring) | 90.9% |
| Age of infants (Mean, SD),months | 13.5±5.3 |
| Age of introducing other foods, (Mean, SD), months | 3.8±2.2 |
| Infant birth order (Mean, SD), | 3.1±1.9 |
| Sex of infants, Male | 52% |

4.3.2 Anthropometric measurements

The mean weight, height and MUAC were 9.0±1.6 kg, 73.5±5.8 cm and 14.5±1.9 cm respectively. The average head circumference was 45.6±2.1cm. Weight, Height, MUAC and head circumference were significantly different between boys and girls (p=0.02, 0.02, 0.03 and <0.001) respectively. Mid-upper arm fat area (mm²) and Mid-upper arm muscle area (mm²) were 497.3 ± 98.7 and 1453.1 ± 141.1 respectively and like the skinfolds measurements, were insignificantly different (p>0.05) between boys and girls. Table 4-2 shows the anthropometric measurements by sex of the study population.

Table 4-2: Anthropometric measurements by sex of the study population (Mean±SD)

| Sex | Weight (kg) | Length (cm) | MUAC (cm) | Head Circumference (cm) | Triceps (mm) | Biceps (mm) | Subscapular (mm) | Suprailiac (mm) |
|---------|------------------|-----------------|-----------------|-------------------------|---------------|---------------|------------------|-----------------|
| | N=611 | N=612 | N=614 | N=613 | N=160 | N=161 | N=160 | N=160 |
| Male | n 317 9.2±1.7 | 318 74.0±5.8 | 319 14.7±2.3 | 319 46.1±2.1 | 87 7.4±2.6 | 88 5.8±2.6 | 87 6.6±1.7 | 87 7.0±2.9 |
| Female | n 294 8.8±1.5 | 294 72.9±5.7 | 295 14.3±1.2 | 294 45.1±2.0 | 73 7.4±1.8 | 73 5.4±1.6 | 73 6.9±1.7 | 73 6.8±2.4 |
| P-Value | 0.002 | 0.019 | 0.027 | <0.001 | 0.891 | 0.355 | 0.172 | 0.760 |

The prevalence of underweight, stunting and wasting together with the mean Z-scores for weight-for-Age (WAZ), height-for-age (HAZ) and weight-for-height (WHZ) for this population are shown in Table 4-3. The overall prevalence of underweight was low. Although not significant, the prevalence of underweight doubled in the 12-23 month age group from 2.5% to 5.4% and remained nearly the same thereafter. The percentage of stunted children increased as the age increased as was with wasting. There was a significant difference between boys and girls for WAZ and LAZ as the ages increased (P=0.001 and <0.001 respectively). The mean (SD) combined WAZ, HAZ and WHZ for the whole group were -0.62±1.30,-1.02±1.63,-0.10±2.6 respectively.

Table 4-3: Prevalence of underweight, stunting and wasting among the children

| Age group(mo) | n | Underweight (%) | WAZ(SD) | Stunting (%) | HAZ(SD) | Wasting (%) | WHZ(SD) |
|---------------|-----|-----------------|-------------|--------------|-------------|-------------|-------------|
| 6-8 | 122 | 2.5 | -0.36± 1.26 | 8.3 | -0.41± 1.63 | 0.8 | -0.08±1.20 |
| 9-11 | 123 | 5.4 | -0.59± 1.36 | 11.4 | -1.14± 1.70 | 1.1 | -0.01± 1.24 |
| 12-23 | 369 | 5.5 | -0.85± 1.29 | 16.3 | -1.44± 1.56 | 3.0 | -0.19± 1.35 |
| Total | 614 | 13.4 | | 36 | | 4.9 | |

Figure 4-1 shows the mean Z scores by age of the study population. At around 6 months, the weighted mean HAZ and WAZ were just below WHO reference median (WHO, 2006). There is however a subsequent sharp decrease in mean Z-scores that continued until the age

of approximately 18 months after which the decrease slowed down and the mean Z-scores levelled off, running parallel to, but substantially below, the reference line. A similar pattern was seen for weight-for-height, with the exception that the mean Z-score values stabilized around the reference median.

The prevalence of stunting was 35.7% overall and showed a downward trend already at the age of six months onwards, peaking in the 18–24-month-old age group. The overall prevalence of underweight was 13.4% and showed a similar age-related pattern to that observed for stunting. Severe wasting was rare ($WHZ < -3$) and may have been also due to the inclusion criteria for the survey. WAZ was significantly predicted by the age, marital status and education level of the caregivers ($P= 0.001, 0.003$ and 0.003 respectively) while HAZ was significantly predicted by education level of the caregiver ($P=0.005$)

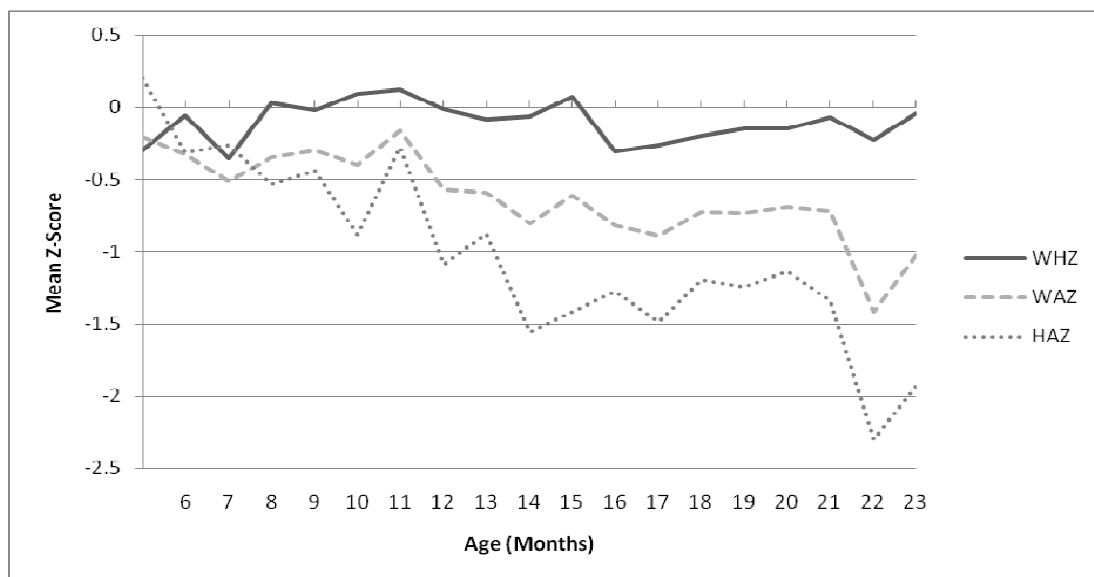


Figure 4-1: Mean Z-scores by age; weight-for height; weight-for-age; height-for-age. The horizontal line at the Z-score value of 0 represents the median Z-score of the reference population. The World Health Organization, 2006 reference was used to calculate the Z-scores.

4.3.3 Complementary feeding practises

Although 83.6% were still breastfeeding at the time of the survey, complementary feeding was introduced at an average of 3.8 (SD 0.3) months. Among those that had introduced other milks apart from breastmilk, 98% used cow milk as the alternative. Commercial

complementary foods were rarely used at only 2.4% and in all the cases it was provided by the health facility for the infant. Most mothers (65.7%) introduced complementary foods mainly in form of porridge from the local cereals (mainly maize) and legumes-either used singly or blended in no specific ratio, with a few cases giving milk, milk tea and black tea, soups (either vegetable or fish-mainly *dagaa*/meat/eggs) and pieces of *ugali*, cooked bananas and rice. Sugar and oil/fat were widely reported among the ingredients used in preparing the complementary foods. In many cases (64.3%), other solid foods used seemed to mirror whole family diet at the time. Fruits were rarely (17.8%) consumed by the children while kales, cabbage, pumpkin leaves and cowpeas leaves mixed with *mrenda* were the main vegetables consumed.

4.3.4 Dietary intake

The energy intake from the traditional complementary foods were 1.8 ± 0.5 , 2.2 ± 0.4 and 2.6 ± 0.8 MJ/day for age ranges 6-8, 9-11 and 12-18 months respectively. The energy intake from breastmilk has been given as 1.7, 1.6 and 1.4 MJ/day respectively by Dewey and Brown, (2003) for the same age ranges. Taking the average iron absorption from non-breast milk to be 4.2% from the range given (Lynch and Stoltzfus, 2003; Cook et al., 1997) of 2.4-6% for iron absorption for maize-based meals, the daily iron, calcium and zinc intake from the complementary foods were 0.4 ± 0.2 mg, 130.0 ± 90.0 and 2.0 ± 0.6 mg respectively for the age range 6-18 months. Vitamin A intake was 205.1 ± 100.0 μ g per day for the same age range.

4.3.5 Morbidity

In the week prior to the survey, 48.3% of the children had experienced moderate illness that required drugs or visits to the clinic to see a nurse as reported by the caregivers. About 30% had experienced watery loose diarrhoea with 53% experiencing both a cough and a fever

within the same recall period. There were no cases of thin flaky skin texture, visible severe wasting, light thin hair colour or bipedal oedema.

4.4 DISCUSSIONS AND CONCLUSION

This study set out to determine the prevalence of malnutrition in the resource limited malaria endemic Kakamega Sub-county region of Western Kenya. About a third of the infants and young children 6-24 months of age were stunted, about one tenth underweight and one in twenty were wasted. The levels of prevalences are considered high as per the WHO 2006 classification of worldwide prevalence ranges for the levels of stunting and underweight. This is however a slight improvement from the situation 10 years before when a study found up to 44.7% of preschool children were stunted, 10.4% were wasted and 27% were under-weight in the same Sub County (Mbagaya et al., 2004). According to Mbagaya et al. (2004), stunting was more prevalent (26.1%) among children from households whose main enterprise was sugarcane farming and where men controlled income from this enterprise. Households keeping dairy cattle as additional farming activities had lower stunting prevalence among children and were better off in terms of food security, increased milk consumption and improved nutritional status especially of the young children (Mbagaya et al., 2004). This could have been due to an enhanced dietary intake measured by a diversity score of 6 compared 4 in those households without the dairy animals.

As observed globally, there was doubling in prevalence of stunting from the first to the second year of life in these children. Moreover, the prevalence of 4.1% for wasting in the 12-24 months age group should serve as a warning sign as the WHO recommends a prevalence of more than 4% wasting in children under five to be considered high (De Onis et al., 1993).

The prevalence estimates are comparable with 2008-09 KDHS for the counties in the region (KDHS, 2008-09). The similarity in the age pattern of the HAZ and WAZ, and the low prevalence of wasting, suggest that deficits in weight-for-age, which is influenced by both

acute and long term processes, were more closely related to long-term processes. The Z-scores were however decreasing already at 6 months and continued to decrease until they reached their nadir at approximately 18–22 months and remained low until the age of 24 months. This age pattern in mean Z-scores is consistent with other reports from sub-Saharan Africa (WHO, 2012) and could have been due to materno-foetal factors. Analysis of the distribution curves for the HAZ and WAZ illustrated a downward shift of the entire distribution curve; i.e. the impact on protein energy nutritional status was a generalized phenomenon and was not restricted to children at risk, but also included children not classified as malnourished. Stunting is a measure of long term deficiencies indicating previous or ongoing nutritional deficiencies (Brown et al., 1995; Hodge et al., 1991; WHO, 2006). This increased prevalence persisted throughout the age range studied. Martorell (1990) argues that once present, stunting remains for life and that there is no catch-up growth. This is supported by Schroeder (1995) and the WHO (2008) with the first 2 years of life identified as the “window of opportunity” for catch up growth (the first 1000 days). If stunting is not reversed it will lock children in a lower growth trajectory with a lower potential for future growth (Martorell, 1995; Grantham-McGregor et al., 1996; WHO, 2008). Hence the importance of addressing nutritional deficiencies at a very young age (Castello, 1989). This suggests that most children did not reach their maximum growth potential and that interventions should be directed at all children in the age range at risk, not just those already considered malnourished. It also suggests that mean Z-scores, rather than the prevalence of stunting or being underweight, should be the primary end points to assess the efficacy of population based interventions.

The high prevalence of stunting reflects the compromised overall health in this population, which is consistent with its very high infant and under five mortality rates (McElroy et al., 2001). There is a strong association between the severity of weight-for-age

deficits and mortality rates, but even mild malnutrition, which is much more common, augments case-fatality rates of disease. This synergism has been found to be strongest in populations with high morbidity and malnutrition (Pelletier et al., 1993; Lartey et al., 1999). Although determination of the main causal factors for stunting and underweight was beyond the scope of this work, it was noted that the time frame of the greatest decrease in mean Z-scores found in this study (6–18 months of age) coincides with the time of weaning (83% by 4 months of age), as well as that of the peak burden of malaria morbidity and mortality in a nearly similar population (Aidoo et al., 2002; Noor et al., 2009) and overlaps with the highest risk period for iron deficiency (Hallberg, 2001).

This study thus demonstrates a high overall prevalence of stunting and underweight in this poor rural area with intense malaria transmission. Although there could have been seasonal variation influence and especially because the study population was not randomly obtained, one could argue that the most probable cause for these deficiencies would be explained by undesirable feeding practices during infancy and early childhood as observed from the energy and key nutrients intake data. Although majority of mothers in this area encouraged breastfeeding, additional milk feeds and solid foods were introduced at an early age as also confirmed in other studies in the same area (Mbagaya, 2009). In this community the first food given to infants is usually maize meal porridge as in other resource similar communities (Faber et al., 1997) which contains inadequate energy and micronutrients if used on its own and given that children consumed a limited variety of foods, this needs to be addressed.

The contributing role of diarrhoea to malnutrition should not be underestimated. From this study up to 30% experienced watery diarrhoea within the week of the study. The water for household use is collected from the unprotected streams, rivers and untreated shallow wells. This makes the water a potential health hazard given that very few mothers treated

water before use increasing the risk of diarrhoea. From information gathered during the survey it was clear that the treatment of diarrhoea was also not sufficient as only 48.3% combined sought treatment of all illnesses experienced with 30% having watery diarrhoeas within the same period. This is a poor community and mothers have a low educational level, both major determinants of malnutrition (Tonglet et al., 1992; Ngare and Muttunga, 1999). This emphasises the need to prevent severe stunting and its associated consequences. Given both the acute and long-term consequences of malnutrition in this vulnerable age group, community-based interventions such as complementary feeding education aimed at reducing child malnutrition in such populations should focus on all children less than two years of age.

4.5 ACKNOWLEDGEMENTS

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4.6 CONFLICT OF INTEREST

None of the authors had a conflict of interest to disclose.

4.7 FUNDING

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CHAPTER FIVE

5. EFFECTS OF IMPROVED, CENTRALLY PROCESSED COMPLEMENTARY FOODS ON GROWTH OF INFANTS AGED 6-15 MONTHS IN A RURAL SETTING, WESTERN KENYA- A RANDOMIZED, COMMUNITY-BASED TRIAL

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ABSTRACT

Background: Traditional complementary foods mainly based on staple cereal-legume blends and served mainly as gruels are used as the primary complementary food in Kenya. Due to low energy and nutrient density of the gruels they have been implicated in the high prevalence of child malnutrition. Corn Soy Blend plus (CSB+), a cereal-legume blend developed by the World Food Program has been promoted as an alternative in food insecure populations as a supplement for children with moderate acute malnutrition without evidence that it works. Animal source foods (ASFs) improve the bioavailability of nutrients from complementary foods. Since ASFs like meat are expensive, alternative sources as edible termites and *dagaa* small fish can be cheaper and more accessible thus useful in addressing undernutrition.

Objective: To assess the effect of CSB+ and 2 locally formulated and improved based on edible termites and *dagaa* small fish and multi-nutrient fortified centrally processed complementary foods on the length and weight gain of infants and young children in resource limited setting, Western Kenya.

Design: Infants were randomly assigned to receive from 6mo for 9 months of age 1 of the 3 study foods as: 50 g/d for 6-8 months old, 75 g/d for 9-11 months old and 125 g/d for 12-15 months old. The foods were: WINFOOD Classic (WFC) with edible termites (10%) and *dagaa* small fish (3%); WINFOOD Lite (WFL) with vitamins and minerals premix and CSB+. Dietary and morbidity data based on maternal recall and anthropometric data were collected monthly.

Results: Over 98% of the children were still breastfeeding at 6 months of age although complementary foods were already introduced around a mean age of 3.5(SD 3.0) months. The mean length of the infants at 6 months of age was 65.9 (SD 2.9) cm, 65.3(SD 2.8) and 65.1 (SD 3.0) for infants in the WFC, CSB+ and WFL respectively (p=0.05). The mean length gain between 6 and 15 months was 9.5 (SD 2.4) cm, 9.8 (SD 2.0) cm and 9.7 (SD 2.2) cm in WFC, CSB+ and WFL groups, respectively (p = 0.39). The difference in length were however not significant at 15 months (p= 0.09). There were no significant differences in weight among the groups at 6 and 15 months (p = 0.35).

The energy intakes at 6 months were 380.0±75.5, 336.0±77.2 and 351.0±52.0 kcal for WFC, CSB+ and WFL respectively (p=0.61). At 15 months the energy intake was 465.1±33.8, 427.0±35.4 and 410.0±23.7 kcal respectively (p=0.36). Coughing, audible wheezing and running nose were significant across all the food groups in the first 2 months (p=0.02, 0.01 and 0.01 respectively). Only a small proportion of mothers fully adhered to WINFOOD regimen across all three arms (14.8% - 19.4%).

Conclusions: All the 3 improved complementary foods improved growth with no significant difference between the foods.

KEY WORDS: Complementary Feeding, Infant Growth, Western Kenya, CSB+, WINFOOD, Edible Termites, Grain Amaranth, *Dagaa* Small Fish, Indigenous foods.

5.1 INTRODUCTION

Malnourished children have poor cognitive performance that ultimately compromise their ability and overall development resulting in loss in physical productivity and loss of resources from increased healthcare costs (Stein et al., 2010; Kuzawa et al., 2012; Carba et al., 2009; Hoddinott et al., 2008; World Bank, 2006). Reaching an acceptable nutritional status is a fundamental prerequisite to improve educational attainment (MDG 2) (World Bank 2006; Stein 2008; Martorell 2010; Waage 2010) and interventions aiming at reducing malnutrition have the potential to reduce poverty and to develop national economies (MDG1) (World Bank, 2006; Waage, 2010; UNICEF, 2013).

More than 2.5 billion people in Africa and Asia eat insects and collecting edible insects for human and livestock feeding is a potential income generating activity in the rural areas (FAO, 2010). Edible insects are highly nutritious and are increasingly becoming a healthy food source with high fat, protein, vitamin, fibre and mineral content comparing well with other forms of protein such as beef, fish and poultry (Quinn, 1959; Dufour, 1987; Gullan and Cranston, 2000). **Tables 5-1 and 5-2** show the proximate composition and the mineral content respectively of some species of edible termites from Western Kenya. There is also a relatively small ecological footprint of farming insects compared with farming conventional livestock (Ooninx et al., 2010; Ooninx and de Boer, 2012).

In Kenya, almost all the edible insects are traditionally collected from their habitat in the forests using traditional methods and one most seasonally collected edible insect in Western Kenya is termites (Ayieko, 2007; Ayieko et al., 2010). Abila et al 2003 reports that '*dagaa*' has been consumed as a source of protein among the low and medium income fish consumers in Kenya since many local people can afford to buy it (LVEMP, 2005). Between 89-95 percent of rural households consume *dagaa* (Abila and Jansen, 1997; Abila et al., 1998)

mainly as an important source of animal protein in their diet (Abila, 2003). Its high protein content and the mineral rich skeleton composition important especially those likely to be in danger of facing micronutrients malnutrition (Bille et al., 2006). Given that edible insects and *dagaa* have high quality proteins, minerals and even polyunsaturated fatty acids among other nutrients (Van Huis et al., 2013; Rumpold and Schlüter, 2013; Kinyuru et al., 2013), incorporating them in complementary foods for IYC will be of interest due to the increasing interest in insects as alternative animal source foods especially in resource limited settings.

Table 5-1: Proximate composition of the edible winged termites (g/100 g). Adapted from Kinyuru et al, 2013

| Termite | Moisture | Protein ¹ | Fat ¹ | Total Ash ¹ | Dietary fibre ¹ | Available Carbohydrate ¹ |
|--|-------------|----------------------|------------------|------------------------|----------------------------|-------------------------------------|
| <i>Macrotermes subylanus</i> ; dewinged | 6.50 ± 0.02 | 39.34 ± 0.12 | 44.82 ± 2.89 | 7.58 ± 0.05 | 6.37 ± 1.18 | 1.89 ± 0.76 |
| <i>Pseudocanthotermes militaris</i> ; dewinged | 5.04 ± 0.15 | 33.51 ± 0.85 | 46.59 ± 2.13 | 4.58 ± 0.06 | 6.59 ± 0.07 | 8.73 ± 1.87 |
| <i>Macrotermes bellicosus</i> ; dewinged | 5.13 ± 0.18 | 39.74 ± 0.61 | 47.03 ± 1.04 | 4.65 ± 0.09 | 6.21 ± 2.04 | 2.37 ± 0.98 |
| <i>Pseudocanthotermes spiniger</i> ; dewinged | 8.76 ± 1.61 | 37.54 ± 0.12 | 47.31 ± 0.13 | 7.22 ± 0.38 | 7.21 ± 0.44 | 0.72 ± 0.01 |

Values are mean ± SD; n=6

¹Values are on dry weight basis

Table 5-2: Mineral composition of the edible winged termites (mg/100 g). Adapted from Kinyuru et al, 2013

| Termite | Calcium | Iron | Zinc |
|--|--------------|---------------|--------------|
| <i>Macrotermes subylanus</i> ; dewinged | 58.72 ± 1.29 | 53.33 ± 1.46 | 8.10 ± 2.80 |
| <i>Pseudocanthotermes militaris</i> ; dewinged | 48.31 ± 7.09 | 60.29 ± 1.11 | 12.86 ± 0.92 |
| <i>Macrotermes bellicosus</i> ; dewinged | 63.60 ± 6.53 | 115.97 ± 3.46 | 10.76 ± 1.93 |
| <i>Pseudocanthotermes spiniger</i> ; dewinged | 42.89 ± 1.75 | 64.77 ± 2.66 | 7.10 ± 1.82 |

Values are mean ± SD on dry weight basis; n = 6

In Kenya, the traditional complementary foods are mainly from the staple cereal-legumes blends whose availability, minimal cost, and ease of preparation make them the preferred complementary food for the majority (Mbagaya, 2009). However, like many traditional complementary foods in developing countries, they have low energy and nutrient density and have been implicated in the development of IYC malnutrition since starch-based complementary feeding provides inadequate protein and micronutrients (WHO, 2008).

In an effort to improve the management of acute malnutrition, currently the WFP is advocating for use of Corn Soy Blend plus (CSB+), a cereal-legume blend of corn and soybeans with a pre-mix of micronutrients added, as an alternative and especially as a supplementary food particularly for children with moderate acute malnutrition. Although CSB+ is an improvement over the traditional complementary foods with respect to its energy and protein density, it has high phytate and fiber contents (estimated phytate-iron and phytate zinc molar ratios of 7.3 and 17, respectively), which may impair iron and zinc absorption (Gillooly et al., 1993). Moreover its efficacy has not been tested before. In addition, it is low in protein of high quality-mostly the animal source foods (ASFs). Inadequate complementary feeding leads to stunting of linear growth and long term health consequences with prevalence of stunting at 18 months at about 35% (KDHS, 2008-09) in Kenya. Traditional ASFs are expensive therefore a novel substitute can be the edible termites that are already a staple in the diet in Western Kenya and almost all in the population consume edible termites including children. This study therefore aimed to assess the potential of using edible termites and *dagaa* to improve the nutrition composition in complementary foods thus improve growth of Kenyan infants 6-15 months of age.

5.2 MATERIALS AND METHODS

5.2.1 Study site

The study was based at the Child Welfare Clinics in Western Kenya, Mumias Sub-county of Kakamega County in three health facilities namely Makunga, Khaunga and Lusheya as detailed in Konyole et al. (2012). Briefly, the study sites were chosen since they constitute resource limited settings and the food ingredients used to prepare the complementary foods are already widely consumed in the locality.

5.2.2 The study participants and recruitment

All infants from the age of 6 months of age were eligible to be included in the study and those included were supplied with the improved complementary foods for 9 months until they were 15 months of age. Mothers and infants (6 months old) pairs were recruited in the study as they came to Child welfare clinics at Khaunga, Makunga and Lusheya for diphtheria, whooping cough and tetanus vaccination.

The **inclusion criteria** for infants at 6 months were for mothers accepting and ready to:

- 1) Prepare and feed their infants with the improved complementary foods and CSB+
- 2) Stay in the study area for the next 9 months
- 3) Consent to participation

Twins were recruited into the study if both were healthy and randomized to receive the same intervention to avoid contamination that could have resulted from confusing the foods during feeding and sharing.

All infants had to weigh at least 2500g at birth and have MUAC >11.5 cm and WHZ > -3.

Exclusion criteria

Children with WHZ < -3, MUAC <11.5 cm, and/or bilateral pitting oedema, or with anaemia (Hb < 80 g/L) or clinical signs of vitamin A deficiency (xerosis or Bitot spots) were excluded and referred for treatment. Infants with chronic illness requiring medication, genetic disorders interfering with normal growth were also excluded, and given CSB+ as substitute together with referral for care by qualified medical personnel.

5.2.3 Study Design

This was a community-based double blind randomized trial in which infants were individually randomized in 6 blocks at 6 months of age to receive one of the three study foods, namely: WFC, WFL or CSB+ for 9 months from January 2012 to January 2013. The

study was designed to assess the effects of improved complementary foods on growth in children 6 to 15 months old.

5.2.4 The intervention

5.2.4.1 Description of Foods

Details of recipe formulation, acceptability and safety of the study foods are discussed elsewhere (Konyole et al., 2012; Kinyuru, 2012). The complementary foods were all acceptable and were industrially processed by extrusion cooking at the Kenya Industrial Research and Development Institute (KIRDI), Nairobi and packed in opaque food grade plastic containers labelled with computer generated random numbers corresponding to WFL, WFC and CSB+. Two types of complementary foods (“WINFOOD Classic” (WFC) and “WINFOOD Lite” (WFL)) based on grain amaranth and maize had been developed. WFC had edible termites (10%) and *dagaa* (3%) which are rich sources of iron and zinc (Kinyuru et al, 2013; Van Huis et al., 2013) with the germinated grain amaranth to enhance micronutrients bioavailability (Kinyuru, 2012). WFL did not contain the ASFs but the micronutrients bioavailability was enhanced by germinating the grain amaranth and fortified with micronutrients (vitamins and minerals premix) at the same rate as CSB+ (WFP, 2010). The minerals and vitamin premix and tricalcium phosphate were added to the blends (where applicable) after extrusion cooking to avoid vitamin losses at high processing temperatures. **Table 5-3** shows the formulations of the study foods (WFC, WFL and CSB+ respectively) while **Table 5-4** shows the theoretical nutrients composition of all the foods.

Table 5-3: Formulations for WFC, WFL and CSB+- percentage (w/w)

| | Germinated amaranth | Whole maize | <i>Dagaa</i> fish | Termite | Soybean oil | Sugar | Premix 1* | Premix 2** | Whole soybean |
|-------------|------------------------|----------------|----------------------|---------|----------------|-------|--------------|---------------|------------------|
| WFC | 71.00 | 10.40 | 3.00 | 10.00 | 0.60 | 5.00 | 0.00 | 0.00 | 0.00 |
| WFL | 82.52 | 10.22 | 0.00 | 0.00 | 0.59 | 4.91 | 0.20 | 1.56 | 0.00 |
| CSB+ | 0.00 | 74.24 | 0.00 | 0.00 | 0.00 | 5.00 | 0.20 | 1.56 | 19.00 |

*Mineral/vitamin premix - FBF-V-10

**Mono-calcium phosphate and Potassium chloride

Table 5-4: Nutrient composition of WFC, WFL& CSB+ per 100g of dry weight

| | Moisture | Energy | Protein | Fat | Fe | Zn | Ca |
|------|----------|--------|---------|--------|---------|---------|---------|
| | g/100g | Kcal | g/100g | g/100g | mg/100g | mg/100g | mg/100g |
| WFC | 8.7 | 423.6 | 19.1 | 12.3 | 12.2 | 6.3 | 48.2 |
| WFL | 9.4 | 407.2 | 14.6 | 9.0 | 12.5 | 5.5 | 139.4 |
| CSB+ | 9.7 | 391.7 | 15.1 | 5.8 | 7.7 | 5.1 | 141.9 |

All the study foods (WFC, WFL and CSB+) were given to the children in monthly rations adjusted to the age of the child; children in the age-group 6-8 months, 9-12 months and 13-15 months received 50g/day, 75g/day and 125g/day respectively based on the recommendations of Dewey and Brown, (2003). The food ration was a complement to breast milk and other foods. The daily rations packets were delivered in monthly rations followed with instructions to mothers/caregivers that the food was not to be shared with other young children not in the study and on how to correctly measure the quantities for daily use. An intervention regimen compliance study was also done once mid-way the intervention study in a sub-sample of 254 participants to confirm compliance with the prescribed feeding regime.

5.2.5 Randomization

Individual randomization stratified by sex was used with the children randomized to receive WFC, WFL and CSB+ when the infant was 6 months old. The study included the children with MUAC \geq 11.5 cm. There were an equal number of boys and girls recruited. Random numbers used in the labelling were generated using Microsoft Excel™.

5.2.6 Allocation concealment, blinding and follow-up

Blinding was achieved by assigning secret barcodes to the foods. To ensure complete blinding, there were 2 different codes for each group resulting into a total of 6 codes each in separate envelopes for boys and girls. The key of codes were kept in a sealed envelope until

preliminary analyses were completed. The list of random numbers assigned to the treatments was kept away from study team who distributed the foods to mothers. The staffs assessing the outcome were unaware of treatment allocation as well as the recipients at the time of distribution, because the packaging was similar for all three foods. Participants who defaulted were followed up and accommodated during another session. Some infants were lost to follow up due to exit from the study area, death or unwillingness to continue with the study by the caregiver. **Figure 5-1** shows the flow chart of the recruitment and follow-up of subjects detailing the loss to follow ups.

5.2.7 Training and quality assurance

The research assistants were trained until they were able to carry out the standard operating procedures of data collection. A pre-test was also done at the end of the training by the researcher. Quality checks were done daily to ensure correct filling of the information.

5.2.8 Data collection methods

5.2.8.1 Socio-demographic and dietary intake data

At baseline, the general demographic and socio-economic information for the family was collected. Dietary intake measurement was conducted monthly using a 24-hour mother recall method to estimate the amount of food eaten. Energy and nutrient intakes from the foods were calculated by using Food Processor PlusTM software (ESHA Research, Salem, OR, USA) as detailed in Lartey et al., 1999. The calculation was based on local and regional food-composition tables (Sehmi, 1993; Lukmanji et al., 2008) and values published elsewhere (Dewey and Brown, 2003).

An average breast milk intake (565 g/d) was assumed based on average breast milk energy intakes in developing countries of 413 kcal and 379 kcal, respectively for infants 6-8 months and 9-11 months old and, 346 kcal for young children 12-23 months old (Dewey and

Brown, 2003; Owino et al. 2007a). Data for iron absorption from complementary foods and breast milk and the recommended dietary allowance (RDA) for absorbable iron were taken from the WHO estimates (Lynch and Stoltzfus, 2003). Iron absorption of about 6% from maize-based diets was assumed. Daily amounts of absorbable iron obtained from breast milk were assumed to be 0.11mg/day and 0.09 mg/day for children 6-12 months and 13-18 months, respectively (Lynch and Stoltzfus, 2003). The daily intake of calcium from breast milk was assumed to be 130 mg, the estimate for children 6-23 months old, and calcium retention from breast milk and solid foods was assumed to be 50% and 20-25%, respectively (Lutter and Dewey, 2003).

5.2.9 Compliance to the intervention regimen

Compliance to food intervention may be defined as adherence to the intervention regimen including recommended daily dose and frequency and method of food preparation and feeding (WHO, 2008). The role of compliance in efficacy trials is important and failure to comply may be due to sharing or non-acceptance of intervention among others. Compliance data was thus obtained by asking the mother how much of the WFC, WFL and CSB+ were consumed by the child while checking for any spoilages, spillages, adequacy in amounts measured and the frequency of feeding among others. The degree of sharing of the assigned diets with other household members was also assessed by interviewing the mother at the health facility during the monthly visits and by making home visits. The mothers were asked to keep all the distributed packets after these were empty so they could be collected and counted on a monthly basis.

5.2.10 Morbidity

Morbidity data were collected by asking mothers about specific symptoms and clinic visits in the past seven days. Mothers were continuously encouraged to bring their children

to the clinic in the event of severe illness prior to the next visit. Generally, morbidity data were collected by assessing overall morbidity in the last month (scored as healthy; mild, self-limited illness; moderate illness requiring symptomatic treatment at the clinic; severe illness requiring antibiotics or other medical intervention) for IYC during their monthly visits to clinic using questionnaires.

5.2.11 Anthropometry

Anthropometric measurements were carried out monthly by the trained assistants who had previous experience in growth monitoring at the clinic's Maternal and Child Health department. Measurements of growth (nude weight and recumbent length) and body composition (subscapular skinfolds, head and mid-upper arm circumferences) were made in triplicate using standardized anthropometric techniques and calibrated equipment as detailed in Lohman et al. (1988). In order to minimize inter-observer variation in measurements each assistant took daily measurements of weight, height, circumferences (mid upper arm) and skinfolds (triceps, biceps, subscapular and suprailiac) of the same volunteer until these agreed within the allowable error margin during the training period.

Length was measured to the nearest 0.1 cm using calibrated length board with the child lying down on a length board and two trained staff measuring the child supported by the mother. Weight was measured to the nearest 0.01 kg, using Seca-UNICEF scales (UniScale). The Triceps, biceps, subscapular and suprailiac skinfolds were measured to the nearest 0.1 mm using Happender skinfold calipers (Crymych, United Kingdom) while the head and mid-upper arm circumferences (MUAC) were measured to the nearest 0.1 cm using non-stretchable measuring tape. Health personnel then examined the child for different symptoms which could be related to diseases and malnutrition as the child sat with the mother throughout the examination.

5.2.12 Sample size consideration

According to the data on the standard deviation (SD) of the increase in length, Dewey et al. (2011) observed differences in prevalence between interventions for stunting at 0.5 SD in LAZ while Lartey et al. (1999) observed length gain of 0.1(SD 1.2) cm. Since the primary outcome was length, the sample size n per arm was 165 resulting to a total of 495 including compensation for 10% loss to follow up observed in similar studies elsewhere (Faber et al., 2005).

5.2.13 Data analysis

Case record forms were checked daily and entered within 2 weeks. The initial analysis was done before breaking the code. Quantitative data was double entered with length and weight measurements converted to Z-scores using WHO Anthro™ v3.2.2 (WHO, 2009). Frequencies, means and median values were calculated using SPSS™ software version 19 (Chicago, Illinois, USA). Analysis of variance (ANOVA) was done to examine differences in baseline characteristics between the 3 study groups and to determine any significant differences occurred between the intervention groups in length and weight gain from 6 to 15 mo. with the independent variables being intervention group and values at 6 mo of the respective dependent variable. Baseline characteristics between the pooled intervention groups and the pooled cross-sectional study infants were compared by using t tests and chi-square tests while growth status (weight-for-age and length-for-age) of the pooled intervention and pooled cross-sectional study infants were compared at each age (6–15 months) using Student's t tests. The differences in baseline characteristics and the potential influence of seasonality were controlled for at every analysis stage using multiple regression analysis.

Stunting, underweight and wasting were defined as length-for-age, weight-for-age and weight-for-length, respectively, < -2 standard deviations of the WHO reference standards

(WHO, 2006). Moderate-to-severe and severe malnutrition in infants were defined as MUAC < 12.5 cm and MUAC < 11.5 cm, respectively (Cogill, 2003).

5.2.14 Ethical considerations

Written and oral information were given to the caregivers of all eligible children in the local language before obtaining written consent. All data obtained in the study were kept anonymous while mother had an option to discontinue from the study at anytime while still receiving the monthly food ration and other health facility services without taking part in the study. The study was cleared by the Kenyatta National Hospital-University of Nairobi Ethics Review Committee (KNH-UON ERC-P436/12/2010) and the trial was registered with the International Standard Randomized Controlled Trials; at Controlled-trials.com. No: ISRCTN30012997. Permission to implement the study was obtained from relevant local authorities in the study area.

5.3 RESULTS

5.3.1 Introduction

Five hundred and twenty seven (527) children were invited for the study, 520 were screened and 499 met the inclusion criteria and were randomized to one of the three food groups as detailed in [Figure 5-1](#). Four hundred and twenty eight children (428) equivalent to 85.7% completed the study. Of the 14% children lost to follow-up, 63 (12.6%) relocated from the study area while 8 (1.6%) died from illness. The dropout rate did not differ significantly between groups as shown in [Figure 5-1](#) ($p=0.69$).

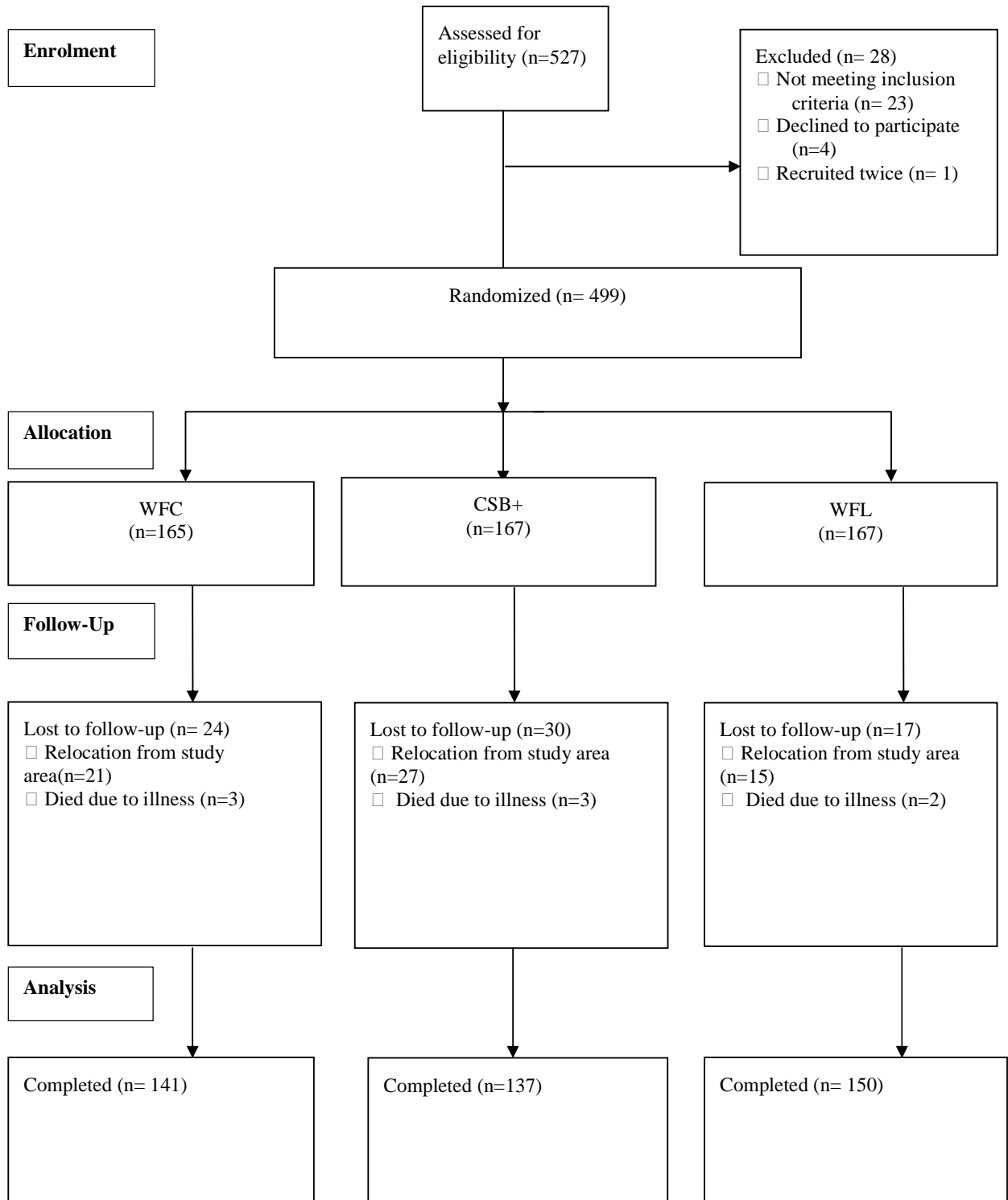


Figure 5-1: Flow chart of the recruitment and follow-up of subjects. Infants were randomly assigned aged 6mo to receive for 9months CSB+, WFC or WFL.

Table 5-5 details some of the baseline characteristics of the study participants by food groups. The distribution by sex and age was similar across the food groups thus randomization resulted in baseline equivalence. The households had a mean of 5 members and about 2 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 at 48.4%, 46.6% and 41.2% for WFC, CSB+ and WFL respectively. Generally, there were no significant differences between the intervention groups with respect to weight at 6 months neither of age, nor in household characteristics such as parental age and education, household income, and household size confirming randomization was successful.

Table 5-5: Baseline socio demographic characteristics of study participants by food group

| | WFC | CSB+ | WFL | P |
|---|------------|------------|------------|------|
| Number of children | 165 | 167 | 167 | |
| Child characteristics | | | | |
| Sex, boys, n (%) | 76 (46.1) | 83 (49.7) | 81 (48.5) | 0.80 |
| Infant birth order | 3.5±2.2 | 3.1±1.9 | 3.3±1.9 | 0.23 |
| Child age (months) | 6.1±0.2 | 6.1±0.3 | 6.1±0.2 | 0.53 |
| Currently breastfeeding, n (%) | 162 (98.2) | 166 (99.4) | 166 (99.2) | 0.25 |
| Age of introduction to food other than breast milk (months) | 3.1±2.1 | 3.1±2.0 | 3.4±2.0 | 0.87 |
| Caregiver characteristics | | | | |
| Age of main caregiver(years) | 26.4±6.8 | 25.6±6.7 | 25.3±5.5 | 0.30 |
| Education level | | | | 0.89 |
| Unable to read and write n (%) | 14 (38.9) | 15 (41.7) | 7 (19.4) | |
| Primary incomplete, n (%) | 62 (28.4) | 73 (33.5) | 83 (38.1) | |
| Primary completed, n (%) | 68 (37.0) | 50 (27.2) | 66 (35.9) | |
| High school completed, n (%) | 18 (34.6) | 26 (50.0) | 8 (15.4) | |
| University/ College graduate, n (%) | 3 (33.3) | 3 (33.3) | 3 (33.3) | |
| Total number of children delivered by the caregiver | 3.6±2.2 | 3.3±1.9 | 3.3±2.0 | 0.41 |
| Household characteristics | | | | |
| Total household members | 5.9±2.2 | 5.6±2.2 | 5.4±2.1 | 0.18 |
| Children <5 years | 1.8±0.7 | 1.9±0.8 | 1.9±0.7 | 0.48 |
| Access to protected well, n (%) | 77 (48.4) | 75 (46.6) | 68 (41.2) | 0.70 |
| Use of insecticide treated net, n (%) | 161 (97.6) | 159 (95.2) | 161 (96.4) | 0.50 |
| Primary income | | | | |
| Farming, n (%) | 90 (55.6) | 76 (47) | 72 (44) | 0.22 |

Data are mean±SD, unless stated otherwise and n (percent))

5.3.2 Anthropometric measurements

5.3.2.1 Growth

As shown in [Tables 5-6 and 5-7](#), the mean length at 6 months was 65.9 (SD 2.9) cm, 65.3(SD 2.8) cm and 65.1 (SD 3.0) cm for infants in the WFC, CSB+ and WFL respectively. There was a significant difference in length between the groups at 6 months ($p=0.05$) although this may have been due to chance. The mean length at 15 months was 75.3 (SD 3.0) cm, 75.3 (SD 3.0) cm and 74.6 (SD 3.0) cm for infants in the WFC, CSB+ and WFL respectively. The mean length gain between 6 and 15 months was 9.5 (SD 2.4), 9.8 (SD 2.0) and 9.7 (2.2) cm in WFC, CSB+ and WFL groups, respectively ($p = 0.39$). There was a trend ($p = 0.08$) towards greater length in CSB+ and WFL than in WFC groups. The length difference were however not significant at 15 months ($p= 0.09$).

The mean weight at 6 months was 7.6 (SD 1.0) kg, 7.4 (SD 1.0) kg and 7.3 (SD 1.1) kg for infants in the WFC, CSB+ and WFL groups, respectively ($p=0.12$). The mean weight at 15 months was 9.7 (SD 1.2) kg, 9.7 (SD 1.2) kg and 9.5 (SD 1.2) kg ($p=0.42$) for young children in the WFC, CSB+ and WFL groups, respectively. There were no significant differences in weight among the groups at 6 and 15 months. The mean weight gain between 6 and 15 months was 2.0 (SD 0.8) kg, 2.2 (SD 0.7) kg and 2.2 (SD 0.7) kg for infants in the WFC, CSB+ and WFL groups, respectively ($p = 0.35$). This means that there was no significant weight difference ($p>0.05$) in weight gains between 6 and 15 months of age.

Table 5-6: Selected baseline anthropometric characteristics of study participants by food group

| | WFC | CSB+ | WFL | P |
|----------------------------------|------------|------------|------------|------|
| Number of children | 165 | 167 | 167 | |
| Child characteristics | | | | |
| 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 | 65.4 ±3.0 | 0.05 |
| Weight-for-Length Z-score (WLZ) | 0.27±1.20 | 0.23±1.21 | 0.16±1.18 | 0.04 |
| Children with Z-score <-2, n (%) | 4 (2.4) | 5 (3.0) | 1 (0) | |
| Length-for-age Z-score | -0.34±1.30 | -0.62±1.18 | -0.70±1.34 | 0.04 |
| Children with Z-score <-2, n (%) | 11(6.7) | 13(7.8) | 23(13.8) | |
| Weight-for-age Z-score | -0.09±1.16 | -0.28±1.11 | -0.40±1.27 | 0.07 |
| Children with Z-score <-2, n (%) | 5(3.0) | 8(4.8) | 9(5.4) | |

Data are mean±SD, unless stated otherwise and n percent)

Table 5-7: Effects of 9 months complementary feeding on some anthropometric measurements

| | WFC | CSB+ | WFL | P value |
|-----------------------------------|-----------|-----------|----------|---------|
| n baseline | 165 | 167 | 167 | |
| n endpoint | 141 | 137 | 150 | |
| Weight, kg | | | | |
| Baseline | 7.5±1.0 | 7.4±1.0 | 7.3± 1.1 | 0.12 |
| Endpoint | 9.7±1.2 | 9.7±1.2 | 9.5±1.2 | 0.42 |
| Difference weight kg | 2.0± 0.8 | 2.2± 0.7 | 2.2 ±0.7 | 0.35 |
| Length, cm | | | | |
| Baseline | 65.9±2.9 | 65.3±2.8 | 65.1±3.0 | 0.05 |
| Endpoint | 75.3±3.0 | 75.3±3.0 | 74.6±3.0 | 0.09 |
| Difference length, cm | 9.5 ±2.4 | 9.8±2.0 | 9.7 ±2.2 | 0.39 |
| MUAC, cm | | | | |
| Baseline, cm | 14.4 ±1.2 | 14.2±1.3 | 14.0±1.2 | 0.05 |
| Endpoint, cm | 14.8± 0.2 | 14.8± 1.2 | 14.6±1.1 | 0.54 |
| Difference MUAC cm | 0.5±1.0 | 0.6±1.0 | 0.6±0.9 | 0.27 |
| Head circumference, cm | | | | |
| Baseline | 43.4±1.5 | 43.5±1.5 | 43.1±1.5 | 0.03 |
| Endpoint | 47.0±1.6 | 46.8±4.3 | 46.7±1.5 | 0.81 |
| Difference Head circumference, cm | 3.4±1.1 | 3.1 ±4.0 | 3.7±0.9 | 0.18 |

Data are presented as mean ±SD

The Length-for-age z scores did not differ significantly ($p>0.05$) between the 3 intervention groups (**Figure 5-2**). Similarly, both Weight-for-height z scores and Weight-for-age z scores were not significantly different ($p>0.05$) between the 3 intervention groups at any age between 6 and 15 months (**Figures 5-3 and 5-4** respectively). Average weight-for-age z score was slightly below the WHO reference median at 6 mo but not at 15 mo of age (WHO, 2006). These results were not altered when infant sex, energy intake from the study foods, or the total energy intake from complementary foods were controlled for. Length gain

and weight gain between 6 and 15 mo, and other anthropometric indexes (midupper arm and head circumferences, triceps and subscapular skinfold thicknesses, MAFA, and MAMA) did not differ significantly ($p>0.05$) between the 3 intervention groups (**Table 5-8**). Even though there were no significant differences in mid upper arm circumference (MUAC), head circumference, triceps, biceps, subscapular and suprailiac skinfold among the groups, at 6 months there was a significant difference in MUAC and head circumference ($p=0.05$ and 0.03 respectively) which could have been due to chance since randomisation was done at baseline. There was no significant difference ($p>0.05$) in the skinfolds measurements between 6 months and 15 months.

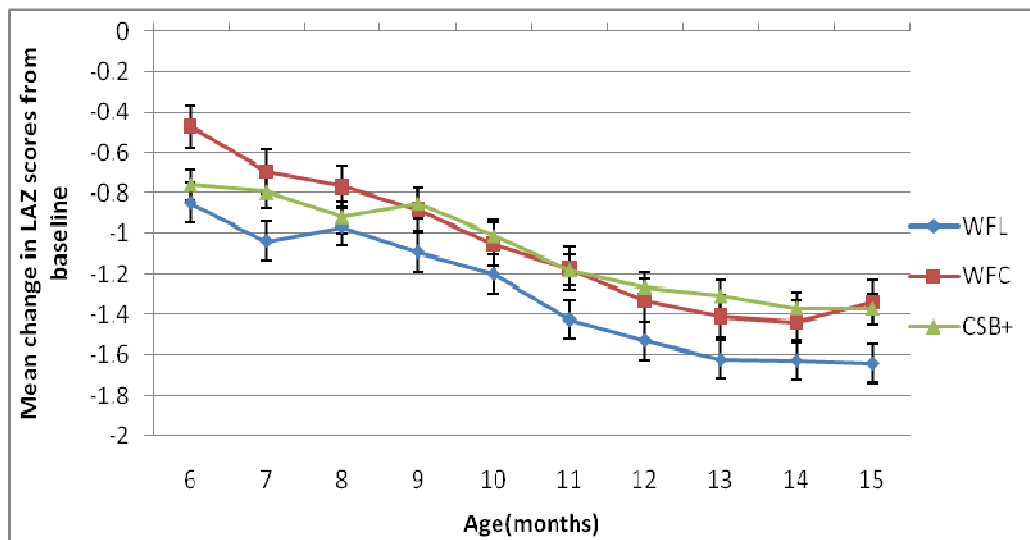


Figure 5-2: Mean LAZ from baseline (6 months) to 15 months by food

Mean Length-for-age z scores of infants in the 3 intervention groups: WFC, WINFOOD Classic, an edible termite-*dagaa* small fish-germinated grain amaranth and maize blend; CSB+, a cereal-legume blend plus vitamins and minerals; WFL, WINFOOD Lite a germinated grain amaranth-maize blend plus vitamins and minerals. There were no significant differences between groups.

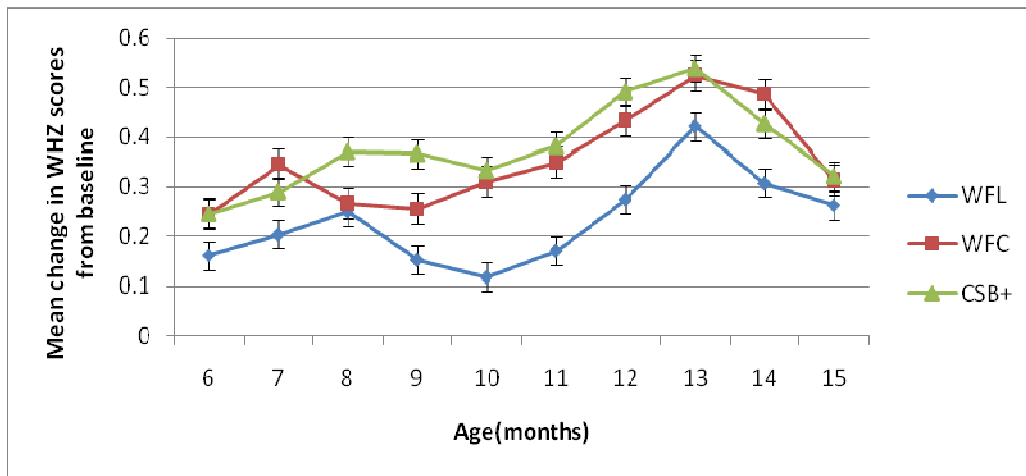


Figure 5-3: Mean WHZ from baseline (6 months) to 15 months by food

Mean weight-for-height z scores of infants in the 3 intervention groups: WFC, WINFOOD Classic, an edible termite-*dagaa* small fish-germinated grain amaranth and maize blend; CSB+, a cereal-legume blend plus vitamins and minerals; WFL, WINFOOD Lite a germinated grain amaranth-maize blend plus vitamins and minerals. There were no significant differences between groups.

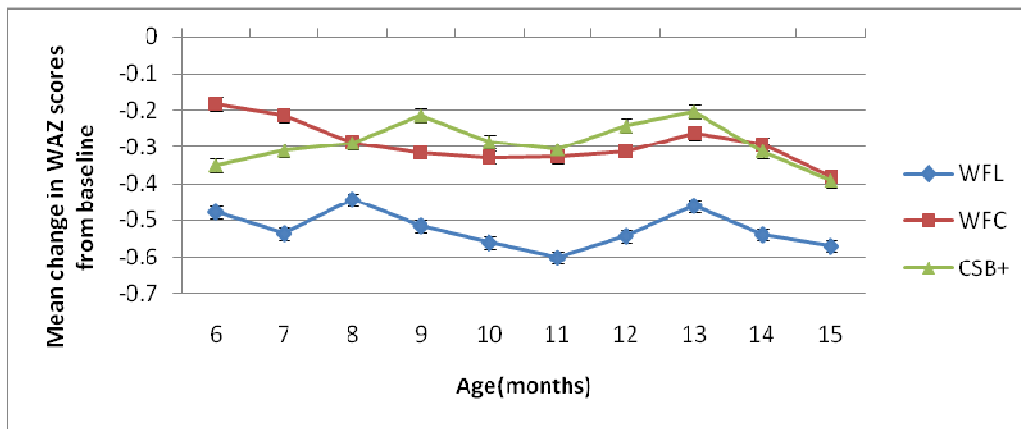


Figure 5-4: Mean WAZ from baseline (6 months) to 15 months by food

Mean Weight-for-age z scores of infants in the 3 intervention groups: WFC, WINFOOD Classic, an edible termite-*dagaa* small fish-germinated grain amaranth and maize blend; CSB+, a cereal-legume blend plus vitamins and minerals; WFL, WINFOOD Lite a germinated grain amaranth-maize blend plus vitamins and minerals. There were no significant differences between groups.

5.3.2.1.1 Body composition

Table 5-8 presents infant skinfold thickness (biceps, triceps, subscapular and suprailiac) and mid arm fat and muscle area at 6 (baseline) and 15 (endpoint) months of age. No significant differences were observed among the food groups ($p > 0.05$).

Table 5-8: Skinfold thickness and mid-arm fat and muscle area at 6mo and 15 months by food groups

| Skinfold thickness | WFC | CSB+ | WFL | P value |
|---|-----------|-----------|-----------|---------|
| Triceps, mm | | | | |
| Baseline | 8.5±1.9 | 8.4±1.8 | 8.5± 1.9 | 0.92 |
| Endpoint | 7.6±2.0 | 7.5±1.6 | 7.6±1.8 | 0.99 |
| Difference triceps, mm | -0.8± 1.7 | -0.8±2.0 | -0.8 ±2.0 | 0.96 |
| Biceps, mm | | | | |
| Baseline | 7.0±1.9 | 7.0±1.7 | 6.9±1.8 | 0.61 |
| Endpoint | 6.0±1.4 | 6.0±1.3 | 6.0±1.3 | 0.95 |
| Difference Biceps, mm | -0.9 ±1.8 | -0.9±1.9 | -0.8 ±1.8 | 0.61 |
| Subscapular, mm | | | | |
| Baseline | 8.0 ±1.8 | 8.0±1.9 | 7.8±1.9 | 0.46 |
| Endpoint | 7.0±1.7 | 6.9± 1.7 | 7.1±1.8 | 0.57 |
| Difference subscapular mm | -0.9±1.8 | -1.2±1.9 | -0.7±1.8 | 0.15 |
| Supriliac, mm | | | | |
| Baseline | 8.7±2.6 | 8.6±2.8 | 8.7±2.8 | 0.92 |
| Endpoint | 6.7±2.2 | 6.7±2.1 | 6.9±2.3 | 0.72 |
| Difference supriliac,mm | -1.9±2.8 | -1.9 ±2.7 | -1.9±2.6 | 0.89 |
| Mid Arm Muscle Area(MAMA) (mm²) | | | | |
| Baseline | 1143±230 | 1125±225 | 1140±240 | 0.56 |
| Endpoint | 1423±111 | 1489±223 | 1500±221 | 0.22 |
| Mid Arm Fat Area (MAFA) (mm²) | | | | |
| Baseline | 594±23 | 556±29 | 574±25 | 0.45 |
| Endpoint | 875±25 | 870±21 | 878±36 | 0.71 |

Data are presented as mean ±SD

5.3.3 Breastfeeding and complementary feeding status at 6 months of age

Over 98% of the children were breastfeeding at 6 months although by around 3.5(SD 3.0) months of age, exclusive breastfeeding had ceased and other foods had been introduced in the infants' diets. The age of introducing these foods however ranged from <1 –7 months (range of about 6 months) with a median value of 4 months. The main foods available for feeding infants included maize meal, rice, groundnuts, beans, fish, milk (mainly cow milk 53.5% at 6 months and 99.8% at 15 months) and fruits (bananas, oranges, pawpaw) and vegetables (pumpkins, green vegetables especially locals like *murenda*, kales and cowpeas). The child's food was determined by the food available in the household for the entire family at the time and seasonality. Maize porridge was the main form of food for infants with varying degree of other foods/ingredients added depending on availability. Twenty percent of all the caregivers reported having special designated flour for infants' porridge. In the cases where the special designated flour for infants' porridge was available, it was either locally made by blending various ingredients in unspecified proportions or the commercially made brand *FAmiLA*TM

with a variant containing citric acid which the caregivers called “sour” were mostly used. Most mothers used cup and spoon (84.6%) to feed the infants with a few mothers resorting to palm feeding. Only 3% used commercial complementary foods to feed the infants and in almost all the cases they sourced it from the health facility as part of a donation attached to some infant feeding program in the facility.

Baseline characteristics and infant-feeding practices of the intervention-study infants were compared with those of the cross-sectional study infants. The findings of the intervention study on breastfeeding and complementary feeding status together with most of the socio demographic information were not significantly different from those of the cross-section study done in the same catchment area.

5.3.4 Dietary and Energy intake

The daily energy intake from traditional complementary foods for infants at 6-8, 9-11 months and young children ≥ 12 months old were 1.6 ± 0.7 , 2.4 ± 0.5 , 2.2 ± 0.5 MJ/day respectively. The calculated energy density for the complementary feeding porridge based on the ingredients given was 2.9 ± 1.3 kJ/ml while the infants and young children aged 6-8, 9-11 and ≥ 12 months consumed about 60g, 142g and 150g respectively of food daily. Almost all the infants were reported to be breastfeeding at 6 months, assuming a daily intake of 635 g/day of breastmilk observed in other studies which was insignificantly different irrespective of the type of complementary foods the infants fed on (Owino et al. 2007a), the calculation was based on nutrient intake from complementary foods only by the food groups for energy. The energy intakes at 6 months were 380.0 ± 75.5 , 336.0 ± 77.2 and 351.0 ± 52.0 kcal for WFC, CSB+ and WFL respectively ($p=0.61$). At 15 months the energy intake was 465.1 ± 33.8 , 427.0 ± 35.4 and 410.0 ± 23.7 kcal respectively ($p=0.36$). There was no significant difference in amounts of energy received from the different solid complementary foods between the groups.

5.3.5 Morbidity

Throughout the study and across the study groups, the infants were well/alert and reported no major symptoms of disease except for coughing, audible wheezing and running nose which were significantly reported across all the food groups in the first 2 months ($p=0.02$, 0.01 and 0.01 respectively). The average respiratory rates were 35.3 ± 6.0 , 35.4 ± 7.0 and 34.9 ± 6.0 counts per minute for WFC, CSB+ and WFL respectively ($p=0.89$). Across all the study groups there were significant differences in reporting for general poor health status in the second month of the study ($p=0.01$), convulsions in the within the first month of the study ($p=0.01$), episodes of loose watery diarrhoea in the 5th month of the study and 8th month of the study ($p=0.02$ and <0.001 respectively). Constipation was also significantly reported during the 6th month of the study ($p=0.02$). These were across all the study groups.

5.3.6 Compliance to the intervention regimen

The daily frequency of feeding the infants ranging from 7-10 months of age with the foods were 3.0 ± 0.8 , 3.1 ± 0.8 and 2.8 ± 0.8 times respectively for WFC, CSB+ and WFL($p=0.09$). In all the cases the mother fed the infant over 86% of the time ($p= 0.30$) and more than 80% of the infants in all the food groups consumed their respective rations daily ($p=0.66$). Most of the mothers reported the infants liked the foods alot at 77.4%, 91.4% and 82.7% for WFC, CSB+ and WFL respectively ($p=0.55$). Only a small proportion of mothers fully adhered to WINFOOD regimen across all three arms (14.8% - 19.4%). **Table 5-9** shows the summary of the compliance of other parameters assessed. There were no significant differences among the parameters assessed across the food groups meaning the compliance to the regimen was similar across the food groups.

Table 5-9: Improved complementary foods supply and household level distribution

| | WFC | CSB+ | WFL | p |
|---|-----------|-----------|-----------|------|
| Number of days since last WINFOOD delivery | 14±8.2 | 15.2±8.2 | 13.5±8.1 | 0.55 |
| Number of WINFOOD tins received at last delivery | 4.5±0.8 | 4.4±1.0 | 4.6±0.8 | 0.73 |
| Number of WINFOOD tins unused at time of last delivery | 0.07±0.2 | 0.09±0.6 | 0.13±0.4 | 0.85 |
| Number of WINFOOD tins shared out with others apart from study infant | 0.05±0.23 | 0.05±0.26 | 0.07±0.26 | 0.95 |
| Number of WINFOOD tins spilled/damaged | 0.06±0.24 | 0.02±0.12 | 0.03±0.1 | 0.42 |
| Daily frequency for feeding child with WINFOOD | 3±0.8 | 3.1±0.8 | 2.8±0.8 | 0.09 |
| Daily amount of WINFOOD fed to the infant (g/d) | 94.9±85.9 | 90.5±41.4 | 106.7±148 | 0.74 |

5.4 DISCUSSIONS AND CONCLUSION

The study aimed to find out the impact of edible termites and *dagaa* small fish and multi-micronutrients fortified improved complementary foods on growth (length and weight) among other anthropometric measurement on infants 6-15 months. Although the study hypothesis predicted differences among the 3 intervention groups, no significant effect was observed on linear growth and weight gain although there were increases in both weight and length among the food groups compared to the cross-section group. The study found no significant difference in linear growth between infants receiving fortified and ASFs enriched complementary foods and infants in the CSB+ group. The absence of significant changes in any of the anthropometric parameters may be explained by a number of factors. Dewey et al., (2011) observed differences in the prevalence between interventions for stunting at 0.5 SD in LAZ while Lartey et al. (1999) observed length gain of 0.1(SD1.2) cm. Based on the observations, the calculated sample size of 165 per group of food was enough to detect a difference of ≥ 0.5 kg in weight gain or 1.0 cm in length gain over the 9-mo period with a power of 0.9. As it turned out, the weight gain from 6 to 15 months of age of the “control” group in the intervention (those fed CSB+) was not as high as initially anticipated, and the maximum difference observed between this group and any of the other intervention groups was only 0.3 cm and 0.2 kg. A much larger sample size per group would have been necessary to show statistical significance for a difference of that magnitude with a power of 0.9.

There are several possible explanations for the growth results. First, all subjects in the intervention were given the study foods free of charge, which may have improved overall food availability for the infants. Second, all 3 intervention foods had a relatively high macronutrient content compared with traditional complementary foods typically fed to infants in the region as shown in an earlier cross section study in the same area and by Mbagaya et al. (2004). It would have been desirable for study purposes to include a group not assigned any of the improved foods, but this was not considered acceptable ethically because of the known nutritional superiority of the improved complementary foods. Third, the attention received by all intervention infants through regular monthly visits may have influenced the mothers' childcare and infant-feeding practices, and the regular morbidity surveillance may have prompted them to seek treatment earlier for their sick infants (Lartey et al., 1999; Vijayaraghavan et al., 1992). All of the above explanations may account for the improvement in growth of the intervention groups compared with the non-intervention (cross section study data) group and the lack of significant differences in growth between the 3 intervention groups (Lartey et al., 1999). Some studies have reported positive growth effects of supplementation with micronutrients such as zinc (Brown et al., 1998; Schlesinger et al., 1992; Castillo-Duran et al., 1995; Ninh et al., 1996), iron (Chwang et al., 1988; Latham et al., 1990; Lawless et al., 1994), and vitamin A (Courtright, 1994; Muhilal et al., 1988), although others have not reported significant difference in growth between the group fed the micronutrient-fortified food and the other groups (Lartey et al., 1999). Elsewhere, a zinc supplementation study of 6 months in a similar age group by Umeta et al. (2000) observed a difference in increase in height over the intervention period between stunted groups of more than 4 cm and between non-stunted groups of 1.6 cm. Oelofse et al. (2005) in a similar study observed weight and length increase of $(2.1 \pm 0.9 \text{ kg})$ and $(10.0 \pm 1.8 \text{ cm})$ respectively. In the current study, zinc supplementation was not considered due to logistics limitations.

Breast-feeding and complementary feeding practices could also have contributed towards explaining the lack of a pronounced effect of the interventions on the outcome measures. The average age of cessation of breast-feeding was 3.5(SD3.0) months. At this age the introduction of complementary food was common and this may have influenced the actual intake of the study foods as observed in other studies of similar settings (Oelofse et al., (2005). Lack of compliance has been cited as a possible reason for lack of expected differences between intervention and control (Bisimwa et al., 2012). Without intentional follow up to measure food intake, several studies have reported little or no impact on primary outcome measures, especially prevalence of stunting (Owino et al., 2007; CIGNIS Study Team, 2010; Manno et al., 2012; Gibson et al., 2011). In cases where intensive food intake monitoring has been done there has been improved linear growth (Adu-Afarwuah, 2008). Monthly follow up was associated with improved physical activity among HIV-infected adults given a chickpea-sesame ready to use therapeutic food (RUTF) in Malawi. Additionally, the number of pots of RUTF consumed was positively correlated with weight gain and body mass index (Bahwere et al., 2009). In some circumstances where food intake has been monitored in sub-set only marginal trends towards better outcome in the intervention group has been observed (Bisimwa et al., 2012). However, intensive food intake monitoring is likely to modify habitual food intake and thus conclusions from such studies may not be generalized to cover real-life context.

The high percentage of completion rate (about 86%) could be explained by a few possible factors. There was improved relationship between the health facility staff and the study infants and this could have made the participants to adhere more. The study acceptance right from the acceptability study was very high while the recruitment was well planned with correct physical address obtained for traceability. The linkages to the community linkage structures through the community health workers also enhanced traceability. On the contrary,

the primary cause of default was the annual migration as caregivers joined their spouses or other relatives in far off locations.

In conclusion, the intervention study showed no significant difference in growth (anthropometric) indices among the study groups. This study suggests the necessity of further effectiveness studies on use of local non conventional ASFs as edible termites and food fortification in infants. The feeding of improved complementary foods to Kenyan infants between 6 and 15 mo of age most likely improved growth compared with the usual growth pattern of infants in the community. The addition of edible termites and *dagaa* small fish (WFC) supported growth just as well as did CSB+ and its equivalent made from germinated grain amaranth and maize (WFL). More research to investigate the bioavailability mechanisms of the nutrients from the edible termites is however needed to help create evidence base and enabling policy environment to support the use of the insects in such improved complementary foods.

5.5 ACKNOWLEDGEMENTS

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5.6 CONFLICT OF INTEREST

None of the authors had a conflict of interest to disclose.

5.7 FUNDING

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CHAPTER SIX

6. EFFECT OF EDIBLE TERMITES AND DAGAA SMALL FISH ENRICHED AND MICRONUTRIENTS FORTIFIED COMPLEMENTARY FOODS ON ANEMIA AND IRON STATUS OF CHILDREN IN RESOURCE LIMITED SETTING, WESTERN KENYA

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ABSTRACT

Background: Iron deficiency and anaemia are global public health problems especially in resource limited settings. Global estimates show that the highest prevalence of anaemia is among children less than 5 years of age with the prevalence rising in Africa. Maize-meal porridge is used for infant feeding in many African countries, including Western Kenya. Two improved complementary foods based on maize and germinated grain amaranth with 10% edible termites and 3% *dagaa* small fish known as WINFOOD Classic(WFC) and the other fortified with vitamin and mineral micronutrient at the rate of CSB+ referred to as WINFOOD Lite (WFL) were compared to CSB+.

Objective: To assess whether the improved complementary foods could reduce anaemia and improve the iron status of infants in a resource limited setting of Western Kenya.

Design: Infants aged 6 mo (n=499) were randomly assigned to receive WFC, WFL or CSB+ for 9 months. The outcomes were haemoglobin, soluble transferrin receptor and ferritin concentrations assessed at 6 and 15 mo. C-reactive protein (CRP) as marker of infection was also measured to allow for more accurate interpretation of iron status. An ELISA test procedure was used while retinol binding protein (RBP) as an indicator for Vitamin A (VA) was included as an outcome.

Results: At 6 mo, 496 were randomised as 164(33.1%) 166(33.5%) 166(33.5%) for WFC, CSB+ and WFL respectively while 428 completed the study. A random sub sample of this total was used for the other outcomes and 268 had at least one value (either baseline or endline value or both) at the end of the study. With reference to baseline values, there was a significant intervention effect of 0.6(SD1.6),-0.3(SD1.8) and 0.2(SD2.0) g/dl for CSB+, WFC and WFL respectively ($p < 0.001$) with respect to Hemoglobin concentration. Transferrin receptors as Ramco assay equivalents and body iron stores at 15mo were significantly different from 6 mo ($P = 0.002$ and < 0.001 respectively) with Transferrin receptors changing from 11.8(SD3.5) to 13.5(SD5.1) mg/l and Iron body stores from 2.0(SD3.8) to -0.6(SD3.7) mg/kg body weight for WFC among others. The proportion of infants with anaemia decreased from 51.8% to 42.3% overall with a larger decrease among the mild and moderate anemic groups. The other outcomes showed insignificant intervention effect including the CRP concentrations.

Conclusions: The improved complementary foods had a significant effect in reducing anaemia and improving iron status and related outcomes of infants in malaria endemic resource limited settings. Further research to investigate the functional quality of the edible termites in such foods is recommended.

KEYWORDS: Complementary foods, Animal Source Foods, infants, micronutrients, Anaemia, ferritin, iron status

6.1 INTRODUCTION

The critical period for developing childhood malnutrition coincides with the introduction of complementary foods, which in many developing countries are nutritionally inadequate (Gibson et al., 1998). Iron, zinc, and vitamin A are problem nutrients because of their low density in plant-based complementary foods common in the developing countries (Brown et al., 1998). Due to the low nutrient density and frequent infections, growth faltering and anaemia are widespread among IYC in the developing world below 24 mo old (Shrimpton et al., 2001; Lutter and Rivera, 2003) and poor nutrition during these critical formative years has both short- and long term consequences (Martorell, 1995; Scrimshaw, 1995; WHO, 2008).

Iron deficiency and anaemia are a global public health problem especially in Sub-Saharan Africa impacting the health and economic potential of many (Zimmermann and Hurrell, 2007; McLean et al., 2009). Iron deficiency leads to anaemia, impaired immune functions, decreased motor activity and cognitive development among young children (Zimmermann and Hurrell, 2007; Cook, 1999). Global estimates show that the highest prevalence of anaemia at 47.4% is among children less than 5 years of age with the prevalence rising to 64.6% in Africa (Zimmermann and Hurrell, 2007; McLean et al., 2009). About 50% of the anaemia is assumed to be iron deficiency anaemia (Zimmermann and Hurrell, 2007). In Kenya, the 1999 national micronutrients survey estimated that seven out of every ten children under five years were likely to be anaemic and nearly half (43.2%) were iron deficient (Mwaniki et al., 1999).

Inadequate dietary intake has been identified as an immediate determinant of micronutrient deficiencies (Black et al., 2008). Studies on dietary patterns of infants and pre-school children in Kenya, found plant foods as the major sources of energy with cereals contributing approximately 62-68% to the total daily energy intake (Macharia-Mutie et al., 2010; Gegios et al., 2010; Ndiku et al., 2010; Grillenberger, 2006). In addition, maize porridge is the major complementary food for children 12-23 months of age contributing to over 50% of iron intake

(Macharia-Mutie et al., 2010; Ndiku et al., 2010). High prevalence of inadequate iron intake among Kenyan children of up to 77% has also been reported (Grillenberger, 2006). According to WHO (2001), the main causes of anaemia are: dietary iron deficiency; infectious diseases such as malaria, hookworm infections, or schistosomiasis infections; deficiencies of key micronutrients such as folate, vitamin B12, or vitamin A; and inherited conditions that affect cell stability such as thalassaemia and sickle cell anaemia. The three main exogenous causes of anaemia are disease, blood loss and diet (Thurnham & Northrop-Clewes, 2007).

Supplementation has been used with various levels of success to tackle the problem of inadequate iron intake and iron deficiency. However, a recent study in Pemba supplementing children aged 1-35 months with iron and folic acid showed a 12% increase in the incidence of serious adverse events in those children classified as iron replete at baseline, presumably due to malaria (Sazawal et al., 2006). This prompted WHO to advise against blanket iron supplementation and use of micronutrient powders (MNP) that provide the entire iron requirement in a single dose in regions where malaria is endemic and infectious disease highly prevalent (WHO, 2007). Therefore, food based approaches including home fortification with low doses of highly absorbable iron or dietary diversification may be better alternatives in malaria endemic zones such as Kenya. Use of these micronutrient powders consumed with food has been found to improve iron status in children within short intervention periods (Osei et al., 2010; Varma et al., 2007; Lundeen et al., 2010).

Dietary diversification is thought to enhance the probability of adequate nutrient intake to alleviate iron deficiency (Zimmermann and Hurrell, 2007; Johns and Sthapit, 2004). Less studied but widely consumed whole grain cereals such as amaranth grain (*Amaranthus spp*) may have a potential to improve iron status among children. Though animal studies have indicated a bio-availability of iron from amaranth grain to be about 3%, amaranth grain is reported to have 2-4 times more iron than wheat, high quality easily digested proteins and is a

drought resistant fast maturing crop able to survive in semi-arid areas (Pedersen et al., 1987; Ologunde et al., 1994). It may thus have a potential to improve iron status among children.

Other possible strategies to enhance iron bioavailability include grains germination (Egli et al., 2002; Marero et al., 1991; Hurrell et al. 1992; Davidsson et al., 1994; Sandberg et al., 1999; Egli et al., 2004), soaking (Hortz and Gibson, 2007; Erdman and Pneros-Schneier, 1994), fermentation (Sandberg et al., 1999; Hurrell, 2004; Sandberg and Svanberg, 1991; Lindsay and Ahluwalia, 1997), mechanical processing such as household pounding of cereal grains (Nout and Ngoddy, 1997) and extrusion cooking (Royal Tropical Institute, 1983; WHO, 2002; Alonso et al., 2000). Iron bioavailability is higher from ASFs than from plant foods (Lartey et al., 1999; Allen, 2003; Hurrell et al., 2006; Hurrell and Egli, 2010). Addition of even a small amount of ASFs is one of the strategies to improve the content and bioavailability of micronutrients in plant-based diets and fish has been used in development of complementary foods leading to enhanced iron, zinc and calcium content and bioavailability (Mosha et al., 2005; Haug et al., 2010).

The objective of this study was to assess the potential of using food fortification and edible termites and *dagaa* as alternative ASFs to improve the nutrients composition in complementary foods thus improve the micronutrients and health status of Kenyan infants. The study was designed to assess the effects of improved complementary foods on haemoglobin and iron status in children between 6 to 15 months old. Two types of complementary foods (“WINFOOD Classic” (WFC) and “WINFOOD Lite” (WFL)) based on grain amaranth and maize were developed. WFC had in addition edible termites (10%) and *dagaa* (3%) which are rich sources of iron and zinc (Kinyuru et al, 2013) with the germinated grain amaranth. WFL did not contain the ASFs but was enhanced by germinating the grain amaranth and fortified with micronutrients (vitamins and minerals premix) at the same rate as CSB+.

6.2 MATERIALS AND METHODS

6.2.1 Study site

The study was based at the Child Welfare Clinics in Western Kenya, Mumias Sub-County of Kakamega County. The study was carried out in three health facilities namely Makunga, Lusheya and Khaunga. Details of the study site are discussed elsewhere (Konyole et al., 2012). Briefly, Mumias sub-county has sugar cane as main cash crop. 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 especially among children thereby increasing family expenditure on health (Mbagaya et al., 2004).

6.2.2 The study participants and recruitment

All infants from the age of 6 months were eligible to be included in the study and those included were supplied with the improved complementary foods for 9 months until the age of 15 months. Mothers and infants (at 6 months) pairs were recruited in the study as they came to the health facility for diphtheria, pertussis and tetanus or oral polio vaccination. Community-based mobilization was also done with the help of Community Health Workers.

The **inclusion criteria** for infants at 6 months were for mothers accepting and ready to:

- 1) Prepare and feed their infants with the complementary foods and CSB+.
- 2) Stay in the study area for the duration of the study (9 months).
- 3) Consent to participation.

Twins were recruited into the study if both were healthy and randomized to receive the same intervention to avoid contamination that could have resulted from confusing the foods during feeding and sharing.

All infants had to weigh at least 2500g at birth and have MUAC>11.5 cm and WHZ>-3.

Exclusion criteria

Children with WHZ < -3, MUAC <11.5 cm, and/or bilateral pitting oedema, or with anaemia (Hb < 80 g/L) or clinical signs of vitamin A deficiency (xerosis or Bitot spots) were excluded and referred for treatment. Infants with chronic illness requiring medication, genetic disorders interfering with normal growth were also excluded, and given CSB+ as substitute together with referral for care.

6.2.3 Study Design

This was a community-based double blind randomized trial in which infants were individually randomized at 6 months of age to receive one of the three study foods; WFC, WFL or CSB+ for 9 months from January 2012 to January 2013.

6.2.4 The intervention

6.2.4.1 Study Foods

The study foods were developed as detailed in Kinyuru (2012) and tested for acceptability and safety (Konyole et al., 2012). The improved complementary foods were industrially processed by extrusion cooking at the Kenya Industrial Research and Development Institute (KIRDI), Nairobi then packed in opaque food grade plastic containers labelled with computer generated random numbers corresponding to WFL, WFC and CSB+. The formulations of the study foods (WFC, WFL and CSB+) are detailed in [Tables 6-1](#) together with the theoretical nutrient composition of the all the foods compared to recommended values ([Table 6-2](#)). All the study foods (WFC, WFL and CSB+) were given to the children in monthly rations adjusted to the age of the child such that children in the age-groups 6-8 months, 9-12 months and 13-15 months received 50 g/day, 75 g/day and 125 g/ day respectively as recommended in Dewey and Brown, (2003).

The minerals and vitamin premix and tricalcium phosphate were added to the blends (where applicable) after extrusion cooking to avoid vitamin losses at high processing temperatures. The WFP technical specification for CSB+, vers.1.6 are given in [Table 6-3](#) with the details for

micronutrient rate and chemical form for CSB+ vers. 1.6. (Table 6-4). The daily rations packets were delivered in monthly rations followed with instructions to mothers/caretakers to ensure that the food was not shared with other young children not in the study and how to correctly measure the quantities for daily use. The compliance study was also done once mid-way in the course of the intervention study to confirm compliance with the feeding regime prescribed. The distribution was tied with clinical examination and micronutrients status assessment of the child.

Table 6-1: Formulations for WFC, WFL and CSB+- percentage (w/w)

| | Germinated amaranth | Whole maize | <i>Dagaa</i> fish | Termite | Soybean oil | Sugar | Premix 1* | Premix 2** | Whole soybean |
|-------------|------------------------|----------------|----------------------|---------|----------------|-------|--------------|---------------|------------------|
| WFC | 71.00 | 10.40 | 3.00 | 10.00 | 0.60 | 5.00 | 0.00 | 0.00 | 0.00 |
| WFL | 82.52 | 10.22 | 0.00 | 0.00 | 0.59 | 4.91 | 0.20 | 1.56 | 0.00 |
| CSB+ | 0.00 | 74.24 | 0.00 | 0.00 | 0.00 | 5.00 | 0.20 | 1.56 | 19.00 |

*Mineral/vitamin premix - FBF-V-10

**Mono-calcium phosphate and Potassium chloride

Table 6-2: Nutrient composition of WFC, WFL& CSB+ per 100g of dry weight

| | Moisture g/100g | Energy Kcal | Protein g/100g | Fat g/100g | Fe mg/100g | Zn mg/100g | Ca mg/100g |
|------|--------------------|----------------|-------------------|---------------|---------------|---------------|---------------|
| WFC | 8.7 | 423.6 | 19.1 | 12.3 | 12.2 | 6.3 | 48.2 |
| WFL | 9.4 | 407.2 | 14.6 | 9.0 | 12.5 | 5.5 | 139.4 |
| CSB+ | 9.7 | 391.7 | 15.1 | 5.8 | 7.7 | 5.1 | 141.9 |

Table 6-3: WFP technical specification for CSB+, vers.1.6

| N ^o | Ingredients | Percentage (by weight) |
|----------------|--|------------------------|
| 1 | Corn (maize white or yellow) | 72.05- 77.05 |
| 2 | Whole soya beans | 20-25 |
| 3 | Vitamin/Mineral FBF-V-10* | 0.2 |
| 4 | CaCO ₃ (calcium carbonate) | 1.19 |
| 5 | Ca(H ₂ PO ₄) ₂ . H ₂ O (mono calcium phosphate) | 0.80 |
| 6 | KCl (potassium chloride) | 0.76 |

*The composition of the micronutrient pre-mix is identical in CSB+

Table 6-4: Micronutrient rate and chemical form for CSB+ per 100g of finished product

| | Target | Chemical forms |
|---------------------------------|----------|--|
| <i>Vitamin/Mineral FBF-V-10</i> | | |
| Vitamin A | 1,664 IU | Dry vitamin A palmitate 250 n.s |
| Thiamine | 0.128 mg | Thiamine mononitrate |
| Riboflavin | 0.448 mg | Riboflavin |
| Niacin | 4.8 mg | Nicotinamide |
| Pantothenic acid | 6.7 mg | Calcium d-pantothenate |
| Vitamin B6 | 1.7 mg | Pyridoxine hydrochloride |
| Folate | 60 mcg | Folic acid |
| Vitamin B12 | 2 mcg | Vitamin B12 – 0.1% spray dried |
| Vitamin C | 100 mg | Ascorbic acid |
| Vitamin D | 4 mcg | Dry vitamin D3 100 CWS |
| Vitamin E | 8.3 mg | Vitamin E 50% CWS |
| Vitamin K | 100 mcg | vitamin K1 5% CWS |
| Iron (a) | 4 mg | Ferrous fumarate |
| Iron (b) | 2.5 mg | Iron-sodium EDTA |
| Zinc | 5 mg | Zinc oxide |
| Iodine | 40 mcg | Potassium iodate (KIO ₃) |
| Carrier | Qs | Malto dextrin |
| Other minerals | | |
| Calcium (a) | 470 mg | Calcium carbonate (CaCO ₃) |
| Potassium | 400 mg | Potassium chloride (KCl) |
| Phosphorus | 200 mg | Mono calcium phosphate Ca(H ₂ PO ₄) ₂ . H ₂ O |
| + Calcium (b) | 130 mg | |

CSB+ must be fortified to provide the above (Table 6-4) net micro nutrient supplement per 100g of finished product:

6.2.5 Randomization

Individual randomization stratified by sex was used with the children randomized to receive WFC, WFL and CSB+ when the infant was 6 months old. The study included the children with MUAC \geq 11.5 cm. There were an equal number of boys and girls recruited. Random numbers used in the labeling were generated using Microsoft Excel™.

6.2.6 Allocation concealment/blinding and follow-up

Blinding was achieved by assigning secret barcodes to the foods. To ensure complete blinding, there were 2 different codes for each group resulting into a total of 6 codes each in separate envelopes for boys and girls. The key of codes were kept in a sealed envelope until preliminary analyses were completed. The list of random numbers assigned to the treatments

was kept away from study team who distributed the foods to mothers. The staffs assessing the outcome were unaware of treatment allocation as well as the recipients at the time of distribution, because the packaging was similar for all three foods. Participants who defaulted were followed up and accommodated during another session. Some infants were lost to follow up due to exit from the study area, death or unwillingness of the caregiver to continue with the study.

6.2.7 Training and quality assurance

The research assistants were trained until they were able to carry out the standard operating procedures of data collection. A pre-test was also done at the end of the training. Quality checks were done daily to ensure correct filling of the information.

6.2.8 Data collection methods

6.2.8.1 Demographic and dietary intake data

At baseline, the general demographic and socio-economic information for the family was collected. Dietary intake measurement was conducted monthly using a 24-hour recall method to estimate the amount of food eaten. Micronutrients intake from the foods was calculated by using Food Processor PlusTM software (ESHA Research, Salem, OR, USA) as detailed in Lartey et al. (1999) and based on local and regional food-composition tables (Sehmi, 1993; Lukmanji et al., 2008) and values published elsewhere (Dewey and Brown, 2003).

6.2.9 Compliance with the intervention regimen

Compliance/adherence to the intervention regimen including recommended daily dose and frequency and method of food preparation and feeding (WHO, 2008) was obtained by asking the mother how much of the WFC, WFL and CSB+ were consumed by the child while checking for any spoilages, spillages, adequacy in amounts measured and the frequency of feeding among others. The degree of sharing of the assigned diets with other household members was also assessed by interviewing the mother at the health facility during the monthly

visits and by making home visits. The mothers were asked to keep all the distributed packets after these were empty so they could be collected and counted on a monthly basis. The role of compliance in efficacy trials is important and failure to comply may be due to sharing or non-acceptance of intervention among others.

6.2.10 Morbidity

Morbidity data were collected by asking mothers about specific symptoms and clinic visits in the past seven days. Mothers were continuously encouraged to bring their children to the clinic in the event of severe illness prior to the next visit. Generally, morbidity data were collected by assessing overall morbidity in the last month (scored as healthy; mild, self-limited illness; moderate illness requiring symptomatic treatment at the clinic; severe illness requiring antibiotics or other medical intervention) for IYC during their monthly visits to clinic using questionnaires. Clinical examinations were also carried out monthly throughout the study. Health personnel examined the child for different symptoms which could be related to diseases and malnutrition as the child sat with the mother throughout the examination.

6.2.11 Blood sampling and analyses

A venous blood sample was collected at baseline and at the 9 months follow-up visit by a qualified phlebotomist using a field-friendly closed vacutainer system in trace-element free tubes and stored at 4°C until separation. Serum was divided into 5 Eppendorf tubes of 200 µL each, and stored at -80°C before analyses. Briefly; the child sat on the mother's lap, while a phlebotomist took 3 ml venous blood, from the inner elbow of the child, using standard hygienic procedures and a paediatric wing-needle. A trained staff assisted the mother and the phlebotomist. Haemoglobin was determined using a HemoCue machine (HemoCue, Sheffield, United Kingdom), in triplicates at baseline and 9 months follow-up visit with a control cuvet measured daily to ensure right calibration of the HemoCue. The blood for the Hemocue test was drawn from the blood left in the wing-needle tube, after the blood tube had been filled. Finger

prick blood was used to measure haemoglobin concentration in case the venous blood could not be drawn (as was in some cases) due to various reasons such as invisible veins, restless children among others. As a measure of the acute phase response, serum acute phase proteins (APP) were determined as both the slow-reacting α_1 -acid-glycoprotein (AGP) and the fast-reacting C-reactive protein (CRP). Retinol binding protein (RBP) was also determined. As measures of iron stores and deficiency respectively, serum ferritin and soluble transferrin receptors were determined. The blood samples analysis detailed above were done based on an ELISA procedure as described in Erhardt et al. (2004) to give insight in the key metabolic mechanisms that underlie the main outcomes of the study. Certain proteins can serve as markers of Vitamin A (VA) and iron status and a sandwich ELISA technique was used for testing the iron status in this study. On the other hand, nearly all VA in the blood is associated with retinol binding protein (RBP). Therefore serum RBP can be used as a surrogate measure for retinol content and, thus, VA status (Gamble et al., 2001). These data were to enable a better understanding of the effects observed when children are consuming nutrient-dense food on micronutrient status, morbidity/immune activation. The analyses were restricted to the key indicators of the key functions only, in order to restrict the blood sampling to an absolute minimum.

6.2.12 Sample size consideration

Because micronutrient deficiencies, especially of iron was expected to be of concern, the sample size was calculated to allow for the detection of differences of equal to or greater than 5 g/L in haemoglobin concentrations at 90% power and 5% significance as observed by Lartey et al. (1999) (n=241) and Faber et al. (2005). Faber et al. (2005) in a similar study detected 9.4microg/L (95% CI: 3.6, 15.1microg/L) for serum Ferritin and 9 g/L (95% CI: 6, 12 g/L) for haemoglobin concentration at 90% power and 5% level of significance with n=361. Two-tailed distribution was assumed because of the fact that giving free nutrient-dense food though unlikely to decrease nutrient intake could still lead to a reduction on some of the outcomes.

6.2.13 Data analysis

Case record forms were checked daily and entered within 2 weeks. The initial analysis was done before breaking the code. Frequencies, means and median values were calculated using SPSS™ software version 19 (Chicago, Illinois, USA). Briefly, analysis of variance (ANOVA) was done to examine differences in baseline characteristics between the 3 intervention groups and to determine whether significant differences occur between the interventions groups in Haemoglobin and iron status biomarkers at 6 and 15 mo. with the independent variables being intervention group and values at 6 mo of the respective dependent variable. Baseline characteristics between the pooled intervention groups were compared by using t tests and chi-square tests while haemoglobin, iron status and anemia prevalence of the study infants were compared at 6 months and 15 months using Student's t tests. The differences in baseline characteristics and the potential influence of seasonality were controlled for at every analysis stage using multiple regression analysis.

Anaemia was defined as haemoglobin concentration <110 g/L (WHO, 2002), moderate- to-severe and severe anaemia as haemoglobin concentration < 90 g/L and < 70 g/L, respectively (Stoltzfus et al., 1998). In summary: Anaemia category were: Normal>11g/dl; Mild=10-10.9g/dl; Moderate=7-9.9g/dl; Severe<7g/dl.

6.2.14 Ethical considerations

Written and oral information were given to the caregivers of all eligible children in the local language before obtaining written consent. All data obtained in the study were kept anonymous while the mothers had an option to discontinue from the study at anytime and still continue receiving the monthly food ration and other health facility services without taking part in the study. The study was cleared by the Kenyatta National Hospital- University of Nairobi Ethics Research Committee (KNH-UON ERC-P436/12/2010) and the trial was registered with

Controlled-trials.com. No: ISRCTN30012997. Permission to implement the study was granted by the relevant local authorities in the study area.

6.3 RESULTS

Among the 499 study participants, 496 were assessed for haemoglobin concentration at baseline while 431 were assessed at endline. For the other outcomes, 268 study participants were included in the endline and 249 at baseline. The disparities at baseline and endline arose from failure to obtain samples from some of the infants at baseline since identifying the veins was more difficult. As the infants grew, identifying the veins became easier and thus more numbers at endline compared to baseline. The biochemical data reported here are for study participants with values either at 6 or 15 mo of age and both. Some of the background characteristics of the study participants are as shown in [Table 6-5](#). There were no significant differences among the baseline characteristics of the study participants.

Briefly, Over 98% of the children were being breastfed at 6 months although by around 3 months of age, exclusive breastfeeding had ceased and other foods had been introduced. The households had an average of 5 members and 2 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. The age of the main caregivers was 26.4(SD 6.8), 25.6(SD 6.7), and 25.3(SD5.5) for food groups WFC, CSB+ and WFL respectively ($p= 0.30$). Education level was not significantly different among the food groups ($p= 0.89$).

Table 6-5: Baseline characteristics of study participants by food group

| | WFC | CSB+ | WFL | p |
|---|------------|------------|------------|------|
| Number of children | 165 | 167 | 167 | |
| Child characteristics | | | | |
| Sex, boys, n (%) | 76 (46.1) | 83 (49.7) | 81 (48.5) | 0.80 |
| Child age, months | 6.1±0.2 | 6.1±0.3 | 6.1±0.2 | 0.53 |
| Currently breastfeeding, n (%) | 162 (98.2) | 166 (99.4) | 166 (99.2) | 0.17 |
| Age introduced to food other than breast milk, months | 3.1±2.1 | 3.1±2.0 | 3.4±2.0 | 0.87 |
| Haemoglobin, g/dl | 10.8 ± 1.5 | 10.7±1.3 | 10.7±1.4 | 0.81 |
| Caregiver characteristics | | | | |
| Age of main caregiver (years) | 26.4±6.8 | 25.6±6.7 | 25.3±5.5 | 0.30 |
| Education level | | | | 0.89 |
| Unable to read and write, n (%) | 14 (38.9) | 15 (41.7) | 7 (19.4) | |
| Primary incomplete, n (%) | 62 (28.4) | 73 (33.5) | 83 (38.1) | |
| Primary completed, n (%) | 68 (37.0) | 50 (27.2) | 66 (35.9) | |
| High school completed, n (%) | 18 (34.6) | 26 (50.0) | 8 (15.4) | |
| University/ College graduate, n (%) | 3 (33.3) | 3 (33.3) | 3 (33.3) | |
| Household characteristics | | | | |
| Total household members | 5.9±2.2 | 5.6±2.2 | 5.4±2.1 | 0.18 |
| Children <5 years | 1.8±0.7 | 1.9±0.8 | 1.9±0.7 | 0.48 |
| Access to protected well, n (%) | 77 (48.4) | 75 (46.6) | 68 (41.2) | 0.68 |
| Use of insecticide treated net, n (%) | 161 (97.6) | 159 (95.2) | 161 (96.4) | 0.51 |
| Primary income | | | | |
| Farming, n (%) | 90 (55.6) | 76 (47) | 72 (44) | 0.22 |

Data are mean±SD, unless stated otherwise and n (%)

6.3.1 Micronutrient intake

Table 6-6 presents the daily intake of some minerals from the diet including the complementary foods and phytate content of diet at 15 months of age by food groups. Infants in combined WFL and CSB+ had significantly greater intake of calcium and iron than infants in the WFC group. Moreover, infants in the CSB+ had significantly higher ($p < 0.001$) phytate: iron molar ratio than infants in the WFC and WFL. There were no significant differences in phytate intake among the groups. The observed trend for infants in the CSB+ and WFL to have higher micronutrient intakes than infants in WFC may be explained by the fact that the two improved complementary foods (CSB+ and WFL) which were fortified, comprised a greater proportion of daily nutrient intake in the groups.

Table 6-6: Selected micronutrients intake from the diet at 15 months.

| Nutrient | WFC | CSB+ | WFL | P |
|---------------------------|-----------------------------|------------------------------|-------------------------------|--------|
| Calcium (mg) | 127 ^b (86, 167) | 179 ^a (114, 243) | 266 ^a (167, 365) | 0.01 |
| Iron (mg) | 4.3 ^b (3.5, 5.1) | 8.0 ^a (5.6, 10.5) | 10.2 ^a (7.4, 13.0) | <0.001 |
| Phytate (mg) | 284 (234, 334) | 238 (194, 282) | 284 (217, 351) | 0.43 |
| Phytate: iron molar ratio | 4.4 ^a (3.2, 5.6) | 7.5 ^b (6.2, 8.8) | 4.1 ^a (2.8, 5.4) | <0.001 |

Row values with same letter superscripts were not significantly different at $p \leq 0.05$ by one-way ANOVA).

6.3.2 Morbidity

There were no significant differences in the state of health among the study groups in the past month as about half of the infants were generally well ($p=0.07$) and morbidity in the pervious three days prior to the return visit for data collection were not significant for most of the conditions/symptoms considered. Throughout the study and across the study groups, the infants were well/alert and reported no major symptoms of disease except for coughing, audible wheezing and running nose which were significantly reported across all the food groups in the first 2 months ($p=0.02$, 0.01 and 0.01 respectively). **Table 6-7** gives a summary of the morbidity data by study groups at baseline.

Table 6-7: Summary of morbidity of the study participants by food groups (%)

| | WFC (n = 165) | CSB+ (n = 167) | WFL (n = 167) | P |
|------------------------------|------------------|-------------------|------------------|------|
| State of health in | | | | |
| Past month [(%)] | | | | |
| Generally well | 55 | 57 | 58 | 0.07 |
| Mild-self-limiting illness | 28 | 15 | 15 | 0.90 |
| Ill and treated at clinic | 14 | 29 | 7 | 0.90 |
| Hospitalization | 3 | 2 | 1 | 0.28 |
| Morbidity in the last 3 days | | | | |
| [(%)] | | | | |
| Diarrhea | 8 | 9 | 10 | 0.98 |
| Vomiting | 6 | 3 | 4 | 0.70 |
| Fever | 14 | 16 | 13 | 0.90 |
| Cough | 76.3 | 61 | 80.5 | 0.02 |
| Wheezing | 37.1 | 35 | 36.1 | 0.01 |
| Running nose | 20 | 20 | 20 | 0.01 |
| Ear problems | 0 | 0 | 0 | |
| Skin problems | 12 | 9 | 16 | 0.57 |
| Excessive crying | 3 | 3 | 4 | 0.84 |
| Eating problems | 14 | 9 | 8 | 0.59 |

6.3.3 Haemoglobin concentration and anaemia prevalence

Table 6-8 shows that there was no significant difference in mean haemoglobin concentration at 6 months among infants in the 3 food groups. WFC, CSB+ and WFL had 10.8 (SD 1.5), 10.7 (SD1.3) and 10.7 (SD1.4) g/dL haemoglobin respectively (p=0.81). The mean haemoglobin concentration at 15 months was 10.5 (SD 1.6) g/dL, 11.3 (SD 1.3) g/dL and 11.0 (SD 1.8) g/dL for young children in the WFC, CSB+ and WFL respectively and this was significant (p<0.001). The same was observed for body iron stores and transferring receptors. Ferritin remained insignificantly different among the three food groups and over time from 6 to 15 months (p=0.77 and 0.06) respectively.

Table 6-8: Summary of some biomarkers at 6 and 15 months of the intervention by food

| Variable | Food ID | n | 6 months | | 15 months | | |
|--|---------|-----|-----------|---------|-----------|-----------|--------|
| | | | Mean±SD | p-value | n | Mean±SD | P |
| Ferritin(ug/l) | WFC | 87 | 45.4±48.1 | 0.77 | 92 | 24.6±29.3 | 0.06 |
| | CSB+ | 83 | 49.5±53.2 | | 87 | 35.5±33.1 | |
| | WFL | 79 | 44.8±33.0 | | 89 | 34.2±36.6 | |
| Transferritin receptors as Ramco assay equivalents(mg/l) | WFC | 87 | 11.8±3.5 | 0.88 | 92 | 13.5±5.1 | <0.001 |
| | CSB+ | 83 | 11.9±4.1 | | 87 | 11.2±4.7 | |
| | WFL | 79 | 11.6±4.1 | | 89 | 11.6±4.1 | |
| Body iron store(mg/kg body weight) | WFC | 87 | 2.0±3.8 | 0.60 | 92 | -0.6±3.7 | <0.001 |
| | CSB+ | 83 | 2.4±3.8 | | 87 | 1.8±3.1 | |
| | WFL | 79 | 2.6±3.5 | | 89 | 1.1±3.7 | |
| RBP as retinol equivalents(umol/L) | WFC | 87 | 0.9±0.2 | 0.76 | 92 | 0.9±0.3 | 0.57 |
| | CSB+ | 83 | 0.9±0.2 | | 87 | 0.9±0.2 | |
| | WFL | 79 | 0.9±0.3 | | 89 | 0.9±0.2 | |
| Creatin Reactive Protein(mg/L) | WFC | 87 | 7.2±13.9 | 0.91 | 92 | 6.0±11.5 | 0.75 |
| | CSB+ | 83 | 7.0±11.5 | | 87 | 6.9±12.6 | |
| | WFL | 79 | 6.4±11.5 | | 89 | 5.6±9.7 | |
| AGP(g/L) | WFC | 87 | 1.1±0.4 | 0.23 | 92 | 1.1±0.4 | 0.36 |
| | CSB+ | 83 | 1.2±0.5 | | 87 | 1.2±0.5 | |
| | WFL | 79 | 1.1±0.4 | | 89 | 1.2±0.5 | |
| Haemoglobin(g/dl) | WFC | 164 | 10.8±1.5 | 0.81 | 142 | 10.5±1.6 | <0.001 |
| | CSB+ | 166 | 10.7±1.3 | | 137 | 11.3±1.3 | |
| | WFL | 166 | 10.7±1.4 | | 152 | 11.0±1.8 | |

For anaemia prevalence, there were no differences in all categories of anemia prevalence among the intervention groups at 6 months (p=0.91) while there was a significant difference at 15 months (p=0.01).

Table 6-9: Categories of anaemia by food groups at 6 months and 15 months

| Anaemia Category | Infants age | N | WFC | CSB+ | WFL | P |
|--------------------------|-------------|-----|------------------------|------------------------|-------------------------|------|
| Normal: Hb>11g/dl, n (%) | 6 months | 239 | 80 ^a (33.5) | 80 ^a (33.5) | 79 ^a (33.1) | 0.91 |
| | 15 months | 247 | 66 ^a (26.7) | 92 ^b (37.3) | 89 ^{ab} (36.0) | 0.01 |
| Mild: Hb =10-10.9g/dl | 6 months | 133 | 44 ^a (33.1) | 40 ^a (30.1) | 49 ^a (36.8) | 0.75 |
| | 15 months | 98 | 41 ^a (41.8) | 22 ^b (22.4) | 35 ^{ab} (35.7) | 0.04 |
| Moderate: Hb =7-9.9g/dl | 6 months | 119 | 38 ^a (31.9) | 45 ^a (37.8) | 36 ^a (30.3) | 0.78 |
| | 5 months | 79 | 31 ^a (39.2) | 22 ^a (27.8) | 26 ^a (32.9) | 0.89 |
| Severe: Hb <7g/dl | 6 months | 5 | 2 ^a (40.0) | 1 ^a (20.0) | 2 ^a (40.0) | 0.95 |
| | 15 months | 4 | 3 ^a (75.0) | 1 ^a (25.0) | 0 ^a (0.0) | 0.25 |

Each superscript letter denotes a subset of categories whose column proportions do not differ significantly from each other at the 0.05 level. (WHO, 2002)

At 15 months the infants had significantly lower ($p = 0.01$) anemia prevalence (Hb < 110 g/L) compared to the groups before intervention. **Table 6-9** shows the proportion of infants with haemoglobin concentration less than cut-off both at 6 and 15 months of age.

6.4 DISCUSSION AND CONCLUSION

The current study aimed to find out the effect of the edible termites and *dagaa* small fish and micronutrients fortified complementary foods on anaemia and iron status of infants 6-15 months in a resource limited setting. The 2 best parameters to assess iron status are ferritin and soluble transferrin receptor (sTfR) (Cook et al., 2003). Ferritin correlates very well with iron stores and sTfR is increased as a result of Iron deficiency (ID). Using both values together it is even possible to calculate body iron stores, previously only possible with bone marrow staining, the “gold standard” for defining iron deficiency (Thurnham et al., 2010). Because ferritin levels are increased and RBP levels are lowered by infection, it is important to identify study participants with infection (Christian et al., 1998; Thurnham et al., 2003 and Tomkins, 2003). For this purpose C-reactive protein (CRP) is a good measure of acute infections (Thurnham et al., 2003; Wieringa et al., 2002). Patients with elevated CRP should be excluded from evaluation or values corrected. If not, the results obtained for iron and VA markers could lead to a false-low rate of ID and a false-high rate of Vitamin A deficiency (VAD). The prevalence of

common illnesses such as URTI were high in these communities. High prevalence of micronutrient deficiency could play a crucial role in the aetiology of these infections (Tomkins, 1981; Sepulveda et al., 1988; Black, 1991; Black, 1998). However, one would expect the prevalence of illness to be higher in the community due to the higher prevalence of marginal VAD (marked by less than 20 micrograms/dL -WHO, 1972) and elevated C-reactive protein concentration (>5mg/L Pepys, 1979) (Nestel et al., 1999).

Fortification with vitamins and minerals clearly prevented the depletion of iron stores in all the food groups. Despite the high iron content of edible termites and *dagaa* small fish, their addition to WFC as ASFs did not improve infant iron body stores. There are several possible reasons for this. One is that diets with phytate: iron molar ratios ≥ 6 have poor iron bioavailability (Wise, 1983). However, only the CSB+ had a phytate-iron molar ratio greater than this (7.5 compared with 4.4 and 4.1 for WFC and WFL respectively). It is therefore unlikely that interference from phytate was the reason for the lack of benefit from WFC. Alternatively, high prevalence of malaria ($\geq 40\%$) in this population (Noor et al., 2009; Githeko et al., 2006) could be an important factor. Malaria parasitemia has been reported to be a common cause of anemia in this age group (Achidi et al., 1996; Binka et al., 1994; Menendez et al., 1997). Besides the increased destruction of red blood cells, reduced iron absorption and disturbed iron metabolism during acute episodes (Molyneux et al., 1989; Weatherall and Abdalla, 1987; van Hensbroek et al., 1995) may increase the requirement for iron above normal physiologic needs. This was however not the case for WFL and CSB+.

Although not intended for investigation initially, the results showed that infants fed the ASFs enriched and vitamin and mineral fortified diets did not have a significant increase in plasma retinol between baseline and 15 months (for WFC, CSB+ and WFL). Typically, complementary foods fed to infants in this population contain little vitamin A. Provitamin A carotenoids in foods such as pawpaw, mangoes, and dark-green leafy vegetables are the main

sources of vitamin A in the adult diet, but are not usually fed to infants and young children or when fed as vegetable soups are usually in diluted forms (results earlier shown). Breast milk is therefore an important source of vitamin A for infants. However, breast milk vitamin A concentrations could be low due to inadequate maternal dietary intake and poor nutritional status (Haskell & Brown, 1999) as shown in rural mothers in southern Ghana where the average breast milk retinol concentration was only 1.0 mmol/L (Lartey & Orraca-Tetteh, 1990), which is much lower than the 2.3 mmol/L value for well nourished mothers (Stoltzfus & Underwood, 1995). Owino et al., (2008) reported 307 micrograms of vitamin A daily intake from breastmilk in Zambia and attributed the observed high intakes of vitamin A to the fact that in Zambia sugar is fortified with vitamin A at a minimum rate of 10 mg/kg (Serlmitos and Fusco, 2001) while sugar formed 20% of porridge dry recipe. The workers (Owino et al., 2008) estimated that vitamin A intake from sugar accounted for 63% of the total vitamin A intake from complementary foods. Lartey et al. (1999) also estimated that the total vitamin A intake (from breast milk and other foods) between 6 and 10 mo of age by infants was only 50–70% of the recommended 350 mg/d (Department of Health, 1991).

C-reactive protein (CRP), an indicator of infection, was not significantly different at baseline and endline for all the food groups. In this study, only CRP was used as indicator for inflammation. Though use of two indicators of inflammation has been recommended (Thurnham et al., 2010; Grant et al., 2012; WHO, 2007; Ayoya et al., 2010) with α 1-acid glycoprotein (AGP) thought to better reflect the change in concentration of ferritin, CRP is currently recommended by WHO as an independent indicator of the acute phase response (WHO, 2007). Furthermore, CRP has recently been shown to correlate with AGP and better explains serum ferritin variance than other markers of inflammation (Ayoya et al., 2010). The high prevalence of elevated CRP levels in the children also explains the slightly higher ferritin levels in these children (>10 ng/mL according to Pilch et al. (1980). Since ferritin is also an

acute phase protein it is known to increase with infection (Larade and Storey , 2004; Beck et al., 2002) , the poor living conditions of the children (evidenced by proxy indicators like source of drinking water) may also contribute to the higher prevalence of infections. The presence of multi-micronutrient (Vitamin A and Fe) deficiencies is an important finding though not intended initially. Many nutrition intervention studies focus on one micronutrient only. In the light of the findings of this study it would be crucial to address micronutrient deficiencies in similar communities with a multi-micronutrient fortification approach. Giving only one micronutrient, e.g. zinc, may potentially be detrimental to a child in creating a higher prevalence of iron deficiency as zinc and iron compete for absorption in the body (Solomons, 1996).

Morbidity was not significantly different among the 3 intervention groups. The incidence rates of diarrhea, fever, and respiratory illness were similar to rates reported for rural Guatemalan infants (Ruel et al., 1997) and Ghana (Lartey et al., 1999). The lack of effect of micronutrient fortification on morbidity differs from the results of some studies in which supplementation with iron, zinc, or vitamin A did reduce morbidity (Sazawal et al., 1995; Angeles et al., 1993; Rosado et al., 1997; Rahmathullah et al., 1990). Inadequate statistical power may account for this. For example, to detect a 30% decrease in diarrheal morbidity with a power of 0.8, a sample size of 200 per group is needed. On the other hand, not all studies have shown an effect of micronutrient supplementation on morbidity (Dibley et al., 1996; Meeks Gardner et al., 1998; Rahmathullah et al., 1991), and it is possible that micronutrient malnutrition in these Kenyan infants was not severe enough to affect those outcomes.

Information on dietary intake provides some explanation for the biochemical deficiency seen in this population. From the repeat 24-hour dietary recall data, the children did not consume food sufficient in iron. The early introduction of complementary food may also contribute to the deficiencies in micronutrients. In the community the average age of

introduction of complementary food was about 4 months of age and the children often received cooked vegetables soups prepared as part of the adult meals. Although the children also received traditional food it was most often only maize meal porridge which has a relatively high energy content, but insufficient in essential micronutrients. However, the 24-hour dietary recall data showed that the majority of mothers use these products in a diluted form, so much so that it cannot possibly supply the required daily needs. If these products are the major source of food for these infants; one can understand the existing deficiencies.

Results from this study show that deficiencies in vitamin A and iron remain a concern among rural setting children and with a bigger rural population (currently estimated at >60%- Grillenberger, 2006); this affects a larger proportion of the entire population. With an increasing population the magnitude of the problem may become a serious public health problem. Efforts to improve complementary feeding in developing countries have often focused on the use of cheap, locally available ingredients as a way to make these foods affordable to poor families. Although these measures may improve the macronutrient content of the diet, the micronutrient quality is often neglected. The results indicate that fortification with vitamins and minerals improves iron stores and reduces anaemia prevalence significantly. Fortification with some nutrients such as iron and vitamin A may be the most effective strategy while further studies are still needed to look at the bioavailability of the micronutrients available in the edible termites since the complementary food with the edible termites in this study did not perform to the level of the micronutrients fortified foods.

6.5 ACKNOWLEDGEMENTS

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6.6 CONFLICT OF INTEREST

None of the authors had a conflict of interest to disclose.

6.7 FUNDING

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CHAPTER SEVEN

7. GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 INTRODUCTION

The primary aim of this thesis was to investigate the effect of consumption of edible termites and *dagaa* small fish enriched and micronutrients fortified germinated grain amaranth grain flours together with Corn Soy Blend Plus (CSB+) on linear growth and weight gain, iron status and anaemia of Kenyan infants and young children 6-15 months of age. To realize this, the research was organized into four studies in Mumias Sub-county; a resource limited setting in Western Kenya which is malaria endemic.

Briefly, The first study (**Chapter 3**) assessed the acceptability of two flours and porridges of complementary foods based on germinated grain amaranth and maize with or without edible termites and *dagaa* small fish named “WINFOOD Classic” (WFC) and “WINFOOD Lite” (WFL), respectively, compared to CSB+ among mothers and young children. While most mothers preferred WFL flour and porridge (63.2% and 70.2%, respectively), all the 3 foods were found acceptable and further developed and tested for efficacy with no adverse effects throughout the study period and follow up lasting 4 weeks among the study participants.

In a cross sectional survey with 618 children-caregivers pairs from the catchment areas of Lusheya, Khaunga and Makunga health facilities in Mumias Sub-county, malnutrition was highly prevalent (**Chapter 4**). The prevalence of stunting, wasting and underweight was 35.7%, 4.1%, and 13.4%, respectively. Height-for-age and weight-for-age deficits increased rapidly in children already at 6 months, continued to 18 months old and were greatest in children 18–23 months old while remaining substantially below the reference median with no evidence of catch-up growth.

Chapters 5 and 6 describe the effect of the improved (a micronutrient fortified and ASFs enriched) complementary foods on growth (length and weight) and the micronutrients status

of 6-15 month old IYC. Four hundred and ninety nine infants were randomly allocated to receive either of the improved foods for nine months while continuing to consume their usual diet. There were no significant differences between intervention groups in length or weight gain values between 6 and 15 mo of age. From 9 to 12 mo of age, Z-scores were lower in cross section study infants than in the combined intervention groups. There was a significant intervention effect of 0.6(SD1.6), -0.3(SD1.8) and 0.2(SD2.0) for CSB+, WFC and WFL respectively ($p < 0.001$) with respect to haemoglobin concentration. Transferrin receptors as Ramco assay equivalents and body iron stores at 15mo were significantly different from 6 mo ($P = 0.002$ and < 0.001 respectively) with transferrin receptors changing from 11.8(SD3.5) to 13.5(SD5.1) (mg/l) and Iron body stores from 2.0(SD3.8) to -0.6(SD3.7) (mg/kg body weight) for WFC among others. The proportion of infants with anemia decreased from 51.8% to 42.3% overall with a larger decrease among the mild and moderate anemic groups. The other outcomes showed insignificant intervention effect including the CRP concentrations. Introducing the improved complementary foods in the diet of 6-15 months old infants seemed to reduce anemia observed over the 9 month follow up period. No benefit was observed in length, weight and other outcomes. In this chapter (**Chapter 7**), a summary of the main findings is presented, some of the methodological issues (internal validity) and key findings (external validity) are presented as well as identification of implications and suggestions for future research are highlighted.

The current study demonstrates that a multi-sectoral collaboration approach can be employed successfully to solve existing nutritional problems in resource limited settings as evidenced by the input of the Universities (JKUAT, UNITID and UoC), the small scale food production enterprise AllGrain Limited and the community in ensuring the success of the collaboration. The involvement of the community prior to the intervention trial in assessing the acceptability of the products contributed to their input in the trial success. The study is

unique having been designed to assess the impact of improved complementary foods on growth and iron status in a resource limited setting. The ingredients used to improve the complementary foods especially the insects (edible termites) have never been tried before in a Kenyan set up.

7.2 SUMMARY OF RESULTS

Overall, although the caregivers continued breastfeeding and practised complementary feeding as recommended by the WHO (2002), these practices were constrained by lack of variety to choose from for complementary feeding and inability to separately prepare meals for the IYC different from the main family meals. Further, the intakes of important nutrients such as iron and calcium were suboptimal. The results further showed that it is feasible to produce an acceptable complementary food based on the utilisation of widely accepted, already-in-use indigenous foods like edible termites. Results from the randomized controlled trial show that there were no significant differences in length gain, weight gain and total energy intake among the foods and that the consumption of fortified and edible termites/*dagaa* enriched germinated grain amaranth-maize blends improved haemoglobin and body iron stores. Compared to the cross section study, the energy and the micronutrients intakes were higher in the intervention groups and it is apparent that traditional complementary foods were displaced by the provision of higher quality complementary foods.

7.2.1 Acceptability of the improved complementary foods and implications for future scale-up and marketing

The results show that it is feasible to develop acceptable complete complementary food based on locally available and already acceptable foods. The dark colour observed for the WFC blend (Table 3-3) by mothers may be attributed to several factors among them the edible insects exo-skeleton with thickened areas in which the chitin is reinforced or stiffened

by materials such as minerals or hardened proteins in parts of the body where there is a need for rigidity or elasticity. The mineral crystals, mainly calcium carbonate, are deposited among the chitin and protein molecules via sclerotisation from where they interpenetrate and reinforce each other, the minerals supplying the hardness and resistance to compression, while the chitin supplies the tensile strength and the hardened proteins are called sclerotin (Richard and Davies, 1977). The sclerotin is hard to neither digest nor mechanically break down by milling and remains as dark particles in the flour and which may have been disliked by the caregivers. The vitamin-mineral premix used for fortification in this study had grey colour and may have caused colour change though insignificant. A study from Mexico (Rosado et al, 2005) found similarly low sensory scores for color in a study designed to assess the effect of fortification on maize flour stability and acceptability. However, there were no observable differences in colour between the fortified blends (CSB+ and WFL) in the current study.

Overall, the observation that extrusion cooked porridges were generally accepted agrees with previous observations that mothers often find improved foods processed from locally available staples attractive (Mensa-Wilmot et al, 2001; Konyole et al., 2012; Owino et al.,2007b). However, the feasibility of local micronutrient premix manufacture should be assessed since the scale up of food fortification activities in developing countries may be constrained by limited capacity e.g. in handling and storage of micronutrient premixes hence safety may not be guaranteed (WFP, 2006). The study blends were well accepted by mothers and infants. The most feasible strategy for the marketing of the developed blends is to distribute them through the health provision system and non-governmental organizations (NGOs) by emphasizing their benefits on micronutrient status.

Infant growth in the study population may also have been affected by high aflatoxin exposure due to the wide use of cereals and legumes for complementary food preparation.

Groundnuts and maize are among foodstuffs that are most susceptible to aflatoxin contamination (Kaaya and Warren, 2005) while consumption of groundnut and maize-based complementary foods has been associated with growth faltering (Egal et al., 2005). Although both aflatoxin exposure and impaired gut permeability are associated with growth faltering, (Gong et al, 2004; Campbell et al, 2003) these factors were not included in the study design due to budgetary and time constraints. Moreover, high levels of aflatoxins occurring in human breast milk have been previously reported (el-Nezami et al, 1995; Abdulrazzaq et al, 2003). Although efforts were made to ensure that the processed blend had very low aflatoxin levels, aflatoxin levels in traditional complementary foods were not assessed. Extrusion cooking used may have also resulted in reduced aflatoxin levels as reported in a study (Castells et al, 2005). Sorting also can be used alone or in combination with other strategies to produce aflatoxin-free foods (Fandohan et al, 2005; Owino et al., 2007b; Turner et al, 2005).

7.2.2 Complementary feeding and nutrient intake of children 6-15 months old

The dietary data show that energy and macronutrient intakes of infants and young children in the study population were generally adequate, but intakes of certain minerals, particularly iron and calcium were inadequate. High levels of breastfeeding may have contributed to the adequate intakes of many nutrients. The high levels of breastfeeding is supported by the observation that over 98% of the mothers were breastfeeding their children at 6 months although some mothers introduced other foods too early or too late. However, in order to improve infant nutrition, it may be necessary to develop and promote fortified complementary foods which are acceptable to and affordable by families. A higher proportion of mothers using traditional complementary foods may have contributed to low nutrients intake thus the need to improve them. There is also need to enhance the frequency of feeding the infants as shown in Malawi (Hotz and Gibson, 2005) where increased energy

intake from complementary foods was attributed to more frequent feeding especially given that low frequency of feeding infants has been observed in similar settings (Mamiro et al., 2005).

The observed daily intake of food solids is within the range (< 25 g to > 250 g) for developing countries (Lutter and Dewey, 2003). The total intake of absorbable iron and calcium is below recommended values (Lynch and Stoltzfus, 2003; Lutter and Dewey, 2003). The observed intake of iron in the current study is consistent with observations from Tanzania (Mamiro et al, 2005) and Zambia (Owino et al., 2007a) that complementary foods provided 15%, 20% and 27% of iron requirements for infants 6-8, 9-11 months and young children 12-23 months, respectively and also support findings from South Africa (Faber et al., 2005) and Zambia (Owino et al., 2007a) that complementary foods were inadequate in iron and calcium. This confirms previous observations that these two micronutrients are among those micronutrients of concern in developing countries even when strategies to improve their bioavailability are employed (Dewey and Brown, 2003; Gibson et al. 1998; Owino et al., 2008).

As detailed in [Table 2-5](#), other studies support these findings for example; in Tanzania Mamiro et al. (2004) showed that improved iron solubility and energy density of a complementary food processed using germinated and autoclaved finger millet-kidney beans, roasted peanuts and mango puree had no effect on growth, haemoglobin concentration and iron status of children 6-12 months old. The workers concluded that the slight improvement in iron solubility as a result of processing was not sufficient to offset the low iron content of the complementary food. In Bangladesh, Kimmons et al. (2005) reported inadequate intake of vitamins and minerals among breast-fed infants 6-12 months old and attributed this to low micronutrient density of complementary foods. The findings of the current study thus strongly support previous recommendations that multi-micronutrient fortification of

complementary foods is required if the needs for most limiting minerals like iron, calcium and zinc are to be met more so after the first 6 months of life.

7.2.3 Randomised Controlled Trial

The results show that consumption of improved complementary foods resulted in improvement in hemoglobin whereas length and weight gain were not affected.

7.2.3.1 Infant growth, haemoglobin concentration and iron status

The observation that the provision of the study foods had no significant effect on growth over 9 months may be explained that energy and protein intake were not that limiting in the study population. Moreover, any benefit of the blends on growth could possibly have been confounded by the fact that some infants may have been HIV-exposed since HIV serostatus was not considered at enrolment. Stunting marked by decreased lean body mass is common in HIV-infected infants (Arpadi, 2000) with HIV-infected infants usually shorter and lighter than uninfected children at birth and remains so several months after birth in both sexes (Moye et al, 1996). HIV infection has been associated with higher prevalence of underweight in developing world e.g. South Africa (Mason et al., 2005) and could also result in poor intestinal permeability thus malabsorption of nutrients (Rollins et al., 2001).

Parasitic infection may also cause impaired gut permeability (Goto et al., 2002) and has been associated with low height-for-age in children under 2 years of age and low weight-for-age in older children (Oberhelman et al., 1998). The decline in WAZ, HAZ and WHZ to below the reference line can be explained by a negative association between the extent of breastfeeding at about 3 months and weight gain in the first 6 months of life that has been observed (Ekelund et al., 2006). Moreover, longer periods of breastfeeding as observed in the current study may be protective against the risk of overweight and obesity in later life (Gillman et al., 2001) as a result of lower weight gain in infancy (Ekelund et al., 2006). Growth faltering despite continuation of breastfeeding combined with the provision of

adequate complementary food in the first year of life may also be attributed to prenatal factors (Dewey, 1998). Indeed Schmidt et al. (2002) demonstrated that neonatal weight and length (proxy of prenatal environment), strongly predict infant nutritional status at 6-7 months of age. Finally, predominantly or partially breastfed infants grow faster than the WHO reference until 6 months of life, after which their growth is slower than the WHO reference (Victora et al, 2010).

The current study shows that macronutrients were not a limiting factor and any differences observed in growth and haemoglobin concentration can be attributed to multi-micronutrient fortification of the study blends. The results of the current study are consistent with Moursi et al, (2003) that found improvement in length between 24-31 weeks in infants given multi-micronutrient fortified maize-soya blend. The main differences between the current and the Congolese study include the fact that in the Congo case complementary foods were introduced at a younger age (13 weeks), the initial stunting rate (15.5%) was higher than that observed at 6 months in the current study and meal frequencies were low with only 21% of infants receiving more than two meals per day. Although an increased energy intake from amylase-treated complementary gruel was observed in the Congolese study, there was no impact on total daily energy intake. Energy intake may have been limiting in the Congolese infants hence the positive effect of nutritional intervention. The same was with Bauserman et al. (2013) in Democratic Republic of Congo (DRC) using a caterpillar-cereal blend for 12 months. Indeed the DRC study concluded that the high initial prevalence of stunting (66% at 18 months) and the non-significant growth response to the micronutrient-rich food supplied suggest that factors other than dietary deficiencies contributed to stunting in the children. Children who consumed caterpillar cereal had higher hemoglobin levels and lower prevalence of anemia (Bauserman et al., 2013).

Owino et al. (2007) in Zambia in a peri-urban mid income setting observed improvement in length using fortified complementary foods for only 3 months unlike other studies that showed no improvement in growth with the provision of fortified blends in infants (Oelofse et al., 2003; de Almeida et al., 2003) among others summarised in [Table 2-5](#). Lartey et al. (1999) found intervention effect on weight-for-age and length-for-age between 9 and 12 months, but earlier. Other multi-micronutrient supplementation studies (Faber et al., 2005; Smuts et al., 2005; Hop et al., 2005) in infants have reported no benefit on growth.

There were no significant differences in baseline body composition data (skinfolds). There was however significant differences at baseline for MUAC and head circumferences for WFC. This was unexpected since randomisation was done and we attribute this purely to chance. However, comparisons of skinfolds at 6 months and 15 months showed a negative difference though insignificant. This is attributed to change in fat mass where infants especially those breastfeeding reduce in non-fat mass as they grow attributed to loss in hydration from the initial about 79% to 73% as they grow normally (Wells et al., 2002). It has been observed that rapid weight gain in infancy is a risk factor for later obesity (Wells et al., 2005; Ekelund et al., 2005) and insulin resistance (Singhal et al., 2003). However, the effect of fatness in infancy on later obesity has not been adequately studied. This is important due to the nutrition transition occurring in developing countries with higher availability of energy-dense complementary foods (Uauy et al., 2001).

The observed significant improvement in hemoglobin concentration with the consumption of the study blends supports what was expected and is attributable to the fact that the blends were adequately fortified with micronutrients including iron. The blends also resulted in improved micronutrient status. The observation in the current study of improved haemoglobin concentration levels of about 6 g/L with the provision of the blends agrees with Faber et al. (2005) in South Africa that fortified maize-meal porridge resulted in 9 g/L

differences in haemoglobin concentration. The differences in effect levels between the two studies may be explained by the fact that although in the South African study the length of follow up was 6 months unlike in current study of 9 months, there was more intense follow up and encouragement to consume the study food in the South African study than was done in the current study. The current study also fortified the blends with iron at a lower rate (5.2mg vs. 11 mg per 40g of dry product) compared to the South African study. The same results were reported for other similar multi-centre studies (Hop et al., 2005; Smuts et al., 2005) assessing the effect of multiple micronutrient supplementation on infant growth and micronutrient status among others detailed in [Table 2-5](#) with a few giving contrary results (Lartey et al., 1999; Oelofse et al., 2001).

7.2.3.2 Nutrient intake

The results show that there were no significant differences in energy and phytate intake from complementary foods, however the CSB+ group had significantly lower intakes of calcium, iron and greater phytate: iron molar ratios compared to WFC and WFL. The observation that study complementary foods resulted in higher micronutrient intake was expected and support results from similar studies (Moursi et al., 2003; Faber et al., 2005) that found increased iron and zinc intake among infants given multi-micronutrient fortified maize-based blend.

7.3 SOME METHODOLOGICAL ISSUES

7.3.1 Selection of study participants

The study participants can be assumed to have substantially increased provision of food supplements, enhanced caregivers attention and compliance to the feeding and completeness of information obtained and consequently internal validity of the study. Children living in the study area who were excluded from the study, e.g., those who did not attend child welfare clinic or did not meet the inclusion criteria might have differed in certain characteristics from

subjects under study with the consequence that results cannot easily be extrapolated to them. However, the study was designed as an efficacy study where external validity is subordinate. Further, it is likely that an effect of the intervention might have even been greater in children who due to less favorable living conditions were more stunted, less well nourished, and/or more prone to infectious diseases.

7.3.2 Allocation of study participants to study groups

The infants were individually randomized into the study groups by sex in six blocks. The treatments having been randomized by study participants meant that each health facility had to be accessed for food delivery. Our study outcomes, i.e., growth (length and weight gain) and iron status, are not likely to have been directly influenced by choosing the unit of randomization because these are outcomes independent of the study participants. Nevertheless, the validity of our results might have been affected by this randomization process if children in the study groups differed in certain characteristics that influence the outcome measures. In fact, children in the WFC group were taller with higher MUAC and Head circumference at baseline than the other children. We however believe this was by chance and that the results obtained are valid. Further, other factors, such as sex, age, and socio-economic status were controlled for in all analyses and we therefore believe that the results are valid.

7.3.3 Blinding

In order to avoid information bias, it would have been preferable to carry out the study with triple-blindness, i.e., study participants, project staff, and data analysts are kept unaware of allocation. The nature of a community-based study of this magnitude and duration providing food supplements made blinding virtually impossible to be maintained throughout the study especially in cases participants in different food groups came from the same homestead. However, the community and project staffs were kept blind to study hypotheses

as much as possible. Lack of total blinding could have led to differences in measurements of outcome variables between groups. Measurements of anthropometry were not likely to have been influenced by subjectivity of the enumerators, as against measurements of morbidity that were more prone to participant and observer effects because they were collected by interview. In order to minimize subjective influence on data collection, a number of procedures were incorporated in the study, such as triplicate measurements of anthropometry by two enumerators, monitoring of intra-team and inter-team measurement error, reliability checks, repeated training, and rotation of enumerators between all health facilities. The lack of complete blinding is therefore not expected to have biased the study results.

7.3.4 Assessment of outcomes

Morbidity was measured as disease events having occurred in the week prior to the interview. This has the advantage that respondents are more likely to remember facts compared with a longer recall period. Interviews are more prone to bias than measurements such as those carried out for anthropometry, because e.g., the educational status of the respondent might influence their knowledge about diseases. However in our study, the educational level of caregivers, who were the main respondents in the interviews, were comparable between groups and this was not likely to have affected the validity of the recall based measurements. Due to financial and logistical restrictions, morbidity interviews were carried out only once per month and children who were found severely ill or anaemic at any time point of the study were referred for treatment, which would have been unethical not to do so. For these reasons, illness prevalence and severity might have been underestimated. Of particular concern are diseases that do not occur frequently and might not have always been captured by the interviews. However time sampling over 9 months and over all seasons should have given a fair estimate of morbidity.

Most importantly assessment of morbidity was similar for all children in all groups and validity of results, which is based on group comparisons, is therefore not likely to be affected. Nevertheless the lack of sensitivity in measuring morbidity might explain our findings on the effect of the food supplements on morbidity though it was not a primary outcome.

7.3.5 Study duration

Food supplements were provided for 9 months and this duration can be expected to have been sufficiently long to detect any changes in growth and micronutrients status. Studies of micronutrient supplementations could already demonstrate an effect on growth for durations as short as 3 months (Owino et al., 2007a). Indeed many studies have been able to record differences in 6 months (Lartey et al., 1999; Faber et al., 2005). However, quantities of micronutrients provided in those studies were much higher than those provided by the food supplements in the current study and impacts expected to be seen faster.

7.3.6 Data analysis

Data analysis was carried out on an intention-to-treat basis and involved all children who were originally included in the study irrespective of their compliance or dropout.

7.3.7 Confounding

The study outcomes can be influenced by various other factors than the provision of the food supplement. Growth as well as micronutrients status is the result of genetic and environmental influences. Growth and micronutrients status can largely be attributed to nutritional wellbeing and exposure to and treatment of infectious diseases. Underlying factors are therefore socioeconomic status and other family characteristics. Randomization should have assured that potential known and unknown confounding factors were balanced amongst groups. However, due to unexplained reasons, study groups might have differed in certain characteristics affecting the outcomes. This problem was addressed by adjusting the

multivariate models for age, sex, socio-economic status, and various others potential confounders.

7.3.8 *Environmental stressors*

Probability of the high prevalence of diseases, such as malaria and intestinal parasites in the study children as has been observed in similar settings (Siekman et al., 2003) may have contributed to anorexic effects, impaired nutrient absorption (Prentice and Paul, 2000), micronutrient losses in urine, increased metabolism, and/or impaired transport of nutrients to target tissues (Stephenson, 1999; Brown et al., 1990). Consequently, energy and nutrient requirements might have been increased in the children and nutrients provided by the food supplements are likely to not have been used to their full potential.

7.4 STUDY LIMITATIONS

Although the right amounts (based on recommended values and manufacturer's instructions) of mineral and vitamin premix was added to the blends and that thorough mixing was done during industrial production, it was not possible to perform micronutrient analyses on the final products. The loss of subjects to follow up at about 14% was larger than the 10% that had been assumed based on previous studies in Ghana (Lartey et al., 1999) and South Africa (Faber et al., 2005) in similar settings. This could have been due to among other reasons the longer intervention time of 9 months unlike in the 2 cited studies where the intervention time was 6 months each. The current loss was however lower than 28% observed in a similar study in a mid-income group though for a shorter period of 3 months (Owino et al., 2007a). Out of the total of 499 mother-infant pairs (165,167 and 167 for WFC, CSB+ and WFL respectively) that were recruited into the study and received the study complementary blend at least once, 63 (89%) mother-children pairs were relocated for various reasons including family reunion with spouses in the cities and separation of spouses due to domestic wars. Few (4) were children of single parents and the mothers left to be married elsewhere.

One mother reported that her infant did not like the blend (WFC) while one mother was dropped upon realising during the compliance visits that she actually sold the blend and did not feed it to her child. One mother could also not be traced at the initial given address nor through phone number as she was actually a visitor to a home in the study catchment area and she did not declare this at recruitment. Eight infants (11%) died due to illness in the course of follow up.

Based on results that showed that energy intake was not limiting in this locality, it was postulated that iron deficiency anaemia would be of more importance in this setting and this is supported by Maberly et al. (1998) that reported micronutrient deficiencies to affect a much larger proportion of the population than those who have evident clinical symptoms and infants and that children in both urban and rural settings are likely to be affected. A sample size that would allow for detection of differences of at least 5 g/L in haemoglobin concentration (Lartey et al, 1999) was calculated. At 80% power and 5% significance we needed at least 58 infants per group. Allowing for 10% loss to follow up, a total of 65 infants were to be recruited per group. Based on the numbers left per study arm at endline we could detect about 1.0 g/L differences in haemoglobin concentration since the numbers recruited were higher than 65 making the findings valid.

The second limitation of the current study is the lack of a pure control group. Ethically the foods having been improved were deemed superior and thus there was no non-intervention group to compare with. Although historical events (Cook and Campbell, 1979) occurring between the cross section study and the end of the intervention period (after 9 months) could have affected any observed differences between intervention and the cross section control group, comparisons in this study are mainly confined to CSB+ as a positive control given that the WFP is currently recommending use of the same in similar settings. The

cross section study results were subject to seasonal variations and lack of randomization when recruiting the subjects.

The other possible source of error in the current study could have been the leakage of porridge to other members within the index infant's family or to families not in intervention thereby reducing the daily ration for the participating child. To prevent intra-household porridge leakage, the mothers were told to feed the porridge to the study child only. Moreover, nutrient intake data in this study may be limited by lack of continuously monitored compliance data. The data obtained was based on mothers' monthly verbal report on the use of the blend and a one off compliance household visit in a sub-sample of the intervention study group. It was difficult for mothers to keep a food diary during this study given the literacy levels.

The inability to assess factors such as aflatoxins, gut permeability and HIV infection that could confound growth and haemoglobin concentration outcomes are also other shortcomings of the present study. Owino et al. (2007a) highlighted some of these factors as issues of concern. The fact that infants who had symptoms of serious illness were excluded at 6 months in the intervention groups may have induced bias. To avoid any bias due to instrumentation, all measuring equipment were calibrated regularly. Weighing scales were calibrated weekly. Batteries for the Hemocue haemoglobin concentration meter were oftely checked and replaced.

In summary the study was faced with a number of drawbacks which should also be considered in future studies. Some of these drawbacks were:

1. Inability to determine the malaria parasitemia due to complications with the slides preparations
2. Haemolysis of the drawn blood samples thus drastically reducing the sample size for the biochemical determinations.

3. Inability to determine intestinal parasites (or control for them eg by deworming) which have been shown to contribute to malnutrition as part of the infections
4. Inability to determine serum zinc as earlier planned making us drop the zinc status from being an initial specific objective
5. Inability to determine IGF-1 which could have further enabled us to explain the growth phenomena observed.

7.5 GENERAL CONCLUSION

The habitual diet of the children in the study area is of poor dietary quality with low intakes of animal source foods and high prevalence of inadequate intakes of certain nutrients, particularly calcium and iron. The linkage between nutrients supply, growth and morbidity is critical, mostly in the very early years of life, but also thereafter. Prevalences of simultaneous multiple nutrient deficiencies, stunting and diseases in the children is high and it was assumed that the provision of food supplements with animal source foods and multi-micronutrients fortification would improve their growth and iron status. The benefits of the porridge blends for infant growth are modest; however, there is greater evidence of benefits on haemoglobin concentration and micronutrient (iron) status which could lead to improved child health. This could mean that adequate nutrient consumption alone will not significantly impact the problem of stunting in the rural areas of Western Kenya. Further, the early stunting (prior to 6 months) among breastfed infants might be caused by factors other than dietary deficiency (e.g., pre-natal factors or environmental enteropathy). The industrially processed fortified complementary foods based on locally available and widely used indigenous ASFs like edible termites and *dagaa* small fish could thus be used to improve growth, haemoglobin concentration and micronutrients (iron) status among infants in resource limited, malaria endemic settings in developing countries. Further work on the effect of the improved complementary foods on growth and body composition is recommended.

7.6 STUDY APPLICATIONS

This study was carried out as part of a larger trial acronymed- The WINFOOD study using the same complementary foods. WINFOOD is funded by the Danish International Development Agency (DANIDA). Apart from growth and micronutrients (vitamin A and iron) status reported in the current study, child development outcomes were measured alongside, body composition (as lean and fat mass), and mental and motor development and results reported elsewhere. These additional measurements may help bridge the limitations identified in the current study when the interpretations are made all inclusive. Moreover, subject to further investigations, the current study provides a model on how generic foods based on the concepts discussed herein can be developed and optimised for the target populations based on their context.

7.7 IMPLICATIONS

The Global Strategy for infant and young child feeding (WHO, 2002; WHO, 2005) recommends that infants be exclusively breastfed for the first six months of life and thereafter should receive appropriate complementary foods with continued breastfeeding for up to two years or beyond. The alleviation of growth impairment is complicated by the complexity of growth mechanisms, however, it is certain that simultaneous multiple nutrient deficiencies play a role. Multiple micronutrient deficiencies seem too common in rural areas in developing countries. When choosing a suitable nutrition intervention strategy to address micronutrient malnutrition it has to be considered that functional deficits may not be alleviated by the provision of single micronutrients.

Although the addition of the edible termites and *dagaa* small fish and the multi-micronutrients fortification did not bring about all the expected effects, study findings indicate that micronutrients contained in high amounts and in a bioavailable form are beneficial for hemoglobin and iron status improvement in the setting of the study population.

The promotion of edible termites and *dagga* fish as animal source food consumption by children therefore seems to be a viable food-based approach to provide highly bioavailable nutrients simultaneously to children and at the same time to improve iron bioavailability. The study findings suggest a possible beneficial effect for health particularly in improving iron status.

Food-based approaches are rather long term solutions aiming at the prevention of micronutrient deficiencies than a short-term correction of the problem and can lead to more diverse diets with an improvement of the overall diet quality. The question arises how feasible it is for low-income households in developing countries to increase production and consumption of edible termites. There are several constraints that might limit an increase in household production of the edible insects but compared to the conventional animal source foods like milk, meat etc they could be cheaper and more friendly to the environment as has been indicated by other workers (Oonincx et al., 2010; Oonincx and de Boer, 2012). A policy environment needs to be created to support such initiatives as this could create jobs and support contribution to value addition. It might also be linked with other positive outcomes, such as increased knowledge about foods, nutrition and health, an increase in dietary diversity, an improvement in nutritional status, and income generation and especially in settings where the indigenous animal source foods are already acceptable (Ayele et al., 2003; Gibson et al., 2003; Maretzki & Mills, 2003; Roos et al., 2003). Processed foods based on edible insects have been developed (Ayieko et al., 2010), such as buns and might be another possibility to increase their consumption especially in older children who can consume the solid products as buns. When developing programs that aim at increasing the consumption of such animal source foods in children, potential constraints have to be evaluated within the specific context and nutrition education components such as social marketing and positive deviance approaches have to be included. Key to any successful and

sustainable food-based program is the involvement of the community through participatory approaches.

Given that the diets of children in developing countries are generally very low in fat, the dietary fat provided by edible termites and such like foods is even advantageous because it is a concentrated source of energy and enhances absorption of fat-soluble nutrients. Further, it is not likely that any additional fat provided by animal foods would result in fat intakes that exceed current recommendations (Haskell & Brown, 1997; Newman et al., 2003; Rivera et al., 2003; Murphy et al., 2003). However, the potential adverse health effects linked with an increased intake of such foods should not be ignored by policies that focus on such production promotion efforts (Popkin & Du, 2003).

Nutrition is interrelated with poverty and environmental factors, and issues of safe water, sanitation and caring practices need to be addressed in any nutrition program. Deworming children has been shown to improve the iron status and physical growth of children (ACC/SCN, 2001) and the prevention and treatment of infections, particularly helminths and malaria, can have immediate benefits for growth and health of affected children.

7.8 FUTURE RESEARCH

The use of locally available ASFs as edible termites and *dagaa* small fish to process improved complementary foods should be further encouraged to enhance the acceptability and to reduce complementary food cost. This study indicates that infants may not receive enough nutrient levels from traditional foods to allow for adequate growth and haemoglobin concentration. There is currently limited data on the benefits of locally processed or commercial fortified indigenous animal source foods (e.g. edible insects) based complementary foods and especially the nutritionally rich but neglected on growth and nutritional status of infants thus need for more work.

Based on the results of this study further research is recommended in the following areas:

1. There is need to further interpret the findings of the current study in the light of other collected data on body composition using the stable deuterium isotope to assess the impact of the nutritional intervention on infant body composition to ascertain what comprises weight gain (fat or fat free mass).
2. Evaluate the contribution of pre-gestational and gestational nutrition on early infant stunting.
3. Examine the aetiology of stunting prior to 6 months of age, including environmental enteropathy
4. Determine if dietary interventions, such as WFC and WFL will be efficacious if coupled with other interventions like deworming, addition of Vitamin C in the diets etc.
5. Assessment of the marketability of industrially processed fortified complementary foods based on locally available ASFs like edible termites and others of the kind in both urban and rural settings. This should be along with the costings to assist in correct pricing.
6. Investigate in a collaborative research the possibility to raise the edible insects for such kind of interventions and what could be the economic benefit to the local populations if the efforts can be done by the locals as an economic activity.
7. What is responsible for rapid decline in weight-for-age in infants from 6 months of age even in the presence of nutritional intervention with fortified complementary foods?
8. Assess what is the likely effect of short-term nutritional intervention with fortified complementary foods between 6 and 15 months of age on later development of adult diseases such obesity, diabetes or insulin resistance?
9. Acquire knowledge about detailed composition of common meals, particularly their content of micronutrients and their absorption promoters and inhibitors if realistic recommendations in dietary modification are to be made to improve micronutrient bioavailability.

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ANNEXES

ANNEX 1: CSB+ FORMULA, MICRONUTRIENT RATE AND CHEMICAL FORM PER 100 GRAMS PRODUCT

WFP technical specification for CSB+, vers.1.6

| N° | Ingredients | Percentage (by weight) |
|----|--|------------------------|
| 1 | Corn (maize white or yellow) | 72.05- 77.05 |
| 2 | Whole soya beans | 20-25 |
| 3 | Vitamin/Mineral FBF-V-10 | 0.2 |
| 4 | CaCO ₃ (calcium carbonate) | 1.19 |
| 5 | Ca(H ₂ PO ₄) ₂ . H ₂ O (mono calcium phosphate) | 0.80 |
| 6 | KCl (potassium chloride) | 0.76 |

The composition of the micronutrient pre-mix is identical in CSB+
Micronutrient rate and chemical form for CSB+

| | Target | Chemical forms |
|---------------------------------|----------|--|
| <i>Vitamin/Mineral FBF-V-10</i> | | |
| Vitamin A | 1,664 IU | Dry vitamin A palmitate 250 n.s |
| Thiamine | 0.128 mg | Thiamine mononitrate |
| Riboflavin | 0.448 mg | Riboflavin |
| Niacin | 4.8 mg | Nicotinamide |
| Pantothenic acid | 6.7 mg | Calcium d-pantothenate |
| Vitamin B6 | 1.7 mg | Pyridoxine hydrochloride |
| Folate | 60 mcg | Folic acid |
| Vitamin B12 | 2 mcg | Vitamin B12 – 0.1% spray dried |
| Vitamin C | 100 mg | Ascorbic acid |
| Vitamin D | 4 mcg | Dry vitamin D3 100 CWS |
| Vitamin E | 8.3 mg | Vitamin E 50% CWS |
| Vitamin K | 100 mcg | vitamin K1 5% CWS |
| Iron (a) | 4 mg | Ferrous fumarate |
| Iron (b) | 2.5 mg | Iron-sodium EDTA |
| Zinc | 5 mg | Zinc oxide |
| Iodine | 40 mcg | Potassium iodate (KIO ₃) |
| Carrier | Qs | Malto dextrin |
| Other minerals | | |
| Calcium (a) | 470 mg | Calcium carbonate (CaCO ₃) |
| Potassium | 400 mg | Potassium chloride (KCl) |
| Phosphorus | 200 mg | Mono calcium phosphate Ca(H ₂ PO ₄) ₂ . H ₂ O |
| + Calcium (b) | 130 mg | |

ANNEX 2: 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:

| | | |
|--|--|--|
| <p>TO The researcher (PI) Attn: (Silvenus Ochieng Konyole), Institute of Tropical and Infectious Diseases, University of Nairobi (UNITID). P.O. Box 19676-00202, NAIROBI. Tel.:0202726765/0723 269 286 Email:konyole2000@yahoo.com</p> | <p>OR The Director, UNITID P.O. Box 19676-00202, NAIROBI. Tel.:0202726765, Email: unitid@uonbi.ac.ke Attn : Prof. B.B.A. Estambale</p> | <p>OR The Secretary, KHN/UON Ethics Research Committee, Kenyatta National Hospital, Hospital Rd, P.O Box 20723, NAIROBI Tel 726300-9. Fax: 725272 Email:KHNplan@Ken.He althnet.org</p> |
|--|--|--|

ANNEX 3: A STUDY FOOD SAMPLE QUALITY 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, 605553

Page 1 of 1

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

PRIVATE SAMPLE

Date: 02 March 2011

| | |
|--|---|
| 1. Description of Sample: Amaranth Based Flours | 6. KEBS Sample Ref.No: BS/03532/11 |
| 2. Sample Submitted by: Jomo Kenyatta University of Agriculture & Tech. | 7. Date of Receipt: 18 February 2011 |
| 3. Customer Contact: John Ndungu | 8. Date Analysis Started: 21 February 2011 |
| 4. Customer's Ref. No: | 9. Sample Submission Form No: 47608 |
| 5. Customer's Address: P.O. BOX 62000-00200, Nairobi Kenya | |
| 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 |

CAUTION:

This report refers to a privately submitted sample, and all details in respect of the source and test results of similar products are not verified or confirmed.


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



The results contained herein apply only to the particular sample(s) tested whose sample submission form serial number is herein quoted, and to the specific tests carried out, as detailed in this Test Report. No exact arrangement or discussion from a Test Report may be published or used to advertise a product without the written consent of the Managing Director, KEBSA. Sample and Report Numbers: If these are not included in the above, contact the Laboratory.

ANNEX 4: FIELD INTRODUCTION LETTER-UNIVERSITY



UNIVERSITY OF NAIROBI
Institute of Tropical & Infectious Diseases

Office of the Director

College of Health Sciences
Kenyatta National Hospital
Tel. 254-20-272 6765
Fax. 254-20-272 6626

P.O. Box 19676 - 00202
Nairobi - Kenya.
E-mail: unitid@uonbi.ac.ke
Website: <http://unitid.uonbi.ac.ke>

27/01/2011

The District Medical Officer of Health,
Mumias District,

Dear Sir,

RE: LETTER OF INTRODUCTION – SILVENUS OCHIENG KONYOLE

This is to confirm that Mr. Silvenus Ochieng Konyole, Registration Number W80/80217/10 is a PhD (Nutrition) student in University of Nairobi. He is preparing to undertake data collection and has applied for ethical clearance to carry out research on the "Effect of improved complementary foods on growth, iron and zinc status of Kenyan infants". The study will be carried out in Mumias District of Western Province.

The purpose of this letter is to ask you to allow him carry out an acceptability test of the intervention foods that will be used later for the controlled randomized trial. The foods have been analyzed in the laboratory of Food Science and Post harvest Technology of Jomo Kenyatta University of Agriculture and Technology and found to be both safe and meeting the nutritional requirements for the target group of infants (6-23 months)

Kindly accord him the necessary assistance.

Regards,

PROF. BENSON B.A. ESTAMBALE.
DIRECTOR

ANNEX 5: FIELD INTRODUCTION LETTER-MINISTRY OF HEALTH



MINISTRY OF PUBLIC HEALTH AND SANITATION

Telephone: 0202121280
When Replying Quote:
OurRéf:

Medical Officer of Health,
Mumias District,
P.O. Box 494 – 50102,
MUMIAS.

Date: 1st March 2011

The Health Officer,
In-charge Makunga Health Centre,
P.O. Box 494,
MUMIAS.

Dear Sir,


RE: SILVENUS OCHIENG KONYOLE.

The above named is a PhD (Nutrition) student in University of Nairobi. He is currently carrying out a data collection on the "Effect of improved complementary foods on growth, iron and zinc status of Kenyan infants" as per the attached letter of introduction.

He has been received by the DMOH and is deployed to your station for data collection and any assistance required.

Thank you.

Yours faithfully,


Mr. Mariga Peter,
FOR: Medical Officer Of Health
MUMIAS DISTRICT

**MEDICAL OFFICER OF HEALTH
MUMIAS DISTRICT**

ANNEX 6: STUDY QUESTIONNAIRES-ACCEPTABILITY AND CROSS SECTION STUDY

**WINFOODS ACCEPTABILITY QUESTIONNAIRE
MARCH 2011**

1.0 INSTRUCTIONS

1. Select children who are between the ages of 6 months to under 24 months (Use vaccination cards, or health card to ascertain age)
2. Explain to each carer that this is a new food and we want to know what they think of it so that we can make it even better.
3. Emphasize that the food is nutritious and that it has been tested in certified laboratory.
4. Ensure friendly environment for each child to taste the food, without other children prompting a response.
5. After explaining the study, ask the parent/guardian to sign the informed consent form.
6. Measure and record child's weight, height/length as per demographic table in page 3 only on the FIRST day
7. Mothers will prepare porridge as a group
8. Each child will be served porridge individually in graduated cups
9. Amount of porridge served and amount left after feeding must be recorded (advise mothers not to pour out the remaining porridge after feeding)
10. During feeding observe child's behavior and record (i.e. 1) child signaling for more, 2) child turning head away at second offer, 3) child spitting food, 4) child smiling, 5) child crying, etc).
11. After feeding, provide mother with 100g porridge flour and instruct her to prepare the porridge at home and feed the child
12. Find out about how the child consumed porridge at home the following day

CHILD ID:- ___/___/___ DAY ___ DATE:- ___/___/2011

**WINFOOD ACCEPTABILITY TESTING
INFORMED CONSENT FORM**

I, being the parent/ guardian of ----- (name of the child), have been given the explanation about this study.

I have understood what has been explained to me and my questions have been answered satisfactorily. I understand that I can change my mind any time and it will not affect the management of my child.

Please fill the boxes as required:

(Please tick) **I have agreed to allow my child to participate**

Parent/guardian's signature----- Date----- (dd/mm/yyyy)

Parent/guardian's name -----Time ----- (24hrs)

(Please print your name)

I certify that I have followed all the study procedures in the S.O.P for obtaining consent.

Investigator's /nominee's signature -----Date -----(dd/mm/yyyy)

Investigator's / nominee's name -----Time ----- (24hrs)

(Please print your name)

The Parent/Guardian's thumb print as named above if he /she cannot write_____

CHILD ID:- ___/___/___ DAY ___

DATE:- ___/___/2011

1.0 DEMOGRAPHIC INFORMATION

| DEMOGRAPHIC INFORMATION AND ANTHROPOMETRY | | | | |
|---|---|---------------------------------|----------------------------|---|
| Age | -----Months | | Location: Sub-location: | |
| Sex | Male <input type="checkbox"/> | Female <input type="checkbox"/> | Length ___ . ___ cm | |
| Birth weight | ___ . ___ Kg(N/A for missing cards) | | | |
| Current weight | ___ . ___ Kg | | MUAC ___ . ___ cm | |
| Others | No of siblings | | Birth order ___ out of | |
| | No of children <5yrs in the HH | | | |
| | Total No of people living in the HH | | | |
| | Is the child breastfeeding | Y <input type="checkbox"/> | N <input type="checkbox"/> | Is the child weaned? Y <input type="checkbox"/> N <input type="checkbox"/> |
| | If, weaned, Is the child receiving any kind of purchased/donated food? Y <input type="checkbox"/> N <input type="checkbox"/> | | | |
| | If Yes, specify the kind of purchased/donated foods | | | |
| | Were you involved in Global Network project/MOMM? probe for the 2. Y <input type="checkbox"/> N <input type="checkbox"/> | | | |
| | If Yes, What benefits/items did you get from the project?----- ----- | | | |
| | What other project are/were you involved in and what were/are the benefits? Project: ----- Benefits: ----- | | | |

Health questionnaire**I. HEALTH**1. Has child been ill in the last TWO days? Yes No Don't know 2. Do you know the name of the illness? Yes No

If yes, specify _____

3. General symptoms:

Fever? Yes No Cough? Yes No Diarrhoea? Yes No Vomiting? Yes No Fits? Yes No Stomachache? Yes No Skin rashes? Yes No Difficulty breathing? Yes No

Other? Specify -----

4. Did you do anything to treat the illness? Yes No

If yes, state:

>Bought medicines from shop? Yes [] No >Went to traditional healer? Yes [] No >Went to clinic/dispensary? Yes [] No >Went to hospital outpatients? Yes [] No 5. Admitted to hospital? Yes [] No

CHILD ID:- __/__/__/DAY __ DATE:- __/__/2011WINFOOD ID:- __/__/__/

3.0 FEEDING OBSERVATION

Preparation

Provide porridge in graduated cup to the parent / guardian together with a plastic spoon.

Ask the mother to feed the CHILD with porridge and take notes.

Ask the mother to give the PORRIDGE to the child.

1. Watch the child's facial expressions as they first see the food. Record the child's expressions according to the scale (1-5) provided below:-
 1. Dislike extremely
 2. Dislike slightly
 3. Neither like nor dislike
 4. Like slightly
 5. Like extremely
2. Ask the mother to feed the baby. Observe and record expression as the child receives food first. Use scale (1-5) as below:-
 1. Dislike extremely
 2. Dislike slightly
 3. Neither like nor dislike
 4. Like slightly
 5. Like extremely
3. Observe and record expression as the child receives food in subsequent offers. Use scale (1-5) below:-
 1. Dislike extremely
 2. Dislike slightly
 3. Neither like nor dislike
 4. Like slightly
 5. Like extremely
4. Observe and record child's behavior during subsequent offers of porridge(Tick all applicable)
 1. Child signaling for more aggressively
 2. Child accepts porridge passively
 3. Child turns head away,
 4. Child spitting food,
 5. Child smiling while eating,
 6. Child crying, refusing to eat
 7. Child crying, but eats
 8. Child vomits
 9. Any other observations on the child's reaction to the food.

-----5. Write down any other comment made by the mother / carer or the child that are not captured above.-----

6. How much porridge did the child consume during this feeding?

| | | | |
|-------------------------|--|--|--|
| Amount served (ml) = A | | | |
| Amount spilt (ml) = B | | | |
| Amount left (ml) = C | | | |
| Amount eaten = A- (B+C) | | | |

4.0 FOLLOW UP ON DAY AFTER OBSERVATION

Adherence Questionnaire (Ask mother the following questions)

1. Were there any problems with the porridge when prepared at home?
If yes, explain-----

2. How much porridge was the child able to eat at home?
None ¼ ½ ¾ All
3. Did anyone else in the household share the porridge?
If Yes, Tick as many as appropriate
Adult Sibling Other child
4. If child, in 3 above, what was the age of the child? _____ months
5. Did the other child (in 4 above) like the porridge? Use scale below (1-5):-
 1. Dislike extremely
 2. Dislike slightly
 3. Neither like nor dislike
 4. Like slightly
 5. Like extremely

Health questionnaire

| | | | | |
|--|------------------------------|-----------------------------|-------------------------------------|-----------------------------|
| I. HEALTH | | | | |
| 1. Has child been ill in the last TWO days? | Yes <input type="checkbox"/> | No <input type="checkbox"/> | Don't know <input type="checkbox"/> | |
| 2. Do you know the name of the illness? | Yes <input type="checkbox"/> | No <input type="checkbox"/> | | |
| If yes, specify _____ | | | | |
| 3. General symptoms: | | | | |
| Fever? | Yes <input type="checkbox"/> | | | No <input type="checkbox"/> |
| Cough? | Yes <input type="checkbox"/> | No <input type="checkbox"/> | | |
| Diarrhoea? | Yes <input type="checkbox"/> | No <input type="checkbox"/> | | |
| Vomiting? | Yes <input type="checkbox"/> | | | No <input type="checkbox"/> |
| Fits? | Yes <input type="checkbox"/> | No <input type="checkbox"/> | | |
| Stomachache | Yes <input type="checkbox"/> | | | No <input type="checkbox"/> |
| Skin rashes | Yes <input type="checkbox"/> | | | No <input type="checkbox"/> |
| Difficulty breathing? | Yes <input type="checkbox"/> | | | No <input type="checkbox"/> |
| Other? Specify ----- | | | | |
| 4. Did you do anything to treat the illness? | Yes <input type="checkbox"/> | No <input type="checkbox"/> | | |
| <i>If yes, state:</i> | | | | |
| >Bought medicines from shop? | Yes <input type="checkbox"/> | <u>frequency</u> [] | No <input type="checkbox"/> | |
| >Went to traditional healer? | Yes <input type="checkbox"/> | [] | | No <input type="checkbox"/> |
| >Went to clinic/dispensary? | Yes <input type="checkbox"/> | [] | | No <input type="checkbox"/> |
| >Went to hospital outpatients? | Yes <input type="checkbox"/> | [] | | No <input type="checkbox"/> |
| 5. Admitted to hospital? | Yes <input type="checkbox"/> | [] | No <input type="checkbox"/> | |

CHILD ID:- __/__/__/ **DAY** __ **DATE:-** __/__/2011 **WINFOOD ID:-** __/__/__/

3.0 FEEDING OBSERVATION

Preparation

Provide porridge in graduated cup to the parent / guardian together with a plastic spoon. Ask the mother to feed the CHILD with porridge and take notes. Ask the mother to give the PORRIDGE to the child.

1. Watch the child’s facial expressions as they first see the food. Record the child’s expressions according to the scale (1-5) provided below:-
 1. Dislike extremely
 2. Dislike slightly
 3. Neither like nor dislike
 4. Like slightly
 5. Like extremely
2. Ask the mother to feed the baby. Observe and record expression as the child receives food first. Use scale (1-5) as below:-
 1. Dislike extremely
 2. Dislike slightly
 3. Neither like nor dislike
 4. Like slightly
 5. Like extremely
3. Observe and record expression as the child receives food in subsequent offers. Use scale (1-5) below:-
 1. Dislike extremely
 2. Dislike slightly
 3. Neither like nor dislike
 4. Like slightly
 5. Like extremely
4. Observe and record child’s behavior during subsequent offers of porridge(Tick all applicable)
 1. Child signaling for more aggressively
 2. Child accepts porridge passively
 3. Child turns head away,
 4. Child spitting food,
 5. Child smiling while eating,
 6. Child crying, refusing to eat
 7. Child crying, but eats
 8. Child vomits
 9. Any other observations on the child’s reaction to the food.-----

5. Write down any other comment made by the mother / carer or the child that are not captured above.-----

6. How much porridge did the child consume during this feeding?

| | | | |
|-------------------------|--|--|--|
| Amount served (ml) = A | | | |
| Amount spilt (ml) = B | | | |
| Amount left (ml) = C | | | |
| Amount eaten = A- (B+C) | | | |

4.0 FOLLOW UP ON DAY AFTER OBSERVATION

Adherence Questionnaire (Ask mother the following questions)

1. Were there any problems with the porridge when prepared at home?

If yes, explain-----

2. How much porridge was the child able to eat at home?

None ¼ ½ ¾ All

3. Did anyone else in the household share the porridge?

If Yes, Tick as many as appropriate

Adult Sibling Other child

4. If child, in 3 above, what was the age of the child? _____ months

5. Did the other child (in 4 above) like the porridge? Use scale below (1-5):-

1. Dislike extremely
2. Dislike slightly
3. Neither like nor dislike
4. Like slightly
5. Like extremely

Health questionnaire

I. HEALTH

1. Has child been ill in the last TWO days? Yes No Don't know

2. Do you know the name of the illness? Yes No

If yes, specify _____

3. General symptoms:

| | | |
|-----------------------|------------------------------|-----------------------------|
| Fever? | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Cough? | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Diarrhoea? | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Vomiting? | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Fits? | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Stomachache | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Skin rashes | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Difficulty breathing? | Yes <input type="checkbox"/> | No <input type="checkbox"/> |

Other? Specify -----

4. Did you do anything to treat the illness? Yes No

If yes, state:

| | | | |
|--------------------------------|----------------------------------|-----------------------------|-----------------------------|
| | <i>frequency</i> | | |
| >Bought medicines from shop? | Yes <input type="checkbox"/> [] | No <input type="checkbox"/> | |
| >Went to traditional healer? | Yes <input type="checkbox"/> [] | No <input type="checkbox"/> | No <input type="checkbox"/> |
| >Went to clinic/dispensary? | Yes <input type="checkbox"/> [] | No <input type="checkbox"/> | No <input type="checkbox"/> |
| >Went to hospital outpatients? | Yes <input type="checkbox"/> [] | No <input type="checkbox"/> | No <input type="checkbox"/> |

5. Admitted to hospital? Yes [] No

CHILD ID:- __/__/__/ DAY __ DATE:- __/__/2011 WINFOOD ID:- __/__/__/

3.0 FEEDING OBSERVATION

Preparation

Provide porridge in graduated cup to the parent / guardian together with a plastic spoon.

Ask the mother to feed the CHILD with porridge and take notes.

Ask the mother to give the PORRIDGE to the child.

1. Watch the child's facial expressions as they first see the food. Record the child's expressions according to the scale (1-5) provided below:-
 1. Dislike extremely
 2. Dislike slightly
 3. Neither like nor dislike
 4. Like slightly
 5. Like extremely
2. Ask the mother to feed the baby. Observe and record expression as the child receives food first. Use scale (1-5) as below:-
 1. Dislike extremely
 2. Dislike slightly
 3. Neither like nor dislike
 4. Like slightly
 5. Like extremely
3. Observe and record expression as the child receives food in subsequent offers. Use scale (1-5) below:-
 1. Dislike extremely
 2. Dislike slightly
 3. Neither like nor dislike
 4. Like slightly
 5. Like extremely
4. Observe and record child's behavior during subsequent offers of porridge(Tick all applicable)
 1. Child signaling for more aggressively
 2. Child accepts porridge passively
 3. Child turns head away,
 4. Child spitting food,
 5. Child smiling while eating,
 6. Child crying, refusing to eat
 7. Child crying, but eats
 8. Child vomits
 9. Any other observations on the child's reaction to the food.

5. Write down any other comment made by the mother / carer or the child that are not captured above.-----

6. How much porridge did the child consume during this feeding?

| | | | |
|-------------------------|--|--|--|
| Amount served (ml) = A | | | |
| Amount spilt (ml) = B | | | |
| Amount left (ml) = C | | | |
| Amount eaten = A- (B+C) | | | |

4.0 FOLLOW UP ON DAY AFTER OBSERVATION

Adherence Questionnaire (Ask mother the following questions)

1. Were there any problems with the porridge when prepared at home?
If yes, explain-----
2. How much porridge was the child able to eat at home?
None ¼ ½ ¾ All
3. Did anyone else in the household share the porridge?
If Yes, Tick as many as appropriate
Adult Sibling Other child
4. If child, in 3 above, what was the age of the child? _____ months
5. Did the other child (in 4 above) like the porridge? Use scale below (1-5):-
 1. Dislike extremely
 2. Dislike slightly
 3. Neither like nor dislike
 4. Like slightly
 5. Like extremely

Health questionnaire

I. HEALTH

1. Has child been ill in the last TWO days? Yes No Don't know
2. Do you know the name of the illness? Yes No Don't know
If yes, specify _____
3. General symptoms:

| | | |
|-----------------------|------------------------------|-----------------------------|
| Fever? | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Cough? | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Diarrhoea? | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Vomiting? | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Fits? | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Stomachache | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Skin rashes | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Difficulty breathing? | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| Other? Specify | ----- | |
4. Did you do anything to treat the illness? Yes No
If yes, state:

| | Yes <input type="checkbox"/> | <u>frequency</u> [] | No <input type="checkbox"/> |
|--------------------------------|------------------------------|----------------------|-----------------------------|
| >Bought medicines from shop? | Yes <input type="checkbox"/> | [] | No <input type="checkbox"/> |
| >Went to traditional healer? | Yes <input type="checkbox"/> | [] | No <input type="checkbox"/> |
| >Went to clinic/dispensary? | Yes <input type="checkbox"/> | [] | No <input type="checkbox"/> |
| >Went to hospital outpatients? | Yes <input type="checkbox"/> | [] | No <input type="checkbox"/> |
5. Admitted to hospital? Yes [] No

Child Feeding Practices: Ask mother/caretaker following questions and record answer as *verbatim as possible*.

1. What are the circumstances under which babies are fed?

2. What infant behavior is regarded as normal during feeding?

3. Which are the foods that your child likes most?

4. Why does the child like the food(s)?

5. Which are the foods that your child dislikes most? _____

6. Why does the child dislike the food(s)? _____
7. What are the reasons that will make the baby NOT to eat as usual? _____
8. Think of words describing feelings of satisfaction of the baby _____
9. How is/ or was child feeding done in times of scarcity? _____

**SENSORY EVALUATION QUESTIONNAIRE FOR WINFOODS FLOUR BY
MOTHERS/CAREGIVERS**

Hedonic Test

Name: _____ Date _____ Panelist No. _____

You are provided with three (3) coded samples of flour.

A. Please rate the samples (1-5) according to the scale provided below by filling in the table against each sample and attribute.

1. Dislike extremely
2. Dislike slightly
3. Neither like nor dislike
4. Like slightly
5. Like extremely

| Sample Code | Color/ Appearance | Aroma | Texture | Overall preference |
|-------------|----------------------|-------|---------|--------------------|
| 841 | | | | |
| 869 | | | | |
| 871 | | | | |

Any other comments: _____

B. Which of the three flours did you prefer most?

C. Why did you prefer the flour?

**SENSORY EVALUATION QUESTIONNAIRE FOR WINFOODS PORRIDGE BY
MOTHERS/CAREGIVERS**

HEDONIC TEST

Name: _____ Date _____ Panelist No. _____

You are provided with three (3) coded samples of porridge.

A. Please rate the samples (1-5) according to the scale provided below by filling in the table against each sample and attribute.

1. Dislike extremely
2. Dislike slightly
3. Neither like nor dislike
4. Like slightly
5. Like extremely

| Sample Code | Color/ Appearance | Aroma | Taste | Mouth Texture | feel/ feel/ | Overall preference |
|-------------|----------------------|-------|-------|------------------|----------------|--------------------|
| 543 | | | | | | |
| 568 | | | | | | |
| 571 | | | | | | |

Any other comments:

B. Which of the three porridges did you prefer most?

C. Why did you prefer the porridge?

WINFOOD PROJECT
CROSS-SECTIONAL STUDY PROTOCOL IN WESTERN KENYA

PROJECT MESSAGE
WINFOOD Project-Cross sectional Study, Nov –Dec 2011

Salutation/Greetings!

We are researchers from the WINFOOD project based at the University of Nairobi and JKUAT. We are studying nutritional status of children 6 months to 2 years (24 months) old. I would like to ask questions about yourself, the eating pattern and health of your child. We will also measure the child's height, weight among others. The results of the study will help us find better ways of feeding children in Kenya and Africa. The information you give us will be confidential. Your acceptance or rejecting of this offer will in no way compromise services/ privileges offered to you in this or another hospital or other projects you are involved in. Do you accept to participate in this activity?

INTRODUCTION AND SOCIO-DEMOGRAPHICS

1. Health centre name: 2. Infant ID
3. Date of dietary assessment : ____/____/____(dd/mm/yyyy)
4. Name of mother (caretaker)
5. Village
6. Household Name:
7. Nearest landmark (e.g. school, market church etc)
8. Mother (caretaker) telephone contact
9. Secondary telephone contact (husbands/neighbour's etc)
10. Name of infant
11. Does (name) have a clinic/growth monitoring card? Yes No
12. Date of birth
13. Sex of infant: M F
14. Infants' birth order
15. Is the infant (name) a twin s p
If No, go to question 18.
16. If Yes, Name of the twin sibling
- 17 Sex of the twin sibling M F
18. Did infant (name) sleep under an Insecticide treated bed net last night? Yes No
19. Age of **main** caretaker (in Years)
- 20 Sex of **main** caretaker M F
21. What is the relationship of **main** caretaker to the infant (name):
 - 1) Mother
 - 2) Father
 - 3) Grandmother
 - 4) Sister/brother
 - 5) other (specify) _____

- 3) Grand mother
22. If answer to question 23 above is mother, how many children has she given birth to? _____
23. Marital status of **main** caretaker:
- 1) Married
 - 2) Widowed
 - 3) Single
 - 4) Separated
 - 5) Divorced
24. Religion of main caretaker:
- 1) Protestant
 - 2) Catholic
 - 3) Indigenous churches
 - 4) Muslim
 - 5) Others (specify) _____
25. Educational status of **main** caretaker:
- 1) Unable to read and write
 - 2) Informal education (e.g. tailoring, mechanics)
 - 3) Primary education completed
 - 4) Primary education and informal course (e.g. tailoring, mechanic, driver etc)5)
 - Secondary/High school completed
 - 6) College/University graduate
26. **Main** source of income for the household:
- 1) Farming
 - 2) Self employed
 - 3) Salaried employment
 - 4) Remittance
 - 5) Other (specify) _____
27. Total number of people in the household _____
28. Number of children under five years of age in the household _____
29. **Main** source of the **drinking** water for the household? _____

30. INFANT MORBIDITY

| | |
|---|---------------|
| A. In the last one week, the baby been.....(CIRCLE ONE) | |
| 1. well | |
| 2. mild illness but recovered without treatment | |
| 3. moderate illness which needed drugs or clinic visit to see nurse | |
| 4. serious illness requiring doctor | |
| B. In the past week, has the baby had: (1=yes, 2=no) | Yes/No |
| Convulsions | |
| Loose watery diarrhea | |
| Diarrhea with blood or mucus | |
| Coughing | |
| Running nose | |
| Fever | |
| Difficulty in breathing | |
| Vomiting | |
| Eye problem (redness, discharge) | |
| Ear problem (discharge)/pain | |
| Feeding poorly | |
| Lethargic child | |
| Skin rashes | |
| Constipation | |
| Other morbidity; specify | |
| Medicine taken – (1=Yes, 2=No) | |

C. Dietary assessment

I will now ask you some questions about breast feeding of your infant

31. Is the infant (name) currently breastfeeding? Yes No
32. If No, at what age did the infant (name) stop breast feeding? _____ Months
33. Do you use any kind of milk apart from breastmilk? Yes No
34. If yes indicate type of milk used? _____
35. Apart from breast milk, does the child (name) eat any other food? Yes No
36. If yes, what was the first food introduced? _____
37. At what age did you introduce the food? _____
38. Is there any type of commercial infant food/formula you use for child feeding? Yes No
39. If yes, what is the source? _____

I will now ask you some questions about how the infant fed since yesterday

E. FOOD RECALL FORM

Village _____ Date of data collection ___ / ___ / ___ Day of week: _____ Time of Recall: ___ / ___

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

For animal source food, food description should include: 1) local name of the animals (be specific with different species of aquatic animals) 2) parts eaten (skin, head, meat, bones etc) 3) Type of serving in general (soup, fried) and in local name.

NB: Every morning calibrate the equipment to ensure accuracy.

40. Infants anthropometric indicators

| Indicator | Reading 1 | Reading 2 | Reading 3 | Average |
|---------------------------|-----------|-----------|-----------|---------|
| Weight (kg) | | | | |
| Length (cm) | | | | |
| MUAC (cm) | | | | |
| Head circumference (cm) | | | | |
| Triceps skinfold (mm) | | | | |
| Biceps skinfold (mm) | | | | |
| Subscapular skinfold (mm) | | | | |
| Suprailiac skinfold (mm) | | | | |
| Oedema (Yes/No) | | | | |

Thank you for your time.

ANNEX 7: STUDY QUESTIONNAIRE FOR THE RANDOMISED TRIAL
RANDOMISED CONTROLLED TRIAL OF THE EFFECT OF IMPROVED
COMPLEMENTARY FOODS ON INFANT GROWTH, BODY COMPOSITION AND
GROSS MOTOR DEVELOPMENT

WINFOODS PROJECT
DATA COLLECTION QUESTIONNAIRE JANUARY 2012

SECTION 1: Enrolment and Admission (6 to 6.5 months)

1. Health centre name:
2. Date of Admission : ____/____/____ (dd/mm/yyyy)
3. Name of mother (caretaker)
4. Village
5. Homestead Name:
6. Nearest landmark (e.g. school, market, church etc)
7. Mother (caretaker) telephone contact
8. Secondary telephone contact (neighbour's etc)
9. Name of infant
10. Does (name) have a clinic/growth monitoring card? Yes No
11. Infant's date of birth: ____/____/____ (dd/mm/yyyy)
12. Age of infant: months
13. Sex of infant: M F
14. Infants' birth order
15. Is the infant (name) a twin? Y No
16. If Yes, Name of the twin sibling
17. Sex of the twin sibling: M F
18. Is the infant (name) currently breastfeeding? No
19. If No, at what age did the infant (name) stop breastfeeding? Months
20. Did infant (name) sleep under an insecticide treated bed net last night? No
21. Age of main caretaker: _____ (in years)
22. Sex of main caretaker:
23. What is the relationship of main caretaker to the infant (name):
 - 4) Mother
 - 4) Sister/brother
 - 5) Father
 - 5) other (specify) _____
 - 6) Grand mother
24. If answer to question 23 above is mother, how many children has she given birth to? _____

25. Marital status of main caretaker:

- 1) Married
- 2) Widowed
- 3) Single
- 4) Separated
- 5) Divorced

26. Religion of main caretaker:

- 1) Protestant
- 2) Catholic
- 3) Indigenous churches
- 4) Muslim
- 5) Others (specify) _____

27. Educational status of main caretaker:

- 1) Unable to read and write
- 2) Informal education (e.g. tailoring, mechanics)
- 3) Primary education incomplete
- 4) Primary education completed
- 5) Secondary/High school completed
- 6) College/University graduate

28. Main source of income for the household:

- 1) Farming
- 2) Self employed
- 3) Salaried employment
- 4) Remittance
- 5) Other (specify) _____

29. Total number of people in the household _____

30. Number of children under five years of age in the household _____

31. **Main** source of the **drinking** water for the household?

| |
|--|
| |
|--|

32. CLINICAL EXAMINATION

| | | |
|----|---|---|
| A. | GENERAL CONDITION | Well/alert.....1 Restless/irritable.....2 Abnormally sleepy.....3 Sleeping, could not be assessed.....4 |
| B. | RESPIRATORY RATE/MINUTE <i>(count for 60 sec and repeat)</i> | 1: <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> 2: <input style="width: 20px; height: 20px;" type="text"/> <input style="width: 20px; height: 20px;" type="text"/> |
| C. | COUGHING | Yes1 No.....2 |
| D. | RUNNING NOSE | Yes1 No.....2 |
| E. | NASAL FLARING | Yes1 No.....2 |
| F. | AUDIBLE WHEEZING OR GRUNTING | Yes1 No.....2 |
| G. | SEVERE CHEST INDRAWING | Yes1 No.....2 |
| H. | BULGING FONTANELLE | Yes1 No.....2 |
| I. | EYE PROBLEM (<i>redness, discharge</i>) | Yes1 No.....2 |
| J. | EAR PROBLEM (<i>e.g. discharge</i>) | Yes1 No.....2 |
| K. | SKIN RASH OR PUSTULES | Yes1 No.....2 |
| L. | JAUNDICE | Yes1 No.....2 |
| M. | TREATMENT REQUIRED | None.....1 Home care without medicine.....2 Home care with medicine.....3 Hospitalization.....4 |
| N. | OEDEMA | Yes1 No.....2 |

| | | |
|----|---------------------|---------------------|
| O. | MUAC | |
| P. | AGE CONFIRMATION |MONTHS |
| Q. | DECISION TO RECRUIT | Yes1 No.....2 |

33. INFANT MORBIDITY-CARETAKERS REPORT

| | |
|---|---------------|
| A. Since the last ONE WEEK, the baby been.....(CIRCLE ONE) | |
| 5. well | |
| 6. mild illness but recovered without treatment | |
| 7. moderate illness which needed drugs or clinic visit to see nurse | |
| 8. serious illness requiring doctor | |
| In the past week, has the baby had: (1=yes, 2=no) | Yes/No |
| B.Convulsions | |
| C.Loose watery diarrhea | |
| D.Diarrhea with blood or mucus | |
| E.Coughing | |
| F.Running nose | |
| G.Fever | |
| H.Difficulty in breathing | |
| I. Vomiting | |
| J.Eye problem (redness, discharge) | |
| K.Ear problem (discharge)/pain | |
| L.Feeding poorly | |
| M.Lethargic child | |
| N.Skin rashes | |
| O.Constipation | |
| P.Other morbidity; specify | |
| Q.Medicine taken – (1=yes, 2=no) | |

37. INFANTS ANTHROPOMETRIC INDICATORS AT RANDOMIZATION

| Indicator | Reading 1 | Reading 2 | Reading 3 | Average |
|-----------------------------|-----------|-----------|-----------|---------|
| A. Weight (kg) | | | | |
| B Length (cm) | | | | |
| C MUAC (cm) | | | | |
| D Head circumference (cm) | | | | |
| E Triceps skinfold (mm) | | | | |
| F Biceps skinfold (mm) | | | | |
| G Subscapular skinfold (mm) | | | | |
| H Suprailiac skinfold (mm) | | | | |

38. DIETARY ASSESSMENT

I will now ask you some questions about breast feeding of your infant

- A. Is the infant (name) currently breastfeeding? Yes No
- B. If No, at what age did the infant (name) stop breastfeeding? Months
- C. Do you use any kind of milk apart from breastmilk? Yes No
- D. If yes indicate type of milk used? _____
- E. Apart from breast milk, does the child (name) eat any other food? Yes No
- F. If yes, what was the first food introduced? _____

G. Is there any type of commercial food you use for child feeding? Yes No
 H. If yes, what is the source? _____

38.FOOD RECALL FORM

Village _____ Date of data collection __ / __ / __ Day of week: __ Time of Recall: __ / __

I will now ask you some questions about how the infant fed since yesterday at such a time

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

*For animal source food, food description should include: 1) local name of the animals (be specific with different species of aquatic animals) 2) parts eaten (skin, head, meat, bones etc) 3) Type of serving in general (soup, fried) and in local name.

NB: Every morning calibrate the equipment to ensure accuracy.

39. COMPLIANCE

I will now ask you some questions about the utilization of the complementary food that you have received before in the household

| | |
|--|---|
| <p>A. Can I please see the food filled WINFOOD ration packs from the monthly food ration?</p> | <p>No. of empty packs <input type="text"/> <input type="text"/></p> <p>Answer refused 88</p> <p>Don't know 99</p> |
| <p>B. Can I please see the empty WINFOOD ration packs from the monthly food ration?</p> | <p>No. of empty packs <input type="text"/> <input type="text"/></p> <p>Answer refused 88</p> <p>Don't know 99</p> |
| <p>C. Yesterday, did you prepare and serve any of the WINFOOD food you received? (If No to this question – end the interview)</p> | <p>Yes 1</p> <p>No 2</p> <p>Answer refused 8</p> <p>Don't know 9</p> |
| <p>D. How many TABLESPOONS did you use to prepare the food yesterday?</p> | <p>No. of used TABLESPOONS..... <input type="text"/> <input type="text"/></p> <p>Answer refused 88</p> <p>Don't know 99</p> |

| | |
|--|---|
| <p>E. Yesterday, who in the household ate the prepared WINFOOD you prepared?</p> <p>Circle ALL applicable answers</p> | <p>(name).....</p> <p>(Another child in the HH under 5 years of age)....</p> <p>(Another child in the HH 5 years or older).....</p> <p>Father</p> <p>Mother</p> <p>Elderly member</p> <p>Other (specify).....</p> <p>Answer refused</p> <p>Don't know</p> |
| <p>F. (Yesterday, what proportion of the prepared WINFOOD you prepared was eaten by (name)?)</p> <p>(Circle ONLY ONE answer)</p> | <p>(None)</p> <p>(Less than 1/2)</p> <p>(About half)</p> <p>(Most)</p> <p>(All)</p> <p>(Answer refused)</p> <p>(Don't know)</p> |

40. BLOOD SAMPLING AND HEMOGLOBIN MEASUREMENT

| | |
|--|--|
| A.Haemoglobin (g/dl) determined on site* | |
| B.Ferritin (mg/dl) | |
| C.Transferritin receptors(mg/dl) | |
| D.Creatine Reactive Protein | |
| E.AGP | |
| F.Plasma Zinc (mg/dl) | |

***To be measured on site USING HEMOCUE MACHINE**

ANNEX 8: STUDY ETHICS CLEARANCE



KENYATTA NATIONAL HOSPITAL
Hospital Rd. along Ngong Rd.
P.O. Box 20723, Nairobi
Tel: 725300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi
Email: KNH-plan@Ken-Healthnet.org
7th April 2011

Ref: KNH-ERC/ A/80

Silvenus Ochieng Konyole
W80/83217/13
UNITID
University of Nairobi

Dear Konyole,

RESEARCH PROPOSAL: EFFECT OF IMPROVED COMPLEMENTARY FOODS ON GROWTH, IRON AND ZINC STATUS OF KENYAN INFANTS" (P436/12/2010)

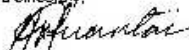
This is to inform you that the KNH/UON-Ethics & Research Committee has reviewed and **approved** your above revised research proposal for the period 7th April 2011 – 6th April 2012.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimens must also be obtained from KNH/UON-Ethics & Research Committee for each batch.

On behalf of the Committee, I wish you a fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely,


PROF. A. N. GUANTAI
SECRETARY, KNH/UON-ERC



c.c. The Deputy Director CS, KNH
The HOD, Records, KNH
Supervisors Prof. B. A. Estambale, UNITID, UON
Dr. Victor O. Owino, Valid Nutrition, Kenya

ANNEX 9: SAMPLE INFORMATION AND CONSENT FORM

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 omema 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+ and CSB++), which is normally distributed by World Food Programme (WFP). Both CSB products contain the important vitamins and minerals, but CSB++ also contains milk powder. 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

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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 stable isotope deuterium-labelled water. First we will sample saliva from your child, about one teaspoon (max. 2 ml), then give your child stable 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 stable 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 is 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 in case of any unexpected observation. In case of any, the patient 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 corn-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 allow the special water to be given to my child to drink when provided on selected days over one day on two occasions. I agree that I will allow the study group to collect saliva/urine from my child and myself over the selected days on both occasions.

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 haemoglobin concentration, iron and zinc status at health facilities

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): Silvanus Ochieng Konyole Institute of Tropical and Infectious Diseases, University of Nairobi P.O. Box 19676-00202, NAIROBI. Tel.: 0202726765 Mobile: +254 723 269 286 Email: - konyole2008@yahoo.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, KHNUON Ethics Research Committee, Kenyatta National Hospital, Hospital Road, P.O Box 20723, NAIROBI Tel 726300-9 Fax: 725272 Telegrams: ME DSUP, 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.



ANNEX 10: ANNUAL ETHICS RENEWAL APPROVAL



UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
PO BOX 1879, CoE, 00202
Tel: 020 273000 ext 4439

KNH/UN-ERC
Email: knh@unbi.ac.ke
Website: www.unbi.ac.ke
Link: unbi.ac.ke/activities/KNH/UN

Ref. No: KNH/ERC/R/16

Silverus Ochieng Konyole
W90/30217/10
UNITED
University of Nairobi

Dear Silverus

Re: Approval for Annual Renewal - study titled "Effect of improved complementary foods on Growth, iron and zinc status of Kenyan infants" (P436/12/2010)

Re: your communication of February 1, 2012

This is to grant you annual extension of approval for ethical research
Protocol P436/12/2010.

The renewal periods are: 7th April 2012 – 6th April 2013.

The approval is subject to compliance with the following requirements:

- Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
- All change (amendments, deviations, violations etc) are submitted for review and approval by KNH/UN-ERC before implementation.
- Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events which/or related or unrelated to the study must be reported to the KNH/UN-ERC within 72 hours of notification.
- 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/UN-ERC within 72 hours.
- Submission of a request for renewal of approval at least 30 days prior to expiry of the approval period. *(Attach a comprehensive progress report to support the renewal).*
- Clearance for export of biological specimens must be obtained from KNH/UN-Ethics & Research Committee for each batch of shipment.

"Protect to discover"



KENYATTA NATIONAL HOSPITAL
P.O. BOX 21725, CoE, 00202
Tel: 726304 9
Fax: 726272
E-mail: MH@KNUH.Nairobi

5th March 2012

ANNEX 11: ETHICS APPROVAL- SAMPLES SHIPMENT FOR ANALYSIS



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
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/SH/ 10

22nd May 2013

Silvenus Ochieng Konyole
University of Nairobi Institute of Tropical and Infectious Diseases
P.O. Box 19676-00202
NAIROBI

Dear Silvenus

RE: APPROVAL FOR SHIPMENT OF SAMPLES -STUDY TITLED "EFFECT OF IMPROVED COMPLEMENTARY FOODS ON GROWTH, IRON AND ZINC STATUS OF KENYAN INFANTS" (P436/12/2010)

Refer to your communication of 20th May, 2013.

The KNH/UON-ERC has reviewed and approved shipment of the following samples to Canada and Germany for analysis:

931 samples of serum – for analysis of serum zinc, ferritin and transferrin receptor cells.
432 samples of whole blood – for analysis of essential fatty acids and lipid profile [cholesterol, high and low density lipoproteins]

The samples will be under the care of the following contact persons and addresses:

Serum: - Dr. Juergen Erhardt,
Kastanienweg 5,
77731 Willstaett, Germany
Telephone : +49-7852-933070
Email: mail@nutrisurvey.net

Whole blood: - University of Waterloo
c/o Ken Stark
Burt Mathews Hall, Room 2417
200 University Avenue West
Waterloo, Ontario, Canada
N2L 3G1
Email: kstark@uwaterloo.ca
Telephone: (519) 888-4567 ext 377738

"Protect to Discover"

ANNEX 12: MINISTRY OF HEALTH AUTHORITY TO SHIP BIOLOGICAL SAMPLES



**MINISTRY OF HEALTH
OFFICE OF DIRECTOR OF MEDICAL SERVICES**

Telegrams: "MINHEALTH". Nairobi
Telephone; Nairobi 2717077 Fax: 2715239

OFFICE OF DIRECTOR OF
MEDICAL SERVICES
AFYA HOUSE
CATHEDRAL ROAD
P.O. BOX 30016
NAIROBI

MMS/ADM/3/8/VOL.V111

16th July, 2013

Prof. B.B. A. Estambale
Director, UNITID
University of Nairobi
Institute of Tropical & Infectious Diseases (UNITID)
P.O. Box 19676 – 00202
NAIROBI

RE: AUTHORITY TO SHIP BIOLOGICAL SAMPLES

Your request for specimen export permit dated 8th July, 2013 refers.

The title of your study is noted to be "*Effect of improved complementary foods on growth, iron and zinc status of Kenyan Infants (P436/12/2010).*"

Authority is hereby granted for shipment of biological samples to this research work which include.

- 931 samples of serum – for analysis of serum zinc, ferritin and transferrin receptor cells
- 432 samples of whole blood- for analysis of essential fatty acid and lipid profile {cholesterol, high and low density lipoproteins}.

The shipment contact details are as follows:

| | | |
|--------------------|---|---|
| Serum | - | Dr. Juergen Erhardt, Kastanienweg 5, 77731 Willstaett, Germany E-Mail: mail@nutrisurvey.net |
| Whole blood | - | University of Waterloo c/o Ken Stark Burt Matthews Hall, room 2417 200 University Avenue West Waterloo, Ontario, Canada N2L 3G1 Email: Kstark@uwaterloo.ca Tel: (519) 888-4567 Ext 377738 |

Dr. Lucy W. Musyoka
FOR: DIRECTOR OF MEDICAL SERVICES

ANNEX 13: CONTROLLED TRIALS NUMBER ASSIGNMENT LETTER

FW: ISRCTN30012997 assigned to your trial (ref: CCT-NAPN-22085) 1

<FF62426E5C774341A2DCEC720B8502190328B0F4@SEGBLOGR0008.springer-sbm.com>, "Kavita Mistry" <Kavita.Mistry@controlled-trials.com> wrote:

ISRCTN30012997 - The WINFOOD Intervention Study: the effect of improved complementary foods on nutrition and health among infants in Western Kenya

Dear All,

I am pleased to inform you that the following ISRCTN has been assigned to your trial:

<http://www.controlled-trials.com/ISRCTN30012997>

When quoting the ISRCTN, please make sure that no space is inserted between the ISRCTN and the actual number. Please refer to http://www.controlled-trials.com/isrctn/sample_documentation.asp for further guidance notes about how to use the ISRCTN. Please also note that once a trial has been registered on the ISRCTN Register and publicly displayed on the website, the study will remain permanently on the register and cannot be deleted (as per our letter of agreement on http://www.controlled-trials.com/isrctn/isrctn_loa)

CCT's sister company, [BioMed Central](#), currently publishes over 220 peer-reviewed open access journals, in particular the journal *Trials*, dedicated to publishing protocols, results and other issues relevant to clinical trials. Selected BioMed Central journals offer a 20% discount on the article processing charge to protocol authors who have registered their trial with the ISRCTN register. Authors should request a waiver during the submission process and provide their ISRCTN trial ID.

If you have any further questions about the use of the ISRCTN, please do not hesitate to contact me. Best wishes,

Kavita Mistry

Database Editor

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ANNEX 14: STUDY BACKGROUND INFORMATION

This was a collaborative study among the Faculty of LIFE, University of Copenhagen, Denmark; the University of Nairobi Institute of Tropical and Infectious Diseases, Kenya; All Grain Products, Kenya; Department of Food Science and Technology, Jomo Kenyatta University of Agriculture and Technology, Kenya. The study titled “Alleviating Childhood Malnutrition by Improved Utilization of Traditional Foods and acronymed “WINFOOD” had the overall aim of developing nutritionally improved foods for IYC in resource limited settings, based on improved utilization of locally available foods including semi-domesticated and wild indigenous foods from uncultivated land or aquatic environment. The study covered 5 broad result areas namely:

1. Identification of consumed traditional foods and processing technologies utilized by local communities,
2. Nutrient composition determination of identified locally available foods and
3. Development and optimization of iron and zinc dense complementary foods for feeding of IYC based on the identified locally available foods
4. Testing the acceptability of the optimized iron and zinc dense complementary foods based on the identified locally available foods
5. Testing the efficacy of the developed and optimized improved complementary foods to address MAM which is currently a problem in resource limited settings.

Results areas 1 to 3 are reported in details in Kinyuru, (2012). The study reported in this thesis is based mainly on the work covering the last two result areas (4 and 5) of the WINFOOD project.

ANNEX 15: OVERALL STUDY STAGES

The study consisted of three successive stages. **Figure 1** presents the main study stages and activities undertaken between February 2011 and January 2013. The **first stage** assessed the acceptability of the three centrally processed complementary foods namely:

1) Maize-germinated grain amaranth-edible termite- *dagaa* fish complementary food naturally enriched with iron and zinc from the ASFs (WINFOOD Classic (WFC))

2) Multi-micronutrient (vitamins and minerals) fortified maize-germinated grain-amaranth complementary food (WINFOOD Lite (WFL)) and

3) Multi-micronutrient fortified corn soy blend plus (CSB+).

To achieve this goal stage one of the study aimed to assess the acceptability of the three foods (WFC, WFL and CSB+) among the study population prior to the RCT.

The **second stage** was cross-sectional survey of basic anthropometric measures (height, weight and age) of non-enrolled IYC 6-24 months conducted in villages adjacent to those selected for the intervention study to assess the prevailing nutritional status to help interpret the results of the main intervention study/randomized controlled trial (RCT). A sample size of 600 children was targeted. Skin fold measurements and a dietary assessment were also carried out in a sub-sample.

The **third stage** involved a RCT of the effect of the above improved complementary foods developed on growth and iron status of infants 6-15 months old.

Expected benefits and potential uses of the study results

The WFC and WFL developed for this study were 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. Potential uses can be in (national) nutrition programs aimed at food-insecure populations, for production by Non-Governmental Organisations working in the mother-child health field and/or income-generating projects, programs targeting especially vulnerable population groups and also commercial production by local food companies. The composition of WINFOODs can also be adapted in recipes for homemade improved complementary foods. These results can also act as good evidence-based guidance for policy-makers to improve and strengthen the national policies on nutrition in Kenya in the future.

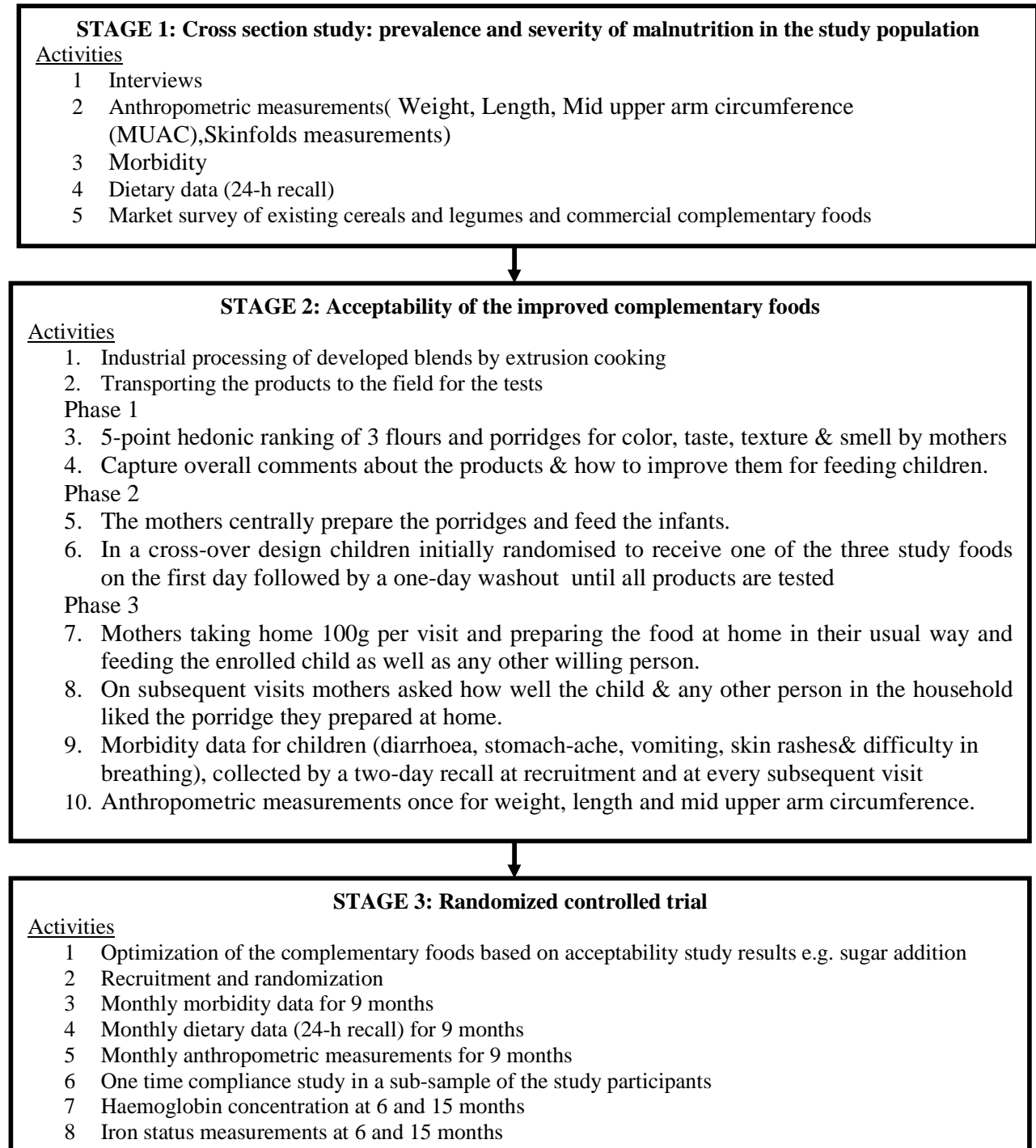


Figure 1: Main study stages and activities undertaken

ANNEX 18: THE STUDY SITE

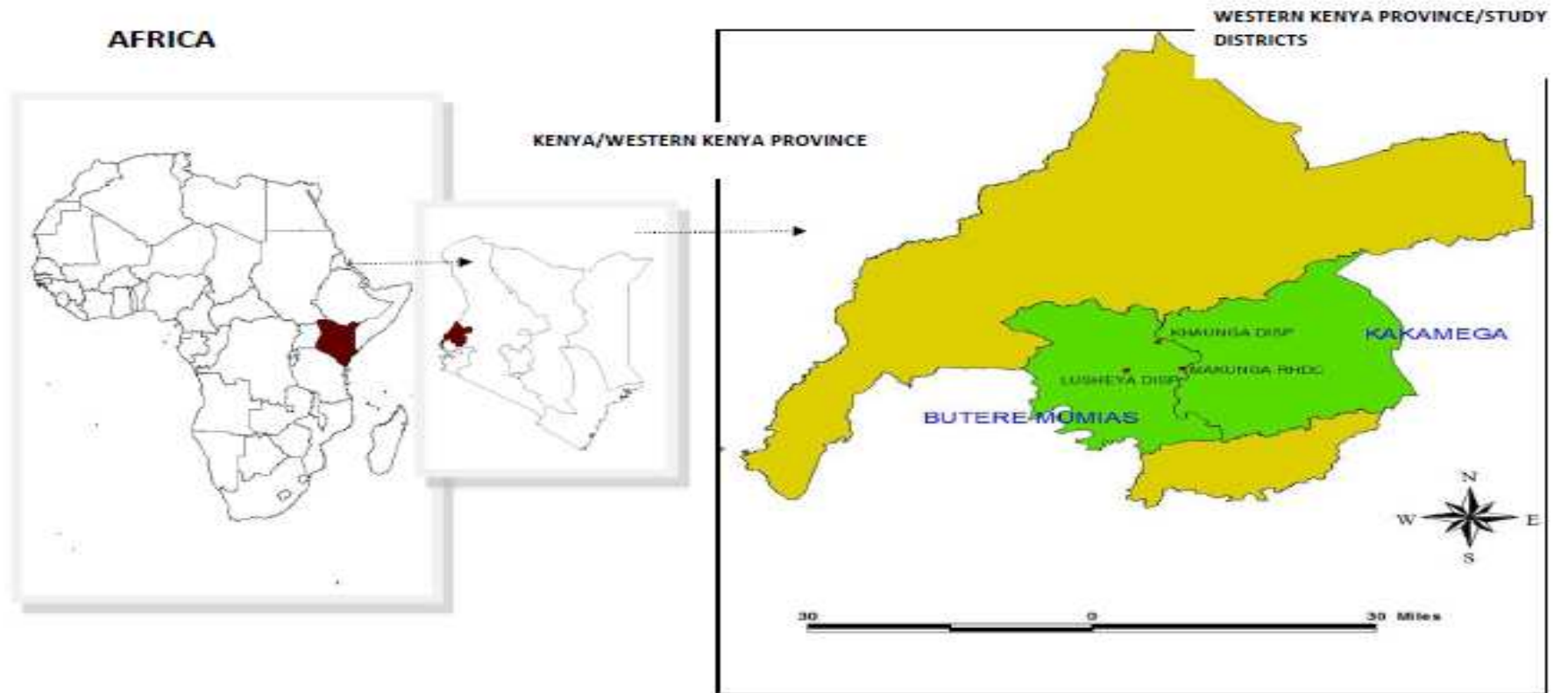


Figure 3: The study area within Kenya

ANNEX 19: SAMPLE SIZE DETERMINATION

The sample size calculation was based on the primary hypothesis that Infants 6-15 months consuming the improved complementary foods (WFC and WFL) would have higher total energy and micronutrient intake thus greater linear growth and micronutrient status (Fe) than those consuming CSB+. According to the data on the standard deviation of the increase in length, Dewey et al., (2011) has observed differences in prevalence between interventions for stunting at 0.5 SD in LAZ while Lartey et al., (1999) observed length gain of 0.1 (SD 1.2) cm. Since the primary outcome was length, the sample size n , determined as per the formula:

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

Where:

n =sample size

$u=0.64$ which corresponds to β for the test of 95% CI

$v= 1.94$, corresponds to an α of 5% two tailed test

- s_1 = the standard deviation of length in group 1
- s_2 = the standard deviation of length in group 2
- m_1 and m_2 = corresponding means for groups 1&2

With (s_1 & $s_2=1.2$ and $(m_1-m_2) =0.1$; $n=150$)

To allow for loss to follow up, 10% added as observed by Faber et al.(2005) and Lartey et al.(1999) to each arm giving $n=165$ for a Total $N=(165 \times 3=495)$. Thus, the sample size per group, n , required to detect a change, d , in a continuous variable with standard deviation, s , with 90% power, was $16 \times s^2/d^2$.

Because micronutrient deficiencies, especially of iron was equally expected to be of concern, the sample size calculated above would allow for the detection of differences of equal to or greater than 5 g/L in haemoglobin concentrations at 90% power and 5% significance as observed by Lartey et al 1999 ($n=241$) and Faber et al 2005. Faber et al. (2005) in a similar study detected 9.4microg/L (95% CI: 3.6, 15.1microg/L) for serum Ferritin and 9 g/L (95% CI: 6, 12 g/L) for haemoglobin conc. at 90% power and 5% level of significance with $n=361$. Two-tailed distribution was assumed because of the fact that giving free nutrient-dense food though unlikely to decrease nutrient intake could still lead to a reduction on some of the outcomes.