CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Adequate nutrition during infancy and early childhood is fundamental to the development of each child's full potential. The period from conception to two years of age is a "critical window" of opportunity for promotion of optimal growth, health and behavioural development. Longitudinal studies have consistently showed that the period between six and twenty-three months of life is the peak period for growth faltering, deficiencies of certain micronutrients, and common childhood illnesses. After a child reaches two years of age, it is very difficult to reverse stunting that has occurred earlier (Martorell , Kettel Khan , Schroeder , 1994; PAHO/WHO, 2003). The immediate consequences of poor nutrition during these formative years include significant morbidity and mortality, and delayed mental and motor development (Black , Allen , Bhutta, Caulfield, de Onis, Ezzati, Mathers, Rivera*,* 2008). In the long-term, early nutritional deficits are linked to impairments in intellectual performance, work capacity, reproductive outcomes, and overall health during adolescence and adulthood. Thus, the cycle of malnutrition continues, as the malnourished girl child faces greater odds of giving birth to a malnourished, low birth weight infant when she grows up (Black et al., 2008; EDHS, 2005). Animal source proteins provide the best proteins for good diets. However, animal proteins are quite expensive in most of the Sub Sahara African (SSA) countries, hence, the high dependence on plant protein. The need to find inexpensive sources of protein of good quality cannot be overemphasized. Plant proteins are known to have limiting amino acids (Okpala & Okoli, 2011; Ihekoronye and Ngoddy, 1985). From about six months of age, energy and certain nutrients supplied by breast milk is no longer adequate to meet an infant's needs, hence, the need for complementary foods (CFs) with a relatively high energy and nutrient density (WHO, 1998). In low-income countries, however, the nutritional adequacy of CFs is often compromised, predisposing infants and young children to micronutrient deficiencies and growth faltering (WHO, 1998; Black *et al*., 2008; Anderson *et al*., 2008a; Lander *et al*., 2008).

1.1.1 Overview of Complementary Feeding

World Health Organization (WHO) defines complementary feeding as "*a process starting when breast milk alone is no longer sufficient to meet the nutritional requirements of infants, and therefore other foods and liquids are needed, along with breast milk*" (WHO, 2001). In order to provide infants with additional nutrients, complementary foods (foods other than breast milk or infant formula) should consequently be introduced to the infants (USDA, 2009). The target age range for complementary feeding is between the age of 6 and 23 months (with continued breastfeeding), where most infants reach a general and neurological stage of development (chewing, swallowing, digestion, and excretion) that enables them to be fed other foods rather than breast milk (WHO/UNICEF, 2003 and Monte & Giugliani, 2004). Complementary foods could be especially designed transitional foods (to meet particular nutritional or physiological needs of infants) or general family foods, and are expected to address the gaps between the daily energy and nutrient requirement of infants and young children and the amount obtained from breastfeeding (WHO, 2009).

In several parts of the developing world, complementary feeding continues as a challenge to good nutrition in children of 6–23 months (WHO/UNICEF, 1998). In India, for instance, 54.5% of children between the ages of 6 and 8 months had received any complementary foods in the previous day, but only 7% of breastfed children between the ages of 6 and 23 months met the minimum acceptable diet criteria.

In Nigeria, only 21% of breastfed children receive the minimum acceptable complementary feeding diet (UNICEF, 2011). However, in Ethiopia, only 4.2% of breastfed children of 6–23 months of age have a minimum acceptable diet (EDHS, 2012). The challenges during complementary feeding are context specific, but many are common across settings. They are often characterized by poor feeding practices and poor dietary quality of homemade complementary foods (Krebs *et al*., 2011; Dewey & Adu-Afarwuah, 2008 and Plessis, Kruger, & Sweet., 2013). Poor feeding practices are characterized by poor timing of complementary foods introduction (too early or too late); infrequent feeding; and poor feeding methods, hygiene, and child care practices (WHO/UNICEF, 2003 & WHO, 2001). Added to these is the poor dietary quality of the foods served, characterized as too little variety; inappropriate consistency (food is too thin or too thick); too few essential vitamins and minerals, especially vitamin A, iron, zinc, and calcium; too few essential fatty acids; and too few calories among non-breastfed infants.

The poor quality and lack of diversity in foods adversely affects the children's growth and nutritional status.

During infancy and early childhood (birth to 2 years), adequate amount of appropriate nutrition has paramount importance for full development of children's human potential. This period is also regarded as "critical window" for child's health, growth, and development (WHO, 2003). It is also peak period for faltering in child's growth, micronutrient deficiencies, and emergence of common childhood ailments as diarrhea. Furthermore, reversing of stunting developed during this period is very difficult after the second anniversary of the children (World Bank, 2005). Complementary feeding should be *timely* (start receiving from 6 months onward) and *adequate* (in amounts, frequency, consistency, and using a variety of foods). The foods should be prepared and given in a *safe* manner and be given in a way that is *appropriate* (foods are of appropriate texture for the age of the child) and applying responsive feeding following principles for psychosocial care (WHO/UNICEF, 2003 and Monte& Giugliani, 2004). During these formative years, poor nutrition has immediate consequences of increased morbidity and mortality and delayed development of the brain and other nervous systems (Krebs, Hambidge, Mazariegos, Westcott, Goco, Wright, 2011).

. The latent impacts of deficits in nutrients in early ages include impaired cognitive performance and reproductive outcomes and reduced work capacity and health status during adolescence and adulthood. Furthermore, malnutrition cycle persists with intergeneration impacts. When malnourished girl child grows up, she faces greater odds of having malnourished, low birth weight infant (Müller and Krawinkel, 2005) where, the failure to consume additional nutritious food in low resource settings has been identified as important risk factor resulting in excess disease and death of young children (Krebs *et al*., 2011).

1.1.2 Common Staple use as complementary foods in developing countries

The typical diet in populations with a high prevalence of malnutrition consists predominantly of a starch-rich staple, such as a cereal (maize, rice) or tuber (cassava), with limited amounts of fruits, vegetables, legumes, and pulses, and little or no animal-source food. Such a diet is bulky, has a low density of energy and nutrients and a low bioavailability of minerals, and will result in impaired growth, development, and host defense to infections.

Unrefined cereals and legumes contain high levels of phytic acid (myo-inositol hexaphosphate) and its associated magnesium, calcium, and potassium salts – termed phytates. Phytate chelates dietary iron, zinc, and calcium in the gastrointestinal tract, making these minerals less available for absorption, while simultaneously complexing endogenously secreted zinc and calcium, making them unavailable for reabsorption into the body (Hurrell, 2004; Hambidge *et al*., 2005; Gibson *et al*., 2006). Moreover, there appears to be no evidence of any adaptation to the inhibitory effect of high-phytate diets on absorption of iron (Brune *et al*., 1989) or zinc (Hunt *et al*., 2008), hence, intakes of iron, zinc, and calcium by breast-fed infants consuming high phytate cereal-based complementary foods (CFs) are often inadequate (Gibson *et al*., 1998; Hotz and Gibson, 2001a; Faber, 2004; Kimmons *et al*., 2005; Anderson *et al*., 2008b; Gibson and Anderson, 2009) when compared with the WHO estimated needs from CFs (Dewey and Brown, 2003).

In addition, introduction of such a diet too early or contamination of the diet will lead to frequent infections, which will further impair nutritional status and, hence, increase the risk of infectious diseases. Young children are also likely to be more sensitive to the effect of antinutrients, e.g., high levels of phytate, which impairs the absorption of several growth-limiting minerals, such as zinc and iron. Infants and young children are especially vulnerable to malnutrition because they have a high growth velocity and also high energy and nutrient needs. Growth velocity up to the age of about 2 years is especially high, and it is also during this period that the brain reaches almost 90% of adult size. Formulation and development of nutritious complementary foods from readily available local raw materials has received a lot of attention in many developing countries (Plahar and Annan, 1994), with several blends making use of legumes singly or in combination (Ekpenyong *et al*., 1977; Plahar *et al*., 1983; Sefa-Dede, 1984).

1.1.3 Challenges to Ensuring Adequate Nutrition at 6–24 Months of Age

Children less than 2years of age have high nutrient needs to support growth and development, yet breast-fed infants typically consume relatively small amounts of foods other than breast milk. As a result, complementary foods need to be high in nutrient density, i.e., the amount of each nutrient per 100 kcal of food. Iron and zinc are generally the most problematic nutrients during the period of complementary feeding (Dewey & Brown, 2003; Vossenaar & Solomons, 2012), largely because their concentrations in human milk are low relative to needs. Because average expected energy intake from complementary foods is lowest at 6–8 months [;200 kcal/d, assuming the average breastmilk intake observed in developing countries (WHO, 1998)], the minimum target nutrient densities in those foods tend to be highest for that age range (e.g., 4.5 mg iron/100 kcal and 1.14 mg zinc/100 kcal) (Dewey & Brown 2003). Target nutrient densities are lower for breast-fed infants at 9–11 months than at 6–8 months, because average expected intake from complementary foods increases to 300 kcal/d at 9–11 months. Expected energy intake from complementary foods increases further to 550 kcal/d at 12–23 months, while at the same time the need for iron is lower than during infancy and the need for zinc stays the same. As a result, the minimum iron and zinc densities of complementary foods are considerably lower in the second year of life (1.0 and 0.46 mg/100 kcal, respectively) than in the first year. Thus, the greatest challenge for meeting micronutrient needs of breast-fed children typically occurs during the second 6 months of life.

1.1.4 Nutrient Gaps in Complementary Food Diets

When actual nutrient densities of typical complementary foods are compared with the target nutrient densities, protein density is generally adequate but several micronutrients are ‘‘problem nutrients’’ (Dewey & Brown, 2003; Gibson et al., 2010) In developing countries, the usual problem nutrients include iron and zinc, and other nutrients may also be low depending on the types of foods consumed (e.g., riboflavin, niacin, thiamin, folate, vitamin B-6, vitamin B-12, calcium, vitamin A, vitamin C, and vitamin E) or the water and/or soil content (e.g., iodine, selenium). If breast-fed infants are given family foods that are nutritionally adequate for the rest of the household, there is likely to be a shortfall in intake of certain key nutrients. For example, using ‘‘best case scenario family food menus’’ from low-income households in Guatemala, (Vossenaar & Solomons, 2012) demonstrated that the nutrient density of the hypothetical infant diet would be far below the critical nutrient density (as defined by the authors) for iron, zinc, and calcium across the entire age range of 6–24 months and below the critical nutrient density for some of the vitamins in certain age intervals. This illustrates that transitioning directly to ‘‘family foods’’ as the sole source of complementary foods may put the infant at risk of multiple micronutrient deficiencies. Even when ‘‘improved’’ complementary food recipes are developed, they usually fall short of providing adequate iron, zinc, and sometimes calcium (Gibson et al., 2010).

1.1.5 Novel and Underused Food Sources of Key Nutrients for Complementary Feeding

Currently, in low-resource settings, the most widely consumed complementary foods are cereal-or legume-based and have low micronutrient density. Meeting the high nutrient needs of infants and young children with diets based on such foods is difficult. Nutrient gaps in complementary food (CFs) diets can be addressed in a variety of ways including supplementation and fortification. There is increasing interest in the use of micronutrient-rich foods that may not be typically consumed by children under two. Depending on local availability and ease of access, underused complementary foods may be affordable and potentially more acceptable than other CF options. Underused complementary foods include the more commonly promoted animal-source foods such as meat, poultry, and eggs; less commonly promoted animal-source foods such as rodents, small fish, insects, and spiders; and foods from indigenous or traditional trees and plants.

1.1.5.1 Meat, Poultry, Eggs, Animal Parts

Meat is generally a rich source of haeme iron, zinc, riboflavin, and vitamin B12. (Dror & Allen, 2011). Poultry and eggs are also good sources of these micronutrients, but contain lower amounts of some of them than found in red meat (such as iron and zinc). Rodents are an example of a less conventional source of meat. They comprise a substantial proportion of wild game consumed throughout the world (NAP, 1991 and Fiedler, 1990). More than 89 species of rodents are consumed including squirrels, guinea pigs, and porcupines. Animal parts aside from meat (i.e., other than the flesh or muscle) can also serve as rich sources of nutrients. For example, heart, kidney, and liver are rich sources of vitamin B-12, iron, and zinc; and liver is also high in vitamin A (USDA). The anthropological literature provides insight into other animal parts that may have sustained child health. For instance, in the 1960s, Jeliffe et al. observed that the Hadza (a Tanzanian ethnic group) fed their children soft fat and bone marrow from zebras as first foods (Jeliffe et al., 1962). Kenyan pastoralists were observed feeding their babies cow's blood and camel fat (Gray, 1996)

1.1.5.2 Small Fish

Although often overlooked as key sources of micronutrients, fish are generally rich sources of various vitamins and minerals (Roos *et al*., 2007) Certain species of small fish, when eaten with bones, heads, and viscera (the parts where most micronutrients are concentrated), can be very high in calcium, iron, zinc, and vitamin A (Kawarazuka & Bene, 2011)

1.1.5.3 Insects and Spiders

Insect-eating is widespread in tropical and subtropical countries, particularly in rural communities (DeFoliart, 1999). Some of the more popular insects and arachnids eaten around the world include crickets, grasshoppers, ants, termites, silkworms, caterpillars, and tarantulas. Insects can be rich sources of energy, protein, vitamins, and minerals. Studies of Angolan insects showed that a termite species had a high energy content (more than twice that of ground beef: 613 kcal/100 g vs. 234 kcal/100 g), and a caterpillar species was high in zinc, thiamine, riboflavin, and iron (Santos et al., 1976)

1.1.5.4 Indigenous Trees and Plants

Various foods from traditional trees and plants are potentially nutritious but often neglected. For instance, the Moringa tree's *(Moringa oleifera)* pods and leaves are particularly nutritious. The pods, which look like giant string beans, are high in protein and vitamin C with significant quantities of vitamin A and B vitamins (Fuglie, 1999, Adepoju, Orobiyi and Eyitayo-Ajayi, 2002; Adepoju, Akinfolarin, Kolapo and Lawal, 2003). The leaves are boiled and eaten like spinach and contain substantial quantities of vitamins A and C, more calcium than most other greens, and high levels of iron (Martin *et al*., 1998). In West Africa, seeds from the néré tree *(Parkia biglobosa)* are used to make a fermented condiment called "soumbala" which is a rich source of iron (16.9 mg/100 kcal) (Ouedraogo et al., 2010). Soumbala is often used as a low cost meat substitute by families (Savadogo *et al*., 2011)

Several processed complementary foods have been designed to include underused foods as key ingredients and are currently being studied in various countries. These include a cereal made from maize and caterpillar flour in the Democratic Republic of Congo (Bauserman et al., 2013) spiders and fish in Cambodia (Chamnan *et al*., 2011) soumbala in Burkina Faso, and sweet potato and fishmeal developed for use in Sub-Saharan Africa. (Amagloh *et al*., 2011)

1.1.6 Nutritional Potential of Edible Insects

Insects represent a significant biological resource that is yet to be fully utilized around the world. Insect bodies are rich in protein, amino acids, fat, carbohydrates, various vitamins and trace elements. Therefore insects offer an important nutritional resource for humans and are worthy of development (Chen and Feng 1999; Yang 1998; Hu 1996; DeFoliart 1992; Mitsuhashi 1992; Comby 1990; Ramos-Elorduy and Pino 1989; Zhou 1982; Zhou 1980).

Besides being a delicious food commodity, the nutritive value of edible insects has attracted the attention of nutritionists, health workers and physicians. In Europe the use of insects for foods has always been very limited. Although frequently mentioned in ancient Greek and Roman literature, there are only very few reports on the use of insects as food in later centuries. Only in times of starvation, insects were eaten. The main reason for the difference between Europe and the other continents is that insects are not so abundant and generally much smaller as in tropical regions. Entomophagy can be divided into two categories: insects used as a source of nutrients and insects as condiments. Some insects are eaten as larvae or pupae, others as adults. Though not insects, arachnids such as spiders, tarantulas and scorpions are also eaten. A total of 1417 species of insects have been recorded as being eaten by over 3000 ethnic groups. These include 235 species of butterflies and moths, 344 species of beetles, 313 species of ants, bees and wasps as well as 239 species of grasshoppers, crickets and cockroaches, amongst others. Other commonly eaten insect are termites**,** bugs, cicadas and dragonflies (Wikipedia, 2010).

The winged termites, *Macrotermes bellicosus* are exopterygotous insects which belong to the order Isoptera of the class Insecta. Due to their eating habits, termites act as scavengers because of their ability to clear wastes from surroundings (Malaka, 1996). They are also useful to man, mainly as food for man and animals. Beyond West Africa, much importance has been attached to termites as food. Hickin (1971) reported that they are on sale in the United States, together with other insects, enveloped in candy. In places where meat and other protein-containing foods are scarce termites constitute a useful source of protein.

1.2 Statement of the problem

Global estimates of undernutrition in 2011 showed that 165 million (26 per cent) children under-five were stunted, 52 million (8 per cent) were wasted and 101 million (16 per cent) were underweight. The global burden of undernutrition is not evenly distributed, with higher prevalence in sub-Saharan Africa compared to other regions. It has been estimated that 56 million (36 per cent) children under-five in Africa are stunted, 13 million (9 per cent) are wasted and 28 million (18 per cent) are underweight (Black et al., 2013). In Nigeria the proportion of children who were stunted, wasted and underweight were 37, 18 and 29 percent respectively (NDHS, 2013). However, the extent of wasting has worsened, indicating a more recent nutritional deficiency among children in the country. Data available on the regional prevalence of diarrhoea, undernutrition and under-5 mortality in Nigeria show a strong interaction among these three factors, with each of them being far more prevalent in Northern than Southern part of Nigeria (UNICEF, 2001). Poor feeding practices and shortfall in food intake are the most important direct factors responsible for malnutrition and illness among Nigerian children (Solomon, 2005). The high cost of fortified nutritious proprietary complementary foods make them to be beyond the reach of most Nigerian families; hence many depend on inadequately processed traditional foods consisting mainly of unsupplemented cereal porridges made from maize, sorghum and millet (Nnam, 2002).

The “1,000 days” period that encompasses pregnancy and the child’s first two years after birth is considered the key window of opportunity for preventing undernutrition and its long-term consequences.(thousanddays.org) The largest part of this window is the complementary feeding period (6-24 months), the period of transition from exclusive breastfeeding to consuming a wide range of foods in addition to breast milk (Dewey *et.al*,2003; WHO,1998) Although considerable growth faltering can occur during the prenatal period and the first 6 months after birth, a large proportion of stunting in low-income countries occurs during this 18-month interval (Dewey *et.al,*2009; Shrimpton *et.al.*,2001; Victora *et.al.*,2010). Inadequate nutrient intake from complementary foods and the high incidence of infections during this age interval in disadvantaged populations are major causes of stunting and other adverse health and developmental outcomes (Dewey *et.al,*2011). Consequently, ensuring adequate nutrition during the complementary feeding period is a major global health priority.

Globally, 1 out of 3 children under five are estimated to suffer growth faltering (stunting) and 1 out of 5 have low weight-for-height, mostly as a consequence of poor feeding and repeated infections, while 43 million are overweight. (Black et al., 2008) In many countries, only 1/3 of breastfed infants 6-24 months of age meet the criteria of dietary diversity and feeding frequency that are appropriate for their age. It is estimated that optimal breastfeeding and complementary feeding practices can save the lives of 1.5 million children under five every year. (Black *et al*., 2008) After six months of age, optimal complementary feeding becomes paramount and locally-available foods need to be used appropriately and adequately as part of an infant's complementary foods. However, research findings revealed that in developing countries providing older infants and young children with locally formulated diets in addition to breast-milk is not enough to meet the growing energy and nutrients requirements that support optimal growth (PAHO/WHO 2003). Fortified nutritious commercial complementary foods are unavailable especially in the rural areas and where available, they are often too expensive and beyond the reach of most of families in Nigeria.

1.3 Justification for the study

Micronutrient needs are high during the first 2 years of life to support the rapid growth and development during this period. The percentage of the recommended nutrient intake needed from complementary foods varies widely, depending on the concentration of each nutrient in breast milk. The nutrients that are most problematic – for which at least 75% must come from complementary foods – are iron (97–98%), zinc (80–87%) and vitamin B6 (80–90%) (Dewey 2005). Thus, complementary food diets need to contain foods rich in these nutrients (generally animal-source foods), or be fortified in some way. Previous studies did not lay much emphasis on these important micronutrients, but rather dwell more on protein and energy need of under-five children (Dewey 2005). In Nigeria, most complementary foods are inadequate in protein (Nnam, 2002). About 40% of the Nigerian population live below poverty line and therefore cannot afford commercial infant formula for their children (Wardlaw, 1999) or good quality animal source proteins. Also, most of these infants are fed with high carbohydrate gruels made from cereals which are not nutritious and too watery to meet the nutritional needs of a growing infant. Adequate processing and judicious blending of the locally available foods could result in improved intake of nutrients to prevent malnutrition problem (Nnam, 2002). Therefore, Simple and adequate methods to improve the quality of the complementary diet in low-income countries are urgently required.

In Nigeria, various authors have used different blend of materials to formulate complementary foods (Adebayo et al., 2012; Ijarotimi and Keshiro, 2012; Ikpeme et.al. , 2012; Sodipo and Fashakin, 2011; Adenuga, 2010; Lalude and Fashakin, 2006 etc.) The authors cited incorporated blend of different food materials, but did not include any form of animal as protein source. Studies carried out on complementary foods between 2005 and 2012 in Nigeria, only one incorporated animal (crayfish) as protein source (Mariam, 2005). Few of previous works done outside Nigeria on formulating complementaryfoods incorporated animal sources (fishmeal, crayfish, full-fat milk) protein into their preparation. The absorption and utilization of iron and zinc in complementary foods of plant origin are significantly lower than those from animal sources. The bioavailability of non-haeme iron found in plant sources is 2 - 8%, compared to 25% bioavailability of haeme iron found in animal products (Brown *et al*., 1998). Phytic acid is a potential inhibitor of both iron and zinc in plant food materials. It affects the bioavailability of minerals by forming an insoluble complex with the metals of Iron, Zinc, and Calcium, inhibiting their absorption (Gibson *et al*., 1998). Most ofthe works done did not test for the bioavailability of the micronutrients as well as the presence and effects of antinutrients in the preparations. Also, majority of the review literatures failed to do follow up study to find out the level of acceptance of the newly developed complementary food among the mothers of under-five children. Previous studies carried out on *M. bellicosus* in Nigeria by Adeyeye et.al. , 2011; Ekpo *et.al.,* 2009; Banjo et.al. , 2006 and preminary investigation by Adepoju and Omotayo (2014) confirmed that *M. bellicosus* is rich in both micro- and macronutrients. It was also reported that the energy content of *M. bellicosus* is high while the level of antinutrients is very low, hence its suitability for use in formulating complementary foods.

Research Questions

1. What are the proximate nutrient compositions of *Marcroterms bellicosus* and the formulated complementary foods?
2. What are some of the important minerals and vitamins present in *M. bellicosus* and CFs?
3. What are the level of antinutrients in *M.bellicosus* and CFs?
4. How bioavailable are the nutrients in the formulated CFs?
5. How acceptable is the formulated CFs among mothers of 6-24 months old infants?

1.4 Objectives of the study

1.4.1 General Objective

The general objective of this study was to enrich four of the commonly used locally formulated complementary foods (CFs) - maize pap, sorghum pap, rice pudding and yam porridge with *Marcrotermes billicosus* powder at various levels of inclusion (10%, 15% and 20%).

1.4.2 Specific Objectives

The specific objectives of this project are to:

1. Carry out the consumption survey on *M. bellicosus* in the community where it is available and consumed
2. Determine the proximate composition: moisture, crude protein, crude lipid, ash, total carbohydrates, and gross energy content of *Marcrotermes bellicosus* and formulated CFs.
3. Determine the mineral composition: Sodium, Potassium, Calcium, Magnesium,Phosphorus, Iron, Zinc, Copper, Manganese and Selenium content of *M. bellicosus* and formulated CFs.
4. Assess the fatty acid Profile: Saturated, monounsaturated, and polyunsaturatedfatty acid composition of M *bellicosus.*
5. Evaluate Vitamin Profile: Oil-soluble (A, D, E, K) and water soluble (C, B_1 B_2, B_3, B6, B_{12}) vitamins of M *bellicosus* and formulated CFs.
6. Assess the level of antinutritional factors in *M. bellicosus* and formulated CFs.
7. Determine bioavailability of some nutrients in *M. bellicosus* and CFs using animal model,

8. Carry out acceptability study of formulated CFs using nursing mothers

1.5 Study Hypothesis

$H_0$1: There is no significant difference between the nutrient composition of enriched locally formulated complementary foods and the control

$H_0$2: There is no significant difference between the weight gain in animal fed with enriched locally formulated complementary diet and animal fed with the control and basal diet.

$H_0$3: There is no significant difference between the serum retinol, ferritin and zinc of animals fed with the enriched locally formulated diets and animals fed with control and basal diets

CHAPTER TWO

LITERATURE REVIEW

2.1 Child undernutrition

Infancy is the developmental period that extends from birth until eighteen to twenty-four months. It is a time of extreme dependence on adults. Many psychological activities are just beginning—language, symbolic thought, sensorimotor coordination and social learning, for example. This makes it necessary for conditions that may lead to irreversible faltering in linear growth and cognitive deficits to be addressed. Poor nutrition during this critical period is one such factor and contributes to significant morbidity and mortality in developing countries. Early detection of growth faltering and promotion of appropriate feeding practices are therefore important for prevention of malnutrition and the very survival of such children.

Child malnutrition is the state when the body lacks adequate energy, protein, and micronutrients for body maintenance, growth, and development (Latham, 1997). A chronic shortage of appropriate types and quantities of food, and with disease, may cause children to experience long-term faltering in physical growth. They grow in height more slowly than expected in children of the same sex and age, a classical indicator of undernutrition termed 'stunting' (WHO, 2006). A short-term deficit in physical growth due to acute food shortage and infection/illness is an indicator of undernutrition referred to as 'wasting'. Wasted children gain weight more slowly than expected in children of the same sex and height (WHO, 2006). The third indicator of undernutrition is 'underweight', which is a composite indicator of stunting and wasting and thus an overall indicator of the extent of child undernutrition. Underweight children gain weight more slowly than expected of children of the same sex and age (WHO, 2006). According to the 2006 World Health Organization (WHO) classification standards for child undernutrition, children with a Z-score below – 2 Standard Deviations (SD) of the median for weight-for-height/length, height/length-for- age, and weight-for-age are classified as wasted, stunted and underweight, respectively. Children with a Z-score below − 3 SD of the median are classified as severely undernourished, while those with a Z-score between − 2 SD and − 3 SD are classified as being moderately undernourished. Those with a Z-score between − 1 SD and − 2 SD are classified as mildly undernourished.

Global trends in child undernutrition from 1990 to 2011 indicate declines in all indicators. Stunting decreased by 35 percent, wasting by 11 percent and underweight by 37 per cent (Black et al., 2013). However, the observed global reductions in undernutrition mask significant regional and country variations (Sahn & Stifel, 2003; Stevens et al., 2012). For example, progress in lowering stunting, wasting and underweight in sub-Saharan Africa has stagnated with children residing in rural areas posting higher prevalence as compared to those living in urban areas (Sahn & Stifel, 2003). While the data just cited focus on undernutrition conceptualized as physical growth deficits, which are relatively easy to measure, the untoward manifestations of malnutrition have many facets. Globally, child undernutrition was associated with over 3.1 million deaths of children under the age of five years in 2011 (Black et al., 2013). The magnitude of the underestimate is not known due to incomplete infant mortality reporting in many parts of the world. The effects of undernutrition are not only linked to infant mortality but have pervasive effects on surviving victims (Pelletier, Frongillo, Jr, Schroeder, & Habicht, 1995; Victora et al., 2008).

Undernutrition alters normal brain development, reduces energy levels and limits the rate of motor development in children (Brown & Pollitt, 1996; Pollitt et al., 1996). These biological impairments have been linked to poor cognitive ability and educational development, resulting in over 200 million children in the developing world not achieving their potential (Grantham-McGregor et al., 2007). Poor growth in childhood is also likely to influence adulthood stature (Gigante, Nazmi, Lima, Barros, & Victora, 2008; Rivera, Martorell, Ruel, Habicht, & Haas, 1995). Stunted children in rural Senegal remained smaller in adulthood as compared to their non-stunted peers, while adopted Indian girls in Sweden with less height for their age at childhood posted below the reference mean height at the onset of their puberty (Coly et al., 2006; Proos, Karlberg, Hofvander, & Tuvemo, 1993). Contrary, better nutritional status aids better child development as exemplified by the improvement in height-for-age Z-scores among children residing in rural Zimbabwe that was associated with increased height in adulthood, increased number of school grades completed and earlier age at which children started school (Alderman, Hoddinott, & Kinsey, 2006).

While undernourished children may experience compensatory growth (catch-up growth) later in life, there is a risk involved, as catch-up growth has been associated with chronic diseases in adulthood (Hales & Barker, 2001; Barker, 2002; Bhargava et al., 2004;). By compromising children's physical and cognitive development, undernutrition diminishes their chances of success later in life. Adults who experienced malnutrition in childhood are likely to have less physical work capacity and earn less income as compared to those who were well nourished in childhood (Haas et al., 1995; Hoddinott, Maluccio, Behrman, Flores, & Martorell, 2008). Positive correlations have been documented between physical stature (height and body mass index) with higher wages, with the likelihood of height having a direct effect on wages through strength (Thomas & Strauss, 1997). Lifelong cognitive impairment is another pathway through which undernutrition limits socio-economic progress with research showing associations between undernutrition during childhood and poor educational achievements and lower incomes later in life (Barker, Eriksson, Forsén, & Osmond, 2005). Undernutrition perpetuates intergeneration poverty and exerts a huge burden for nations whose future citizens are likely not to be as healthy and as productive as they could have been (Smith & Haddad, 2014; UNICEF, 2013).

2.2 Infant Growth and Development

The pattern of a child's growth can be resolved into an infant, child and pubertal phases based on endocrine influences. By the first year of life, the child increases in weight threefold and by 50% in length; and in head circumference by 10cm. The growth rate slows in the second year of life, and head circumference increases by 2cm over the year. From 2 to 5 years of age, weight gain occurs at about 2 kg/year and height increases by 7–8 cm/year. When exclusively breastfed, babies grow more quickly than standard growth rates, but after 3–4 months, a relative deceleration in growth velocity becomes apparent (Lutter, 2003)

Growth in infancy is a complicated process; it is affected by factors such as diet, the nutritional status and health of the mother and the occurrence of infections. Also, social factors (family structure and cohesiveness), economic status, cultural practices and biological factors (the sex of the infant, birth weight, birth order, birth interval and genetics) may also play significant roles in growth. The nutritional factors may affect growth in infancy both before and after birth. Maternal and infant nutrition are therefore intimately related. For breastfed infants, nutrition of the mother and that of her young are interrelated from conception until weaning. This makes the dietary intake of pregnant and lactating women crucial to the child.

2.2.1 Assessment of Child Growth

Anthropometric measurements are commonly used for assessing growth and nutritional status of children. These include a weight for age, height for age and weight for height. Low height for age (stunting) reflects the cumulative effects of numerous insults experienced by children during infancy and early childhood. It begins at birth and continues through the initial 40 months, after which time it is irreversible. In contrast, low weight for age (wasting) is reversible and can reflect either acute or chronic malnutrition.

Faltering in length extends through the first 3–4 years of life. In contrast, faltering in weight is concentrated between 3 and 12 months. After 12 months of age, a child may be stunted and of low weight/ age, but his weight/height ratio improves. In other words, weight gain can be adequate even while the process of stunting continues for another two years. While failure to gain weight is a signal of inadequate nutrition, sufficient weight gain does not necessarily mean that a child is growing normally. Thus, differences in the degree of growth failure in weight and height have implications for assessing the actual prevalence of chronic malnutrition. This is also essential for monitoring trends or evaluating the effects of interventions.

2.2.2 Nutritional Requirements during Infancy

Appropriate feeding practices to reduce malnutrition requires that infants should be exclusively breastfed for the first six months of life and after that, should receive adequate and safe complementary foods while breastfeeding is continued for two years or beyond (WHO, 2008) until the child is fully dependent on a family meal. This transition period is the most vulnerable when growth faltering starts in many children and is caused by many factors including late introduction, poor nutritional quality and insufficient amounts of complementary foods. According to the FAO/WHO recommended nutrient intake (RNI) for developing countries, infants 0–24 months need about 400–500 mg calcium per day; for iron, 3.9–6.2 mg/day; zinc, 4.1 mg/day; vitamin A, 400 mg RE/day; thiamine, 0.3 mg/day; riboflavin, 0.4 mg/day; while for pyridoxin (B6) it is 0.3–0.5 mg/day. Poor nutrition in the first two years can slow a child’s physical and mental development for the rest of his/her life (Brown et al., 1998). The nutrient requirement from complementary foods for infants that consume average intake of breast milk after the age of 6 months are: energy, 50–70%; protein, 20–45%; vitamin A, 5–30%; thiamine, 50–80%; riboflavin, 50–65%; calcium, 60%; vitamin B6, 75–88%; zinc, 85%; and almost 100% for iron. Beyond these micronutrients, there is a developmental need for the polyunsaturated omega-3 and omega-6 fatty acids which are major building structures of membrane phospholipids of the brain and are important for visual and intellectual capabilities in infants and pre-schoolers. Similarly, the amino acid composition of dietary proteins contributes to the cerebral function for which tryptophan has a special role. Other essential amino acids are important for the formation of neurotransmitters.

2.3 Food Acceptance, Preference and Intake Patterns in Infants

Infants at birth depend on breast milk only for adequate nutrition during the intensive nursing period (first 6 months) of life. In order to continue normal growth and development beyond this period, additional food of varying nature must be provided (Hendricks & Badruddin, 1992) by the parents to help them make the transition from dependent to independent self-feeding at a time certain motor skills such as chewing and mastication have been developed. This transition occurs gradually over the first 2 years of life (Birch & Grimm-Thomas, 2011) and involves:

- A shift from a single to multiple food sources
- Increased opportunities for self-regulation of food intake
- New social contexts for eating involving peers and adult caretakers

The success of this transition can be attributed largely to the fact that the infant "comes equipped" with a set of predispositions and abilities that facilitate this dietary transition, promote the acceptance of solid foods and shape subsequent dietary patterns (Birch & Fisher, 1995). This is shaped by the availability of dietary variety, the quality of children's early feeding experiences and the ability of parents to accommodate their children's emerging independence. The ultimate food intake pattern of the child therefore depends on the child, the parents and the society in which the child lives.

2.3.1 Food Acceptance Pattern

The overall food acceptance pattern of a child is developed as the child comes into contact with the omnivorous diet in the family and society. It is mainly shaped by three factors:

- Opportunities for repeated exposure to new foods to make the food "familiar"; up to 5–10 exposures may be needed (Sullivan & Birch, 1994) to achieve this.

- The social context of meals: Routine family meals teach children about foods their culture finds edible, the food combinations, meal times and what foods are typically eaten at these meals. Other social contexts include the attitudes of parents and older adults to food
- Associative learning: Children learn to associate foods with the post-ingestive effects of eating those foods. These include conditioned aversion (Garcia et al., 1974) and the association of food cues with the positive consequences of ingestion (Capaldi, 1996)

Other factors which affect an infant's food acceptance pattern include: mother's level of education/literacy, age and household income.

2.3.2 Food Preference Pattern

Human infants are instinctively afraid of anything new and prefer to eat foods that are familiar. This helps them to avoid the ingestion of potentially harmful foods and may also explain why newborns cry at birth. Human infants are however born with a well-developed sensitivity and preference for sweet and fatty foods but reject sour and bitter tastes. The recognition or preference for salt taste begins to appear at about 4–6 months of age and may be due to the maturation of salt-specific receptors on the tongue, early experiences with salted foods (Birch & Grimm-Thomas, 2011) and the salt flavour note in breast milk.

Infants as young as 6 weeks old can regulate their energy intake and can learn to associate the flavour cues in a food with the post-ingestion consequences, and hence regulate their intake accordingly in subsequent encounters with the food. The foods preferred by an infant are conditioned by the parents' attitudes and the foods available to them. But the amount consumed will depend on caregivers' feeding behaviours, internal satiety cues and characteristics of the diet. Additionally, children on average prefer seven different items on their plates, and six different colours which suggest that this preference for diversity could open opportunities for parents to encourage more nutritionally varied diets (Zampollo et al., 2012)

2.3.3 Infant Feeding Practices and Beliefs

Infant feeding practices in developing countries include breastfeeding and the use of complementary foods. The extent to which these are practised depends largely on the cultural norms of the communities.

2.3.3.1 Breastfeeding

Breastfeeding is the culturally accepted method of feeding infants in most developing countries of the world and the initiation rates are very high (Schmidt *et al.*, 2002) . It exceeds 90% in almost every country and exceeds 95% in more than half of the countries. Breastfeeding practices are, however, far from optimal. On average, the proportion of infants under 4 months of age who are exclusively breastfed is the highest in Asia (up to 82% in Nepal) and the Near East/North Africa (63% in Morocco) followed by Latin America and Sub-Saharan Africa. There is significant variation within regions. For example, in Latin America and the Caribbean, the proportion of infants under 4 months of age who are exclusively breastfed ranges from less than 5% in Haiti to more than 50% in Bolivia, Guatemala and Peru. In Ghana, the proportion of those breastfeeding exclusively up to 6 months was below 32%. This figure is woefully below the WHO/UNICEF's aim of achieving exclusive breastfeeding rate of 75% and above in sub-Saharan Africa.

2.3.3.2 Complementary Food Use

Complementary foods are used in addition to breast milk to make up for the deficits in nutrient requirements when the child's needs outstrip that provided by breast milk. The target range for complementary feeding is generally taken to be 6–23 months (WHO, 2008). However, some discrepancies that draw on cultural differences exist. Ghanaian mothers are known to use complementary foods starting with water and glucose solutions in the first few months of life (Davis et al., 2003). Most mothers in Ghana give `koko', a maize-based fermented porridge, to their infants as early as the first month of life, a stage at which the child is supposed to be exclusively breastfed. In other parts of the developing world, complementary foods are similarly used and consist of home-made and factory-processed foods.

2.3.3.3 Breastfeeding and Timing of Weaning

Complementary foods are introduced mostly when the child attains 4–6 months, but may extend from 1 to 9 months. Factors determining the timing of complementary feeding include baby crying, baby not being satisfied, advice from the health clinics, the necessity for some mothers to return to work and maternal HIV status (Owino *et al*., 2010; Omari et al., 2003)

2.3.3.4 Food Beliefs and Attitudes

Foods prepared at home are preferred by Zambian women who feel that they are fresher and contain all the nutrients Cold foods and some foods such as *okra* prepared at home using sodium bicarbonate may cause stomach problems in the child (Omari et al., 2003) as does maize meal porridge. In Ghana, water and glucose solutions are used in the first few months of life to quench the thirst of the newborn after the exhaustive birth process or as a cultural gesture to welcome the child into the world (Davis *et al*., 2003). In Ethiopia, before the initiation of breastfeeding, the newborn is often fed with butter up to 1–2 months of age and in some areas is continued even longer, mixed with a liquid made from fenugreek (*Trigonella faenum graecum*). This is to open up the throat, to grease it or to get rid of dirty things (Abate &Yohannes, 1987). On the day of birth or a few days after birth, a liquid made of boiled fenugreek seeds is given to the child until he/she can walk. This is to get rid of intestinal dirt. Honey is forbidden in some areas in the belief that it will make children to stammer. According to Abate and Yohannes (Abate &Yohannes, 1987), eggs are believed to be the cause of intestinal parasites such as tapeworm; children who eat liver lose their teeth while eating the heart will make one to be forgetful. Sorghum and wheat are believed by some mothers to cause *Ascaris* infection.

Even though 35–40% of Indian families consume eggs and meat, it is traditionally believed that meat products and eggs cannot be given to infants because infants fail to digest animal foods (Paul *et al*., 2010). In Nepal, meat, fish, or eggs are infrequently given to children because of the belief about pure and impure food (Joshi *et al*., 2012). For example, in the Nepalese rural communities, vegetables and fruits are considered dangerous to the health of the infant and young children because it is regarded as cold food for young children (Helen Keller International and USAID, 2010). A widely shared misperception was that infants under 1 year of age cannot digest animal source foods (Gittelsohn *et al*., 1997) . In Bangladesh, food taboos are maintained by older family members especially the grandmothers who do not recommend oils/fats and eggs which are suitable for young children, thus further restricting food diversity (Roy *et al*., 1993) In

Guatemala, mothers prefer thinner complementary foods for children less than 1 year old and thicker foods for children more than 1 year old. When the child has a cough or fever, most mothers prefer thin, liquid complementary foods. When the child has diarrhoea, about half of the mothers believe that thinner complementary foods would replace the water the child lost with diarrhoea, whereas other mothers believe that thicker complementary foods would harden the stool or stop diarrhoea. Infants are also believed to innately prompt mothers to begin introduction of certain foods.

2.4 Complementary Local Foods for Infants in Developing Countries

Complementary foods are food-based sources of nutrients other than breast milk that are provided to infants who are still breastfeeding (Faber, 2004). Such foods are supposed to be nutrient dense because infants have high nutritional requirements relative to body size and consume small amounts of food at a time. Appropriate complementary foods can be readily consumed and digested by the young child from 6 months onwards and provide nutrients to help meet the growing child's needs. The most important nutrients are protein, iron, zinc, calcium, and vitamin A, the absence of which contributes to increased morbidity and mortality in children. In the developing countries, the raw materials used to prepare complementary foods are unrefined cereals or legumes that contain high amounts of phytic acid and phenolic compounds. They are monotonously consumed and contain negligible quantities of animal source foods because of some cultural factors. Fruits and vegetables rich in ascorbic acid and provitamin A carotenoids are not included because of the innate preferences of infants for sweet foods.

In most rural and urban poor settings, mothers are largely housewives and caregivers which restricts the choice of foods they consume and the foods provided for infants. The mothers' poor status and low intake of some micronutrients can affect the concentration of these micronutrients in human milk and by extension determines the infant's status (Black *et al*., 2008). In the case of iron and zinc, the concentrations of these trace elements in human milk are normally low regardless of maternal intake and stores, and therefore the infant's reserves at birth determine infant status (Dallman, 1992; Krebs, 2000). The need, therefore, for complementary foods becomes imperative (Bhutta *et al*., 2008) in order to improve the nutritional quality of intake when breastmilk is no longer enough and to help the child develop independent feeding capabilities based on chewing, mastication and swallowing.

Assuming an average intake of energy from breast milk, infants 6–8 months of age should on average receive 270kcal/day, and those 9–11 months of age should consume 450 kcal/day from complementary foods (Brown *et al*., 1998).Complementary foods should provide approximately 25–50% of total daily requirements for protein, riboflavin and copper; 50–75% for thiamine, calcium and manganese; and 75–100% for phosphorus, zinc and iron (Gibson *et al*., 1998).

2.4.1 Developing World Complementary Local Foods

Cereals, often maize, is the major ingredient for African complementary foods, whereas rice is the major ingredient for the Asian complementary foods (Gibbs & Gibson, 2010). Sorghum and millets are used in complementary foods in countries such as Ethiopia, India, the United Republic of Tanzania, Uganda and some processed ones are based on these raw materials (Seenappa, 1987). Porridge made with maize meal, which is a bulky food low in nutrient density, is used as complementary food in many African countries (Bentley *et al*., 1991; Lartey *et al*., 1999; Huffman *et al*., 2000; Black et al., 2008). Nout *et al* (1987) summarised the preparation of African weaning foods as follows:

(a) Fresh flour + water →boil →consume (sweet porridge)
(b) Fresh flour + water →ferment overnight →boil →consume (sour porridge)
(c) Fresh flour + water →boil →ferment →consume (sour porridge)

Systems (a) and (b) are the commonly used while system (c) is used occasionally. The method of fermentation used in (b) is mostly of the uncontrolled and mixed character with lactic acid bacteria being responsible for the fermentation.

2.4.2 African Local Complementary Foods

In Nigeria, there are two popular indigenous complementary foods; hot-pap (*ogi*) and cold-pap (*agidi*). To prepare paps generally, the maize grains are soaked in cold water for 2–3 days (with 24 hourly change of water) and later ground to paste, wet-sieved through nylon cloth and the starch sediment collected. Water is added and left for days with change of water at 24 h intervals. It is prepared by stirring a desired amount, in a limited amount of cold water before hot water is added and stirred continuously until a semi-liquid porridge (hot pap) is obtained (Essien *et al*., 2010) . There are variations to this process.

For cold-pap, after grinding the grains, the paste is wet sieved using clean, white cloth to get a very smooth paste which is allowed to settle at the bottom of the pot. The top water is removed while the paste is poured into boiling water and stirred to get a semi-solid porridge. This is then put inside banana (*Musa* spp. L., *Musaceae*) leaves, in a characteristic domed shape and steamed to solidification.

In Ghana, the child is first started on water and glucose solution before the main traditional complementary food, *koko* —a fermented maize porridge (Davis *et al*., 2003) is used.

In East Africa**,** *uji* is a cereal gruel or porridge used widely for weaning children (Mbugua, 1981). The traditional manufacture of fermented *uji* varies slightly from area to area. In Kenya, a flour mixture containing about 80% maize and 20% millet or sorghum flours is slurried in tap water at about 30% (w/v) level. The slurry is allowed to ferment spontaneously at room temperature for 1–3 days, diluted to give slurry of about 6–8% flour mixture and boiled for 15–30 min until smooth and thick before sweetening with sugar. It is served hot. Traditionally, fermented *uji* suffers from problems of off-flavour and flavour irregularity and occasionally, insufficient acid production leading to a hazardously high pH product. In some areas, mashed potatoes, bananas, or cassava are also used. Depending on the season and the area, a variety of beans, peas and vegetables are added. The energy contribution from fat is low. The liquid gruel usually contains around 5% dry matter, which results in an energy density of 0.2 kcal per gram of prepared gruel. For children above 1 year old, mothers may prepare thicker gruels. The upper limit for dry matter, however, is normally 20%, because, beyond this concentration, the gruel becomes difficult to stir. This provides an energy density of about 0.7–0.8 kcal per gram.

Obusera is the traditional fermented millet porridge of Uganda and is processed by one of three methods. The processing involves a combination of germination and fermentation resulting in porridge with low viscosity, high nutrient density and the desirable sour taste (Tomkins et al., 1987).

In Ethiopia, traditional weaning practices start immediately after birth (Abate & Yohannes, 1987): (a) before the initiation of breastfeeding, the newborn is often fed with butter. Water is also given, either alone or mixed with butter. Butter feeding continues up to 1–2 months of age and in some areas, is continued even longer, mixed with a liquid made from fenugreek (*Trigonella faenum graecum*). (b) On the day of birth or a few days after, a liquid made of boiled fenugreek seeds is given the child until he can walk. In some areas, fenugreek water is mixed with milk, butter or spices; (c) diluted milk (from cow and less frequently, goat and camels) and milk products are given as from 2 to 3 months and continued to at least 1–2 years of age. Apart from these, grain-based complementary foods are used in Ethiopia and are summarised in Appendix.

Maize or maize meal with groundnuts, *nshima* and *okra* are used in Zambia as complementary foods, According to Owino et al., (2010) maize meal-based porridge is the main form of food for infants. *Nshima* is prepared from the flour of maize, sorghum, or millet which is boiled into a stiff porridge. The amount of flour in such porridge is about 30% to give an energy density of 1 kcal/g and is given to children about 1 year in age (Luhila & Chipulu, 1987). To improve intake, it is diluted with water to a thin porridge containing 5% dry matter and giving 0.2 kcal/g. Porridge may be prepared with the addition of any or more of the following ingredients to maize meal: pounded groundnuts, cooking oil, sugar, fresh milk, salt, and eggs. In addition, from about 3 months onwards, cassava porridge with a little salt and/or sugar (Dirorimwe, 2007) may be used.

The traditional complementary food in Tanzania is based on maize, sorghum and finger millet. They can be classified into four groups (Mosha & Lorri, 1987):

- Complementary foods composed of single foods, usually cereal flours made into thin porridges or gruels (*uji*)
- Double mixes of cereal or root/tuber flour or banana prepared as a mash, mixed usually with a little milk or animal fat
- Triple mixtures of cereal, fruit and vegetable mash
- Multimixes including all the ingredients mentioned, plus any other food that the mother finds suitable and that makes the food palatable and attractive.

In Zanzibar of the Tanzanian Republic, the primary complementary food is maize porridge (*Ugali*), although foods made from local tubers and fried dough (*maandazi*) are also introduced early (Kung'u, 2009). More children are fed starchy staples consisting of rice, porridge, cassava, *shelisheli* (breadfruit), potatoes, pancakes, sweet potatoes, pumpkins, plantains, or maize-flour. At 10–15 months, food made from local tubers and green vegetables may also be incorporated, but to a small extent. Meat and large fish are expensive and are, therefore, not regularly consumed. Fruits (mainly mangoes, pineapples and oranges) tend to be seasonal.

The traditional gruel in the Republic of the Congo is made from sugar, maize, sorghum and maize flours (Donnen *et al*., 2011). The high-energy-density gruel used for comparison (treatment) was prepared from mixed flours (maize, soya, sorghum) to which industrial amylase was added (Legros & Treche, 1993). The energy densities obtained are 0.5kcal/g for the traditional gruel and 1.0 kcal/g for the high-density gruel.

In Lesotho, the complementary foods include a fermented thin porridge known as *Motoho* and an unfermented porridge, *Leshele-shele* (Sakoane & Walsh, 1987). *Leshele-shele* is prepared by mixing a portion of finely ground sorghum or maize flour with water and boiling for 15 min to make a thin porridge. *Motoho* on the other hand, is prepared by mixing finely ground mealie (sorghum or maize flour) with lukewarm water and inoculated with a bacterial culture or inoculum from a previous preparation. The mixture is wrapped in a blanket and left overnight (11–13 h) to ferment. The following morning, a sieved mixture of the porridge is cooked in boiling water for about 20–30 min. alternatively; a rough grind is incubated overnight with the starter culture, then reground finely and cooked as described.

Botswana has an interesting deviation from the all-cereal weaning diets: the traditional complementary food is milk from cow or goat, depending on availability. Weaning porridges include a fermented porridge, *ting* and the unfermented (*mosokwena*). Sorghum is the preferred grain; to make *ting*, sorghum is mixed with warm water to form a thick paste which is left in a warm area. To accelerate the fermentation, left-over from a previous fermentation may be used to start a fresh fermentation. The mixture is left overnight after which it is ready to use (Motwena, 1987). In South Africa, a soft porridge is made with maize meal (dry matter content approximately 14%) with usual addition of margarine, peanut butter, sugar, formula milk powder and eggs. In a survey by Faber (2004), only 4% of the infants had nothing added to their porridge. Eighteen per cent of the infants consume fruit and vegetables, pumpkin and butternut during the 24-h recall period.

Nasha is the traditional Sudanese porridge for weaning babies (Tomkins *et al*., 1987). It is made from sorghum and millet. Fermentation is both lactic and alcoholic. Suckling infants are also given *Furesa* (butter)
obtained by churning *kit rob* . It is very soft and is given in small quantities to suckling infants (Dirar, 1983)

Beverages Used in Africa for Complementary Feeding In Africa, a number of beverages are used in complementary feeding. These include *Kunun zaki*, a millet-based non-alcoholic beverage widely consumed in the Northern parts of Nigeria. The traditional process (Adeyemi & Umar, 1994) for the manufacture of *kununzaki* involves the steeping of millet grains, wet milling with spices (ginger, cloves, pepper), wet sieving and partial gelatinization of the slurry, followed by the addition of sugar, and bottling (see appendix).*Um gufufu* is used in Sudan and is prepared by milking cow directly into a bowl containing *rob* (milk from which fat has been removed) to give a foamy product appreciated by children (Dirar, 1983).

2.4.3 Asian Complementary Foods

Local complementary foods in Asia are mainly cereal-based (Jani *et al*., 2009) but the following are also used:

(a) Fruits (mashed bananas, boiled and mashed apples and other seasonal fruits such as papaya, *chikoo*, apples, and bananas). They are mashed and given as such or mixed with *malai* (cream) or milk

(b) Vegetables are started as weaning foods after fruits. Vegetables are boiled, mashed in a blender and then strained. After the baby is 7 months, straining is not required because vegetable fiber is very desirable. Dark green leafy vegetables, carrots and pumpkin are very healthy. Ghee, butter, or cooking oil can also be added for flavour as well as for calories

(c) The first or introductory solid foods for infants and toddlers are gruels made from staple flours such as rice, wheat (*Triticum aestivum*), *bajra* (*Pennisetum typhoideum*), maize (*Zea mays*) and sorghum (*Sorghum vulgare*) (Jeliffe,1968) . Cereals are introduced in the baby's diet gradually after proper cooking with vegetables to enhance the nutritive value. The cereal complementary foods include *Suji Upma* (*rava upma) and Suji kheer* made from suji, *Dalia* (from broken wheat) and rice preparations

Rice preparations include (i) *Khichri* (*moong dal*) in which the rice is cooked well till tender. To make it a very nutritious and filling full meal, vegetables and pulses are added. When the baby is still young, the entire mixture is mashed in a blender so that it is easy for the baby to swallow. The consistency can gradually be increased to a semi-solid state so that the child's palate starts getting used to regular foods; and (ii) *Pulav* which is prepared with vegetables.

Other preparations include Pumpkin *Malagutal* and Spinach *Malagutal* which can be served with rice, and are very nutritious for an expectant mother as well as the baby.

Home-made baby foods used in diarrhoeal situations include: rice *kanji* and sugar, salt and water solutions. Orange juice may be added depending on the child's preferences.

In China, congee is used to feed young infants. It is a thick porridge or soup of rice which has disintegrated after prolonged cooking in copious amounts of water. The congee is not seasoned with salt or any other flavouring. Often it is mixed with steamed and deboned fish. Congee made from other grains, such as corn meal, millet, barley and sorghum are common in the north of China where rice does not grow as well as other grains suited for a colder climate. Multigrain congee mixes are used as health foods. Congee with mung beans is usually eaten with sugar.

In Nepal, Vaidya (Vaidya, 1987) reported that *kheer*, a special rice meal with milk and sugar is served to the infant at 5–6 months of age as a first solid food. After this, the infants are fed with *lito* , a traditional bland rice porridge made with clarified butter (*ghee*) and sugar (if these are available). Rice *lito* is deficient in protein and vitamins. *Jaulo* is a traditional complementary food made from rice, lentils and green vegetables for convalescing young children. *Sattu* is an instant food made from roast cereals and legumes, ground into a powder and mixed with water to make a thin gruel or cake.

In other East Asian countries and China, babies are started on rice porridge called *xifan* , then moved on to mashed fruits, soft vegetables, *tofu* and fish which are usually added to the porridge babies eat. In Indonesia, combinations of semi-solids and solids are offered to increase the energy intake of six- to 12-month-old infants (Husain *et al*., 1991). In Bangladesh, a complementary food called *chop-chop*is made from a mixture of wheat flour, oil and brown sugar (Brown et al., 1992).

In the Palestinian homelands, majority of mothers provide their children with some types of fluids such as herbal tea, *sage*, water and other liquids in addition to breast-feeding. Majority of mothers (95%) prefer homemade over commercial complementary food because it is cleaner, free of preservatives, fresher and more economical. Rice, vegetables, fruits and soups are introduced at 1–6 months. At 7–12 month, milk, meat and eggs are used more frequently. When the child reaches 1 year of age, all the foods have already being introduced.

2.4.4 Latin American Complementary Foods

Foods most often used to complement breastmilk include locally produced cereal-based gruels and porridges (*atols*) , coffee with sugar, tortillas and bread dipped in coffee, bananas and broths. *Atole* , a high-energy moderate protein, micronutrient-fortified drink is a preferred weaning drink (Schroeder *et al*.,1993) . Atole is prepared from boiled fresh maize ground into nixtamal and boiled with a variety of ingredients including sugar, milk and water to produce *atole* . In Mexico, when *atole* is mixed with chocolate it is called *champurrado*.

Maize-based non-alcoholic beverages and porridges include *acupe* from Venezuela; *cachiri* and *fubá* from Brazil; *champuz* and *napú* from Colombia and Peru; and *pozol* , *sendechó* and *atole* from Mexico. When producing *pozol*, water and lime are mixed in a suitable container to which maize is added and boiled. Once nixtamal has been prepared, the by-product is washed and ground into maize dough, which is then shaped into small balls and covered with banana leaves to ferment. The production of pozol lasts from one to fourteen days.

In Guatemala, mothers prefer thinner complementary foods (*atolls*) for children less than one year old and thicker foods (*masa de maiz)* for children more than one year old. When the child has a cough or fever, most mothers prefer thin, liquid complementary foods. When the child had diarrhoea, about half of the mothers believe thinner complementary foods would replace the water the child lost with diarrhoea, whereas other mothers believe that thicker complementary foods would harden the stool or stop diarrhea. Foods most often used to complement breastmilk include locally produced cereal based gruels and porridges (*atols*), coffee with sugar, tortillas and bread dipped in coffee, bananas and broths. Mothers gave their children mostly soft, smooth complementary foods, such as soups or puddings, because they were easy to swallow. Simple herbal infusions, thin gruels or sweetened water are given to infants and toddlers. Early introduction (within first 2 month) of *agüitas* was strongly associated with more reported instances of diarrhea and respiratory infections irrespective of ethnicity.

In Peru, mothers prefer to give children with diarrhoea mashed rather than fried potatoes, toasted or roasted cereals and legumes rather than the coarser fresh, dried, or whole-grain forms and hard foods that had been peeled or ground (Creed de Kanashiro *et al*., 1991).

2.5 Nutritional Quality of Complementary Local Foods in Developing Countries

Home-prepared complementary foods available in low-income countries frequently lack sufficient quantities of selected essential nutrients (iron, zinc and calcium) which have been designated by the World Health Organization as problem nutrients. Deficiencies of these minerals can lead to adverse health consequences and restricted child growth and development. Inadequate intake of these nutrients occur most commonly when the local complementary foods are based primarily or exclusively on plant-derived products. This is because plant-based complementary foods usually have low mineral contents relative to young children's physiological requirements, and these foods often have high levels of inhibitors of mineral absorption, such as phytic acid (phytate).

The indigenous complementary food recipes based on starchy roots and tubers or rice contains very low amounts of iron, zinc and calcium unless they also include animal source foods. By contrast, the recipes prepared from maize and legumes or other cereal mixtures and legumes have higher iron and zinc content. But they also have considerably greater phytate content. Only those recipes enriched with liver, eggs, powdered fish (with bones) or milk powder have adequate (or near adequate) mineral contents (Helen Keller International, 2011). Energy density in these foods is usually low because of the absence of a significant fat content, thus contributing to early growth faltering (Dirorimwe, 2007).

2.5.1 Improvement of Complementary Local Foods in Developing Countries

Most of the habitually used complementary foods in developing countries are unfortified cereal-based gruels characterized by low energy and nutrient density. They are often inadequate in iron, zinc and pyridoxine and in some populations, may be deficient in riboflavin, niacin, calcium, thiamin, folate, ascorbic acid and vitamin A (Dewey & Brown, 2003; Hotz & Gibson,2001; Lutter, 2003) even in cases where strategies to improve their bioavailability are employed (Luhila & Chipulu, 1987). Incidentally, multiple micronutrient deficiencies have been reported in developing countries; the most important "problem nutrients" are iron and zinc, which requirements are difficult to satisfy without the incorporation of animal foods into the diet or by fortification (Michaelsen, 2000). The main responses in the control of these micronutrient deficiencies are dietary diversification and modification approaches that optimize trace element bioavailability and increase trace element and vitamin density to enhance the nutrient intake from plant-based local complementary foods. The options to improve trace element (iron and zinc) bioavailability include:

Addition of animal source foods (muscle tissue) and ascorbic acid sources (fruits, fruit juices) to complementary foods.

Increasing dietary diversity which is often constrained by a lack of resources for producing and purchasing higher quality foods in resource poor setting

Degradation of phytic acid by adding exogenous phytase or by fermentation, germination and soaking to activate native phytase Malting generates hydrolytic activity which is used to predigest and hence reduce the viscosity of food products, thus increasing the energy and nutrient density of weaning foods (Ralison, 2003). It also aids easy dehulling (Uvere et al., 1999) It is necessary, however, to ensure that growth of mycotoxin-producing microorganisms does not occur. Contamination of grains with pathogenic bacteria like *Bacillus cereus* and *Staphylococcus aureus* can lead to multiplication of both species in kidney beans and of only *B. cereus* in finger millet during germination.

Fermentation enhances bioavailability of iron and zinc by reducing the content of phytic acid (Gibson & Ferguson, 1998) to below 1:1 and preferably below 0.4:1 to achieve a two fold increase in iron absorption (Hurrell, 2003). For zinc, dietary phytate:zinc molar ratios below 18:1 are desired to markedly improve zinc absorption (Gibson *et al*., 2010). Phenolic compounds are also strong inhibitors of trace element absorption (Hurrell, 2003; Tuntawiroon et al., 1991; Petry *et al*., 2010) which are affected by fermentation. Besides, the associated probiotic properties, increased vitamin content, diversity of flavours, aromas and textures and preservative effects may promote infant health especially in the high risk ones

Fortification

Fortification is especially important to meet the infants' needs for energy, protein and the problem micronutrients (Austin *et al*., 1981) especially for non-breastfed infants after the first 6 months of life (Owino *et al*., 2010). The impact of fortification is, however, doubtful as the experience with commercially available, cereal-based manufactured complementary foods from Indonesia, the Philippines, Thailand, China and Mongolia (Gibbs & Gibson, 2010) has shown. Complementary foods have, however, been fortified by using food wastes such as cattle bones and foods rich in certain micronutrients (Uvere *et al*., 2010) but no bioavailability studies were conducted.

Supplementation

Higher intakes of foods from animal sources are usually associated with greater nutrient intake and higher dietary quality; it is therefore recommended that meat, poultry, fish, or eggs should be eaten daily, or as often as possible (Fairweather-Tait & Hurrell, 1996; PAHO/WHO, 2003; Yamashiro, 2006). An unidentified component of muscle tissue from meat, poultry or fish significantly enhances non-heme iron bioavailability, especially from phytate-containing cereal- and legume-based meals fed infants and adults (Bach Kristensen et al., 2005, Hurrell et al., 2006; Engekmann et al., 2006;). In addition, meat (in particular red meat) provides highly bioavailable heme iron and zinc. Ascorbic acid is a good enhancer and may be added in the home as fruit/fruit juices. Ascorbic acid substantially enhances iron absorption, primarily by reducing ferric iron to the ferrous state and thus preventing its reaction with inhibitors (Hallberg & Hulthen, 2000). Human milk can be an alternative source of ascorbic acid for breastfed children in settings where fruits and fruit juices are rarely included in the young child's diet due to limited availability, affordability or tradition. Vitamin A intake could potentially be increased by regular consumption of B-carotene-rich fruits and vegetables.

2.6 Complementary Feeding

Complementary feeding is giving infants other foods or fluids than breastmilk. Complementary food is any food other than breastmilk given in the complementary feeding period WHO/UNICEF, 1998). Complementary foods can be especially prepared for the infant or can be the same foods available for family members, modified in order to meet the eating skills and needs of the infant. In the first case, they are called transitional foods, and in the second case, there is no specific nomenclature (WHO/UNICEF, 1998).The terms related to infant feeding used in the present write up are those currently recommended by the World Health Organization (WHO) and internationally adopted, WHO/UNICEF, 1998; PAHO/WHO, 2003) The terms 'weaning foods' and "supplementary feeding"., widely used for a long time, are not recommended as synonyms for complementary feeding, since their use is incorrect, (Piwoz et al., 2003) giving the impression that foods are introduced to replace breastmilk, instead of complementing it (Anderson et al., 2001). The use of the term weaning is not advisable, since in many Countries (Daelmans, 2003), it may be understood as total cessation of breastfeeding and cause problems in breastfeeding promotion. The term weaning was used to indicate the transition between exclusive breastfeeding and the cessation of breastfeeding. Nowadays, the term full weaning is used to indicate the total cessation of breastfeeding (WHO/UNICEF, 1998).

2.6.1 Complementary Feeding of the Breastfed Child

Infant feeding from birth up to the first years of life influences an individual whole life. It is common knowledge that breastfeeding is important for optimal infant feeding. Breastmilk alone can be used to properly feed infants in the first six months of life, but from then on, complementary feeding is necessary. The nutritional adequacy of complementary foods is essential to the prevention of infant morbidity and mortality, including malnutrition and overweight (ACC/SCN/WHO, 2000). The linear growth retardation acquired early on in infancy cannot be easily reversed after the second year of life (Martorell *et al*., 1994)

Age of introduction of Complementary Foods

It is difficult to pinpoint the ideal time for starting provision to infants of diet other than breast milk (Lanigan *et al*., 2001). There is high risk of harmful effects (possibility of choking, food allergies, and decrease in breast milk intake or formula) through the early introduction of complementary foods. Delayed introduction may miss developmental readiness of infants (and difficulties learning to eat at later ages) while risking malnutrition at the same time (USDA, 2009).

According to pediatric nutrition authorities, developmental readiness in most infants and the ability to tolerate foods consumed would occur around 4 and 6 months of age (USDA, 2009, Issaka, 2015). During this period, the intestinal tract will have well-developed defense system that minimizes or averts risk of allergic reaction in infants following intake of foods containing foreign proteins, while its ability to utilize proteins, fats, and carbohydrates improves. Similarly, the infant’s kidney develops to a state where it can successfully eliminate waste products emanating from foods such as meat with characteristic high renal load. Furthermore, their neuromuscular system matures enough leading into development of abilities for recognizing food, accepting spoons, masticating and swallowing foods, and, even, distinguishing and appreciating varieties in food tastes and colors (USDA, 2009; Cohen *et al*., 1994 and Health Service Executive, 2008). There is no evidence for harm when safe nutritious complementary foods are introduced after 4 months when the infant is developmentally ready. Similarly, very few studies show significant benefit for delaying complementary foods until 6 months.

Introduction of appropriate diet corresponding to developmental stage of the infants allows provision and intake of sufficient nutrients as per their requirements and facilitates proper development of eating and self-feeding skills. Recommendations for complementary food introduction should follow assessment of infant's developmental readiness, nutritional status, and health status; the family's economic and socio cultural issues toward diet and food preferences; and other findings viewed important for consideration (USDA, 2009 and Issaka, 2015).

Consistency of Complementary Foods

The minimum age at which infants develop the ability to swallow particular type of food is highly dependent on their level of neuromuscular development (WHO, 1998). Thus, failure to account such abilities for mastication and swallowing when preparing and serving diets to infants may result in consumption of only trivial amount or extended feeding time (USDA, 2009). Starting at 6 months, infants can eat pureed, mashed and semi-solid foods prepared from infant cereal, vegetables, fruits, meat, and other protein-rich foods. By 8 months, most infants will become capable of eating "finger foods." In line with the changing oral skills and emerging new abilities (such as munching, chewing, etc.), the thickness and lumpiness of the foods can gradually change from pureed to ground, fork-mashed, and eventually diced foods (WHO, 200). Introduction of lumpy solid foods should occur around a critical age window of 10 months so as to avoid latent risk of feeding difficulty associated with late introduction (EFSA, 2009). Evidences suggest that most infants are able to consume solid consistency "family foods" by 12 months, even if they frequently are still served semi-solid foods (EFSA, 2009). To enhance optimal growth of the child, it is highly advisable to increase the consistency of the foods gradually with age of the infants even when it would result in longer feeding time for the caregivers. Foods that may cause choking by getting into or blocking airways should be avoided. The risk of chocking from ingesting certain food is often subject to its *size* (small, but hard, pieces that may get into the airway and larger more difficult to chew pieces that may block airways), *shape* (sphere or cylinder shaped that may block airways), and *consistency* (firm, smooth, or slick foods that may slip down the throat; dry or hard foods; sticky or tough foods that may not break apart easily and may be hard to remove from the airways) (WHO, 2001; USDA, 2009 and WHO, 2009).

Disadvantages of early or late complementary feeding

Several studies carried out in developing countries and in industrialized countries showed that the early introduction of complementary foods increases infant morbidity and mortality, as a result of the reduced ingestion of protective factors present in breastmilk, in addition to the fact that complementary foods are an important source of contamination for infants (Dewey *et al*., 2001). From the nutritional viewpoint, the early introduction of complementary foods can bring some disadvantages, since these foods, in addition to replacing part of breast milk, even when breastfeeding frequency is maintained, (Drewett et al., 1993) often have a lower nutritional value than breast milk (WHO/UNICEF, 1998) for instance, foods that are extremely diluted. A shorter duration of exclusive breastfeeding does not protect infant growth so well as exclusive breastfeeding for six months does (Kramer et al., 2002) and neither does it improve it (Dewey *et al*., 1999).

After the sixth month, the replacement of breastmilk with complementary foods is less problematic (PAHO/WHO, 2003).Moreover, the early introduction of complementary foods shortens the duration of breastfeeding (Zeitlin & Ahmed, 1995) interferes with the uptake of important nutrients found in breastmilk, such as iron (Oski & Landaw, 1980) and zinc (Bell et al., 1987) and reduces the efficiency of lactation in preventing new pregnancies (McNeilly et al., 1985). More recently, the early introduction of complementary foods has been associated with the development of atopic diseases. Exclusive breastfeeding minimizes the risk of asthma and this protective effect seems to persist for at least during the first decade of life, which is particularly evident in children with a family history of atopic diseases (van Odjik *et al*., 2003) Exclusive breastfeeding also, seems to protect against the development of type 1 diabetes mellitus. It has been described that early exposure to cow.s milk (before the fourth month) can be an important determinant factor for this disease and that it can increase the risk for diabetes by 50%. It is estimated that 30% of the cases of type 1 diabetes mellitus could be avoided if 90% of the infants aged up to three months were not fed cow.s milk (Gerstein, 1994). In some countries, there is a recommendation to only introduce some specific foods, considered highly allergenic, after the second year of life. Cow.s milk (responsible for 20% of food allergies) ranks on the top of the list, being not recommended before 9-12 months. In case of important family history of food allergy, it is recommended that foods such as eggs, peanuts, nuts and fish not be given in the first year of life. There is a recommendation that honey should be avoided in infants younger than 12 months in order to prevent botulism (Dewey, 2000).

When infants exclusively breastfed for six months do not develop properly, before considering the introduction of complementary foods, a careful assessment should be made to verify whether they are not ingesting too little breast milk due to a poor breastfeeding technique, which leads to improper emptying of the breasts and, consequently, to a low milk production. In these cases, the usual recommendation is that mothers receive instructions and support so that the baby can increase the intake of breastmilk and complementary feeding is not introduced unnecessarily (WHO/UNICEF, 1995).

The late introduction of complementary foods also is disadvantageous, because infant growth stops or slows down and the risk of malnutrition and micronutrient deficiency increases (PAHO/WHO, 2003).

2.6.2 Energy and Nutrient Composition of Complementary Foods

Complementary foods are expected to bridge the gaps in energy and nutrients between daily requirements for infants and young children and the amount consumed through breastfeeding. As such, the diets should be high in energy density, with balanced protein composition (containing all essential amino acids), required vitamins and minerals (iron, folic acid, and calcium), no (safe level) antinutritional components, and while retaining the qualities for palatability (UNICEF, 2011)

A proper complementary feeding consists of foods that are rich in energy and in micronutrients (especially iron, zinc, calcium, vitamin A, vitamin C and folates), free of contamination (pathogens, toxins or harmful chemicals), without much salt or spices, easy to eat and easily accepted by the infant, in an appropriate amount, easy to prepare from family foods, and at a cost that is acceptable by most families (WHO, 2000)

2.6.2.1 Energy Requirement

Complementary foods are expected to have sufficient energy density to provide a growing child with adequate daily energy requirement. Energy density is the number of kilocalories of energy in certain food per milliliter per gram of that food. Breast milk contains an energy density of about 0.7 kcal/ml (WHO, 2002). The recommended minimum energy density in complementary foods is 0.8 kcal/g higher compared to that of breast milk. In reality, the energy density in complementary foods usually is between 0.6 and 1.0 kcal/g and may even drop to as low as 0.3 kcal/g in watery and dilute foods. Consequently, the amount of complementary food required to cover the energy gap corresponds to the level of energy density in the diets served (WHO, 2009). Energy-dense foods are most important for children with wasting, as they have an increased energy need for catch-up growth. Low energy density complementary foods have long been implicated in PE malnutrition (Daelmans & Saadeh, 2003).

The total energy requirement estimated for healthy breastfed infants is approximately 615 kcal/day at 6–8 months, 686 kcal days at 9–11 months and 894 kcal/day at 12–23 months (Dewey, 2003). For infants in developing countries with “average” breast milk intake, the energy need from complementary food increases from 200kcal/day at 6–8 months to 300 and 500 kcal/day at 9–11 and 12–23 months, respectively. This accounts for 29, 55, and 71% of total daily energy needs, respectively, coinciding with the decreased intake of human milk at older ages. These values could vary based on the level of breast milk intake per day (Dewey, 2003 and WHO, 2001).

The amount of food needed per day to satisfy their energy requirement is a function of amount of energy needed from complementary foods and the energy density of the foods (i.e., kilocalories per gram) (WHO, 2009 and Caballero & Allen, 2005). For complementary foods with energy density range of 0.6–1 kcal/g, the amount (gram or volume) of food needed to provide energy requirement is 200–333 g/ day for 6- to 8-month-old, 300–500 g/day for 9- to 11-month-old, and 550–917 g/day for 12- to 23-month-old children. Energy- dense foods have energy density of 1.07–1.46 kcal/g. For such foods, the approximate quantity of complementary food that would meet the energy needs described above is 137–187 g/day for 6–8, 206–281 g/day for 9–11, and 378–515 g/day for 12- to 23-month-old children (WHO, 2004). The number of meals per day is dependent on the energy gap for stated age, gastric capacity of the child, and energy density of the meal (kilocalories per gram). Thus, for a given age interval and level of breast-milk intake, calculating the recommended number of meals requires information about the energy density of the foods. For older children requiring larger quantity of food in a day, the food needs to be sub-divided multiple servings compared to their younger counterparts (WHO, 2001). For foods containing recommended minimum energy density (0.8 kcal/g), assuming gastric capacity of 30 g/kg body weight, the meal frequency expected to provide adequate daily energy requirement is two to three times for 6–8, and three to four times for 9–11 and 12- to 24-month-old children, with one to two nutritious snacks (WHO, 2001). Transition from two to three meals or smaller to larger meals between the ages should happen gradually based on appetite and development of the child (WHO, 2009).

2.6.2.2 Protein Requirement

The recommended protein content (grams of protein per 100 kcal of food) for complementary foods is of 0.7 g/100 kcal, from 5 to 24 months. In most countries, the protein requirements of infants are met when the energy intake is appropriate, except if there is a predominant intake of low protein foods (e.g: sweet potato and cassava) (WHO/UNICEF, 1998). It is of paramount importance that infants eat high quality and easily digestible proteins, which are found in breastmilk and in animal products. Alternatively, high quality protein can be provided by properly mixing some vegetables or cereals (ex: rice and beans). (Cameron & Hofvander, 1983)

Protein makes important nutrient composition in complementary foods. They are major sources of essential amino acids and energy at times of energy deprivation. Adequate supply of dietary protein is vital for maintaining cellular function and integrity and for ensuring normalcy of health and growth. On the other hand, the combined effect of protein deficiency and low energy intake leads to protein energy (PE) malnutrition, the commonest forms of malnutrition worldwide (Rolfes et al., 2008). The protein requirement of infants and young children increases with their age. The amount of protein (in grams per day) required to satisfy their daily nutritional requirement is 9.1 g for 6–8 months, 9.6 g for 9–11 months, and 10.9 g for 12–23 months. Breast milk provides a significant portion of daily protein requirement of infants and young children. When average breast-milk intake is assumed, the amount of protein needed from complementary foods is 1.9 g/day at 6–8 months (21%), 4.0 g/day at 9–11 months (42%), and 6.2 g/day (57%) at 12–23 months (Dewey, 2001; WHO, 2005 and WHO/UNICEF, 1998).

The PE ratio, which is the ratio of energy from protein to total energy contained in certain amount of food, is one of the indicators used to assess the quality and adequacy of protein in complementary foods. Based on the protein quality of the food (high quality, such as milk, or lower quality, such as plants) and age of children, the recommendations for PE ratio vary. Expressed as a percentage of estimated energy requirements coming from protein, some countries set a PE ratio range of 8–15% energy from protein (i.e., 23.75 g protein per 100 total kilocalories). The PE ratio is approximately 7.5% in human milk and 8–8.5% in infant formulas. These ratios are adequate for a normal rate of growth (EFSA, 2009 & Reeds and Garlick, 2003). The minimum recommended PE ratio to come from complementary foods of children between 6 and 23 months of age lies between 4.3% (for foods with high protein quality, such as milk, and for child of age 23 months) and 6.3% (for foods with low protein quality, such as plants, and for a child of age 6 months) (Caballero et al., 2005).

2.6.2.3 Fats/Lipids Requirement

Lipids in complementary foods should provide approximately 30 to 45% of the total energy required (Dewey & Brown, 2003; Bier et al., 1999) which is enough to guarantee the adequate intake of essential fatty acids, good energy intake and uptake of fat soluble vitamins (PAHO/WHO, 2003). Fat in the diet affects the general intake of nutrients (WHO/UNICEF, 1998) and, if excessive, may exacerbate micronutrient malnutrition in vulnerable populations (PAHO/WHO, 2003). Anecdotal evidence suggests that excessive fat intake predisposes to childhood obesity and cardiovascular diseases (Milner & Allison, 1999)

Dietary fats constitute an important portion of nutrients obtained from foods. For infants and young children, they are source of energy, essential fatty acids, and fat soluble vitamins (A, D, E, and K). In addition, dietary fats have an important role in promoting good health and enhancing the sensory qualities of the foods (Rolfes *et al*., 2008). Fat accounts for about 50% of breast-milk’s energy and serves as primary energy source for infants during the first 6 months of life. With the introduction of complementary food, however, fat is gradually overtaken by carbohydrate as the chief energy source. Even so, fat remains important source of energy, and together with carbohydrates, they meet the energy needs of the growing child (WHO, 2001 and Monte & Giugliani, 2004). There is often a debate on the optimal amount of fat in the diets of infants and young children. Although limited evidences exist, average daily fat equivalent to 30–45% of energy intake is often suggested to balance the compromise in risks from little intake (especially essential fatty acids and lowered energy density levels), and excess intake and the likelihood of childhood obesity and cardiovascular diseases (WHO, 2001 and Monte & Giugliani, 2004). This recommendation also guarantees adequate intake of essential fatty acids, fat soluble vitamins, and improved energy (WHO, 2001 and WHO, 2009). If the percentage of energy from fat is accounted to be at least 30%, the amount of fat needed from complementary foods to satisfy daily requirement for infants and young children depends on the level of breast milk intake. For those with low breast milk intake, complementary foods should provide dietary fats appro-priating to 34, 38, and 42% of daily energy requirements for 6–8, 9–11, and 12–23 months, respectively. With adequate breast milk intake, however, the requirement from complementary foods is 0 g/day (0%) at 6–8 months, 3 g/day (5–8%) at 9–11 months, and 9–13 g/day (15–20%) at 12–23 months (WHO, 2001).

2.6.2.4 Carbohydrates

Starch is likely to be a major constituent of many complimentary foods for older infants and young children. To ensure that its energy value is realized, this starch should be provided in a readily digestible form. Increasing the intake of dietary fibers increases stool bulk, causes flatulence, and decreases appetite. Lack of agreement on the definition of fiber and differences in analytical techniques make it difficult to compare recommendations from different sources. Infants consume a very low-fiber diet, although oligosaccharides in breast milk are thought to have fiber-like properties. Fibers should be introduced gradually into their diet from the age of 6 months. Use of large quantities of whole grain cereals and pulses or nuts during infancy is not recommended as they are likely to affect bioavailability of micronutrients and result in a low-energy diet (Caballero *et al*., 2005). The extent to which foods produce satiation and sustain satiety depends, in part, on their nutrient composition. Proteins and lower energy density foods are considered much satiating. Similarly, high-fiber foods effectively provide satiation by fill-ing the stomach and delaying the absorption of nutrients. Such attributes of the complementary foods may also lower the child’s feeding ability (Rolfes *et al*., 2008).

2.6.2.5 Micronutrients

Micronutrients are essential for growth, development, and prevention of illness in young children (WHO, 2009). Adequate intakes of micronutrients, such as iron, zinc, and calcium, are important for ensuring optimal health, growth, and development of infants and young children (Caballero *et al*., 2005 and Rolfes *et al*., 2008). Breast milk makes substantial contribution to the total nutrient intakes. In well nourished mothers, breast milk contains generous amounts vitamin A, B, C, folate, iodine, and selenium. As a result, the amount needed from complementary foods before 12 months is 0 (or close to 0) (Dewey, 2001 and Dewey & Brown, 2003). However, breast milk is relatively low in several other micronutrients, even after accounting for bioavailability. The percentage of total daily requirement for micronutrients needed from complementary foods ranges from 30 to 97%. For instance, 97% of iron, 86% of zinc, 81% of phosphorus, 76% of magnesium, 73% of sodium, and 72% of calcium during 9–11 months are expected from complementary foods (Dewey, 2001; WHO, 2001 and Dewey & Brown, 2003). Thus, added to the fact that infants bear only limited gastric capacity to consume adequate quantity of food, the diets need to have very high nutrient density (WHO, 2001).

2.6.2.6 Mineral content

To meet the nutritional mineral requirements of infants, a variety of mineral-rich complementary foods should be offered, since the consumption of these foods is relatively small among infants/children aged 6 and 24 months (WHO/UNICEF, 1998). From 9 to 11 months of life, the amount of minerals that should be provided by complementary foods is high: 97% for iron, 86% for zinc, 81% for phosphorus, 76% for magnesium, 73% for sodium and 72% for calcium (Dewey & Brown, 2003).

Iron

The recommended iron intake is of 4 mg/100 kcal from 6 to 8 months, 2.4 mg/100 kcal from 9 to 11 months and 0.8 mg/100 kcal from 12 to 24 months. In developing countries, due to low iron intake and bioavailability (only approximately 11 to 18% of uptake), iron requirements often cannot be totally met (WHO/UNICEF 1998, Dewey *et al*., 1998; Allen & Ahluwalia, 1997). Infants aged between six and 12 months cannot eat enough iron-rich foods to meet their requirements (WHO/UNICEF 1998, Gibson et al., 1998) in addition to the fact that the price of these foods can be prohibitive to low-income families (WHO/UNICEF 1998; Lutter, 2003; Lutter, 2000). The availability of iron-fortified foods is larger in industrialized countries than in developing countries (WHO/UNICEF 1998; Lutter, 2000). This is why iron-deficiency anemia is highly frequent among infants younger than two years in developing countries. Foods of animal origin have a better iron bioavailability (up to 22% of uptake) than those of vegetable origin (1 to 6%). Meats (especially red meat) and some animal organs (mainly liver) have some advantage over milk and its derivatives due to their iron content and bioavailability. Some foods contain reasonable iron content, but low bioavailability. This is the case of egg yolk, beans, lentils, soybean and dark green vegetables (Swiss chard, kale, broccoli, white mustard, wild chicory). The iron uptake in foods of vegetable origin can be enhanced if some foods such as meat, fish, fructose and ascorbic acid (orange, guava, lemon, mango, papaya, melon, banana, passion fruit, peach, tomato, capsicum, green leaves, cabbage, broccoli, cauliflower) are offered in the same meal. In this case, raw and fresh foods should be preferred, as vitamin C is destroyed during cooking.5 On the other hand, eggs, milk, tea, mate or coffee hamper iron uptake, since they form insoluble precipitates with iron. The inhibitory effect of whole cereals (rice, corn, wheat) is due to the presence of phytates and not of fibers, which do not have an inhibitory effect. Milk inhibits the uptake of heme and nonheme iron due to its calcium content and probably due to the presence of phosphoproteins. The high intake of cow.s milk contributes to the high prevalence of childhood anemia. In a cohort of European infants at 12 months of life, hemoglobin level decreased by 0.2 g/dl to each additional month in which nonfortified cow.s milk was ingested (Male et al., 2001). In São Paulo, Brazil, the risk for anemia is 2.2 times higher in infants/children aged between six and 59 months with a higher milk intake than those with a lower milk intake (Levy-Costa, 2002).

2.6.2.7 Vitamin content

Vitamin A

If the mother's diet has adequate vitamin A content, the offer of vitamin A-rich foods easily meets the requirements of the nursing infant. If the mother lives in a vitamin A deficiency endemic area, she should receive special supplementation (Undeerwood, 1994; Huffman, *et al*., 1998) and her infant should be offered vitamin A-rich foods (Allen & Gillespie, 2001) preferably some time before or after breastfeeding in order to increase the uptake of carotene and retinol from the diet (WHO/UNICEF, 1998). The major food sources of vitamin A are liver, egg yolk, milk products, dark green and leafy vegetables and yellow/ orange vegetables and fruit (carrots, pumpkin, red peppers, yellow peppers, mango, passion fruit and papaya).

Vitamin D

Breastmilk and complementary foods have very little to contribute to the supply of vitamin D requirements, since this vitamin basically depends upon direct exposure of the skin to sunlight. Its dietary intake is only important in case of inappropriate endogenous production or depletion of body stores. In exclusively breastfed babies unexposed to sunlight, vitamin D stores present at birth would probably become depleted within eight weeks (Ala-Houhala, 1985) However, a few hours of sunlight exposure in the summer. 0.5 to 2 hours a week (17 minutes a day) with exposure of the baby face and hands only, and 30 minutes a week (4 minutes a day) if the baby is wearing nothing but diapers produces enough vitamin D to avoid deficiency for several months (Poskitt *et al.*, 1979). Dark-skinned infants require three to six times more exposure than fair-skinned babies to produce the same amount of vitamin D (Specker *et al.*, 1985; Mojab, 2002).

2.7 Food Items Used to Prepare Complementary Foods

From the sixth month onward, complementary foods should be of variety, and balanced mixtures of food items containing cereals, tubers, foods of animal and vegetable origin, and fat should be offered. Only a varied diet guarantees the supply of micronutrients, enhances good eating habits, and prevents the development of anorexia caused by monotonous foods (Monte & Giugliani, 2004; WHO, 2009 and FAO, 2011).

Grain products (whole grain) can serve as sources for carbohydrates, fibers, and micronutrients such as thiamin, niacin, riboflavin, and iron. Protein-rich foods, such as meat, poultry, fish, egg yolks, cheese, yogurt, and legumes, can be introduced to infants between 6 and 8 months of age. Fruits and vegetables introduced over time can provide infants with carbohydrates, including fiber, vitamins A and C, and minerals (Monte & Giugliani, 2004; Northstone, 2001 and FAO, 2011).

Complementary foods usually are of two types: commercially prepared infant foods bought from the market and homemade complementary foods, which are prepared at household level by the caregivers following traditional methods. Commercially, complementary foods can be produced following simple technologies such as malting, popping, fermentation, or using modern food-processing technologies such as roller drying and extrusion cooking. Some of the commonly available commercially prepared infant foods include iron- fortified infant cereal made of food items, such as *rice*, oat, and barley, wheat, mixed-grain infant cereals, and infant cereal and fruit combinations; juices such as infant juices, citrus juices, canned juices, and unpasteurized juices; commercially prepared vegetable or fruit infant foods; and commercially prepared infant food meats (Ng et al., 2012 and Hotz & Gibson, 2007).

2.7.1 Homemade Complementary Foods

Complementary foods could also be prepared at household level by the caregivers following other traditional methods. These are commonly described as homemade complementary foods. The recommendation for specific food type to prepare depends on their age appropriateness and development stage of infants and young children.

For infants and young children of age 6–11 months, for instance, provision of *thick porridge* made of maize, cassava, millet; to which milk, soy, ground nuts, or sugar is added; or *mixtures of pureed foods* made out of potatoes, cassava, *posho* (maize or millet), or rice, being mixed with fish, beans or pounded ground-nuts, and green vegetables added would allow consumption of nutritious foods. Addition of nutritious snacks, such as egg, banana, bread, papaya, avocado, mango, other fruits, yogurt, milk, and puddings made with milk, biscuits or crackers, bread or *chapatti* with butter, margarine, groundnut paste or honey, bean cakes, and cooked potatoes, would suffice their nutritional needs (WHO, 2009, 1998, 2003; FAO, 2011 and FMOH, 2006).

For children of 12–23 months, provision of adequate servings of *mixtures of mashed or finely cut* family foods made out of potatoes, cassava, posho (maize or millet), or rice; mix with fish or beans or pounded groundnuts; add green vegetables or *thick porridge* made out of maize, cassava, and millet; add milk, soy, ground nuts, or sugar, would allow consumption of nutritious foods. Addition of nutritious snacks, such as egg, banana, bread, papaya, avocado, mango, other fruits, yogurt, milk, and puddings made with milk, biscuits or crackers, bread or *chapatti* with butter, margarine, groundnut paste or honey, bean cakes, and cooked potatoes, would suffice their nutritional needs (WHO, 2009, 1998; FAO, 2011, and FMOH, 2006).

The complementary foods should also contain foods rich in *iron*: liver (any type), organ meat, flesh of animals (especially red meat), flesh of birds (especially dark meat), and foods fortified with iron; *vitamin A, K*: liver (any type), red palm oil, egg yolk, orange colored fruits and vegetables, and dark green vegetables; *zinc*: liver (any type), organ meat, food prepared with blood, flesh of animals, birds, and fish, shell fish, and egg yolk; *calcium*: milk or milk products and small fish with bones; and *vitamin C*: fresh fruits, tomatoes, peppers (green, red, and yellow), and green leaves and vegetables (WHO, 2009, 1998, 2003; FAO, 2011 and FMOH, 2006).

In many developing countries, commercial fortified food products are often beyond the reach of the poor. As a result, homemade complementary foods are frequently used during child feeding (USAID, 2010). The basic recipe food items used for the preparation of the complementary food commonly base on locally available staples, while the choice of specific food item differs considerably between populations, owing to tradition, availability, and ease of access (Kuyper et al., 2013). In many developing countries, the staples are cereals, roots, and starchy fruits that consist mainly of carbohydrate and provide energy (USAID, 2010).

Cereals form the staple foods of virtually all populations. Cereals are an important source of energy, providing between 334.4 and 382.2 kcal/100 g of whole cereal, and provide starch and dietary fibers (soluble and insoluble). Grains comprise 70–77% of all cereals, which usually are processed and cooked to make the starch more digestible (Caballero *et al*., 2005). In cereals, 65–75% of the total weight is carbohydrate, 6–12% is protein, and 1–5% is fat. The protein quality, however, is very low compared to animal-based foods (Ng et al., 2012). For instance, the protein composition of maize and guinea corn used in several West African countries is of poor protein quality and low in lysine and tryptophan. In Nigeria, corn gruel contained only 0.5% protein and less than 1% fat, compared to 9% protein and 4% fat in the original corn, and has been indicated to have been too low even to support the growth of rat (Ogbonnaya, et al., 2012 and Onofiok & Nnanyelugo, 1998).

In several West African countries, the first solid food and popular complementary foods are based on thin cereal and are low in foods from meat, eggs, or fish, especially among low-income groups due to socioeconomic factors, taboos, and ignorance (Onofiok & Nnanyelugo, 1998).

In Nigeria, for instance, such foods are made from maize (*Zea mays*), millet (*Pennisetum americanum*), or guinea corn (*Sorghum* spp.) After successful introduction of cereal gruel, other staple foods in the family menu, such as yam (*Dioscorea* spp.), rice (*Oryza sativa*), *gari* (fermented cassava grits), and cocoyam (*Xanthosoma sagittifolium*), are given to the child being mashed, thinned, or pre-chewed. Legumes are rarely used and are introduced much later (after 6 months of age) because of the problems of indigestibility, flatulence, and diarrhea associated with their use (Ogbonnaya, et al., 2012 and Onofiok & Nnanyelugo, 1998).

A commonly shared phenomenon about homemade complementary foods that are based on starchy roots and tubers or rice and available in many low-income countries is their frequent shortfall in amounts of selected essential micronutrients such as calcium, iron, and zinc. In contrast, the recipes prepared from maize and legumes or other cereal mixtures and legumes had higher iron and zinc contents, but they also have considerably higher phytate contents (Gibson, 2010). Under both circumstances, they fail to meet the theoretical mineral requirements of young children due either to their low mineral content or as a result of low bioavailability, unless enriched with animal-source foods such. As a result, WHO designates calcium, iron, and zinc as “problem nutrients” and deficiencies of these minerals can lead to adverse health consequences and restricted child growth and development (WHO, 2001 and Gibson, 2010).

Complementary foods need to be far more nutrient-rich compared to family foods. Yet, the opposite is the case in low- income households. The foods are often known to be of low nutritive value and are characterized by low protein, low energy density, and high bulk (Dewey, 2013). Bulk is one of the major problems of homemade complementary foods, where a problem of high viscosity, low energy density, or both may occur (WHO, 2001). Under such circumstances, it is usually possible to achieve an adequate protein and energy intake for adults and older children by increasing the daily intake. For infants and younger children, however, the volume of the diets may be too large to allow the child to ingest all the food necessary to cover his or her energy needs. For instance, a 4- to 6-month infant would need 62 g of corn gruel to meet daily needs of energy (740 kcal) and protein (13 g), which would be an impossible task considering the size of an infant’s stomach (Onofiok & Nnanyelugo, 1998 and Temesgen, 2013).

2.7.2 Selection of Complementary Foods

The infant can be fed family foods, provided that consistency and energy content are appropriate. Food preparations that do not meet the minimum energy requirements (e.g.: soups, oatmeals and overly diluted milks) should be avoided (WHO/UNICEF, 1998). From the eighth month onwards, foods should vary and balanced mixtures containing cereals, tubercles, foods of animal and vegetable origin, and fat should be offered (WHO, 2000). Only a varied diet guarantees the supply of micronutrients enhances good eating habits and prevents the development of anorexia caused by monotonous foods (WHO/UNICEF, 1998). Infants and adults later on, tend to prefer the foods the way they were initially introduced. Therefore, infants should be initially offered foods containing low sugar and salt contents (WHO, 2000).

It is important to guarantee the offer, every day if possible, of foods of animal origin rich in iron and of fruit and vegetables, especially those rich in vitamin A (WHO/UNICEF, 1998; Butte, 1996; Ala-Houhala, 1985). Nonfortified or nonsupplemented vegetarian diets are not recommend for infants younger than two years because they do not meet the requirements of some nutrients, such as iron, zinc and calcium. (WHO/UNICEF, 1998; PAHO/WHO, 2003; Dewey & Brown, 2003; Gibson *et al*., 1998) It is not advisable to give infants younger than one year unmodified cow's milk, especially if raw and undiluted, because its use is associated with blood loss in the stools and iron deficiency (Ziegler *et al*., 1990; Griffin & Abrams, 2001; Smith & Lifshitz, 1994). Avoid offering sugary beverages (soft drinks and others), as they reduce the infant's appetite for more nutritious foods and may soften the stools (PAHO/WHO, 2003). Tea and coffee are also unadvisable because they may interfere with iron uptake (WHO/UNICEF, 1998; PAHO/WHO, 2003).

The American Academy of Pediatrics recommends a maximum of 240 ml/day of fruit juices, to avoid competition with nutritionally richer foods (PAHO/WHO, 2003). Association between excessive intake of fruit juices and failure to thrive (Smith & Lifshitz, 1994) short stature and obesity (Dennison *et al*., 1997) has been reported, but further studies are necessary in order to confirm these findings (Skinner, 1999). To guide mothers/caregivers in the selection of complementary foods, health professionals must know the nutritional value of local foods, and their use in infant feeding (WHO/UNICEF, 1998; WHO, 2000). If necessary, local food composition tables should be referred to (PAHO/WHO, 2003).

2. 7.3 How to Introduce Complementary Foods

The recommendation is that new foods be gradually introduced, one at a time, every three to seven days. It is common for infants to reject new foods, but this should not be interpreted as permanent aversion to that food. On average, infants need to be exposed to a new food eight to 10 times until they accept it well (Birch *et al*., 1987; Sullivan & Birch, 1994). Breastfed infants tend to accept new foods more easily than non breastfed ones, because via the breast milk, they are exposed to different flavours and scents very early on, which vary according to the maternal diet. Thus, infants are introduced to the family eating habits from the moment of birth (probably during the intrauterine life too). Improper food consistency compromises the appropriate intake of nutrients by the infant (PAHO/WHO, 2003). Therefore, at the beginning of complementary feeding, the foods should be especially prepared for the infant. The foods should be initially semi-solid and soft (in the form of a puree), and should be crushed, never sifted or blenderized. Soups and soft foods do not provide enough calories to meet energy requirements of infants and are therefore not recommended.

Food consistency should be gradually improved, considering the infant's eating skills (WHO/UNICEF, 1998). At eight months, the infant can be offered family foods, provided that they are crushed, shredded, chopped or cut into small pieces. At 10 months, the infant can eat grain foods; otherwise, he/she will be at greater risk for eating disorders at 15 months (Northstone *et al*., 2001). At 12 months, most infants can eat the same foods their family eats, provided that these foods have an appropriate energy content and consistency (Dewey & Brown, 2003). After that, the offer of semi-solid foods should be restricted, and sharp foods and/or foods with a hard consistency should be avoided (e.g.: raw carrots, nuts, grapes), as they can make infants choke (WHO, 2000).

Complementary foods should be given using a spoon or glass (PAHO/WHO, 2003) which are well accepted by infants. Baby bottles should be avoided because, in addition to being an important source of contamination for the infant, they interfere with oral dynamics (Newman, 1993) and may cause "nipple confusion" (Neifert *et al*., 1995) especially during the establishment of breastfeeding, exposing the infant to a greater risk of early weaning (Newman, 1990; WHO, 1998). One should recall that the use of baby bottles is not necessary during the baby's growth (WHO, 2000). Complementary foods can be offered either before or after breastfeeding (WHO/UNICEF, 1998; Drewett et al., 1987)

In some populations, infants are encouraged to eat only when they are sick or when they refuse to eat (Engle & Zeitlin, 1996; Bentley *et al*., 1991; Bentley *et al*., 1992). In other populations mother or caregivers sometimes use inappropriate ways to encourage their infants to eat. Currently, WHO recommends that mothers/caregivers of infants younger than two years follow the responsive feeding practice, which employs the principles of psychosocial care (Engle *et al*., 2000; Pelto *et al*., 2003). This practice includes respect for the physiological mechanism that self-regulates the appetite in infants, helping them to feed until they feel satiated, and requires that mothers/caregivers be aware of the signs of hunger and satiety expressed by the infants. Infants should be fed slowly and patiently until they feel satiated; they should never be force-fed. Meals should be pleasant, with emotional exchange between the person who feeds and the infant, using eye contact, touching, smiling and talking. If infants refuse to eat several foods, different combinations, flavors, and textures should be attempted, and besides, non coercive ways to encourage them to eat and that do not divert their attention during the meal should be used (PAHO/WHO, 2003). There is some evidence that active feeding improves food ingestion and the infant's nutritional status (Ruel *et al*., 1999) and development. (Sternin *et al*., 1997; Creed de Kanashiro *et al*., 2001).

Amount and frequency

The small amount of complementary foods initially offered should be gradually increased with age. The amount and frequency of foods should be based on infant's acceptance, which varies according to individual needs, the amount of breast milk ingested and the content of complementary foods (PAHO/WHO, 2003; Dewey & Brown, 2003). The infant should be encouraged to eat until he/she feels satiated (PAHO/WHO, 2003). The current recommendations regarding the frequency of meals with complementary foods for breastfed infants result from theoretical estimates based on the energy provided by complementary foods, assuming a gastric capacity of 30 g/kg and an energy intake of at least 0.8 kcal/g (PAHO/WHO, 2003; Dewey & Brown, 2003) The minimum frequencies of meals per age were calculated such that the requirements of almost all infants could be safely met (Dewey & Brown, 2003). Thus, WHO currently recommends two to three meals a day with complementary foods for breastfed infants between 6 and 8 months of life and three to four meals a day for those between 9 and 24 months, with additional nutritious snacks (PAHO/WHO,2003) pieces of fruit or bread, couscous, homemade cake, cassava) once or twice a day at 12 months (If energy content or the amount of complementary foods per meal is small, or if the infant has been completely weaned, a higher frequency of meals may be necessary (;PAHO/WHO, 2003; Dewey & Brown, 2003). It should be underscored that meals with complementary foods do not replace (but complement) breastfeeding. The frequency of breastfeeding can be maintained. With the introduction of complementary feeding, the infant will naturally begin to nurse less. Therefore, avoid an excessive number of meals with complementary foods in breastfed infants so as not to substantially decrease the amount of breastmilk ingested by the infant (Rivera et al., 1998) Nutritious snacks are time-saving and contribute less to milk displacement (PAHO/WHO, 2003).

2.7.4 Hygiene practices for complementary foods

Contaminated complementary foods are the major route of transmission of diarrhea among infants (Black et al., 1989). For this reason, the higher incidence of diarrhea in the second semester of life coincides with the increase in the intake of these foods (Martinez *et al*., 1992) Proper maternal practices regarding the management, preparation, administration and storage of complementary foods may reduce their contamination (Feachem *et al*., 1983). Safe food hygiene practices include the following: those who handle the food, during preparation or feeding, should wash their hands properly with soap and water, after using the toilet and before meals, and the infant's hands should be washed likewise; kitchen utensils and cooking surfaces should be kept clean; only healthy-looking foods should be used and they should be kept in a safe place; an amount of food that suffices one meal only should be prepared and it should be served immediately after preparation; the infant should be fed from a glass or cup, spoon and plate, avoiding the use of baby bottles; infants should not be given leftovers from the previous meal; and, if using a fridge, it should be cleaned regularly and any spoilt foods should be thrown away (WHO, 2000)

2.8 Factors That May Affect Consumption of Novel and Underused Complementary Foods

Barriers to consumption of novel and underused complementary foods include cultural perceptions and opportunity costs.

2.8.1 Cultural Perceptions

One barrier to the consumption of underused complementary foods is a pervasive western aversion to the idea of eating insects as well as other non-western foodstuffs, such as blood and spiders. Individuals promoting entomophagy (the consumption of insects as food) have noted this aversion for centuries, with literature accounts dating back to the American Revolutionary War (DeFoliart, 1999). Although western attitudes may not impact traditional practices in more insular societies, these aversions have resulted in reduced funding and research for efforts to increase the consumption of local, underused complementary foods (DeFoliart, 1999).

Although as much as 80 percent of the world's population lives in settings where various insects have long been a component of the traditional diet, populations tend to shift away from consuming these foods as they urbanize and possibly come to view them as "primitive" foodstuffs (DeFoliart, 1999). Even in contexts where these foods are widely accepted, they may not be provided to young children. In Laos, forest foods (including insects, frogs, and bamboo shoots) comprise the largest share of non-rice foods consumed by populations living in certain regions, yet Mennonite Central Committee (MCC) health promotion staff report that many of these foods are reserved for household elders and not prioritized for consumption by young children (Clendon, 2001).

2.8.2 Opportunity Costs

The opportunity costs of proposed new activities should always be considered before undertaking efforts to change specific IYCF behaviors. Opportunity costs can be understood as the trade-offs in use of time and resources associated with one particular choice compared to its alternative. For example, the opportunity costs of choosing to harvest wild insects may include less time to engage in another money-making activity or reduced time spent socializing or caregiving, such as making enriched complementary foods for a young child. With underused complementary foods, opportunity costs for specific foods may vary greatly even within a relatively small geographical area, depending on the habitat availability of foods of interest. Where foods are currently being harvested for consumption by household elders, redistributing these nutrient-rich foods to young children must not adversely impact elders' food security. The ease of collecting wild foods generally varies depending on the season, so efforts to increase consumption must be sensitive to seasonal availability and the potential for preservation of these foods for year-round consumption, for example by drying. The sustainability of harvesting practices also needs to be taken into account. In Zambia, the picking season for a certain type of forest caterpillar is regulated to ensure that adequate numbers of caterpillars remain to reproduce for the following year (DeFoliart, 1999). In places where abundance of wild foods is decreasing, efforts to promote their harvest and consumption should be carefully conducted with experts familiar with the local ecology and with a plan for increasing their supply.

The source of the labour should also be considered. Women are often responsible for gathering wild foods. If increasing their harvest of wild foods competes with their ability to perform other work which may include feeding young children, the end result could negatively impact child nutrition. In other instances, wild food collection can be a useful boon to household livelihoods in addition to improved child nutrition.

2.9 Socioeconomic Context of Child Feeding and the UNICEF Model of Child Care

Child feeding takes place in a broader context of multiple social, political, economic, and cultural forces. Complementary feeding is an essential element in the care of young children. According to the conceptual framework on the causes of malnutrition adopted by UNICEF as a foundation for its country programming (UNICEF, 1990), nutrient intake and the presence or absence of disease are the direct determinants of child survival, growth, and development. Dietary intake and the incidence of illness are, in turn, influenced by the underlying factors of household food security, available health care services, and child-care practices. Child-care practices are themselves determined in part by the ability of caregivers (and others) to gain access to economic, human and organizational resources that are necessary to provide adequate complementary foods and care. The theoretical placement of child care as one of the three critical determinant of adequate nutrition, along with access to food and health services, represents an important breakthrough in the understanding of good nutrition as more than just a problem of food security and health.

2.10 Trends in Development of Complementary Foods

Complementary foods in developing countries have for a long time been cereal based. Recently in Africa, supplementation of cereals with locally available legumes as a protein source has been exploited such as soya bean, groundnut (Martin, 2010; Anigo et al., 2010) cowpeas (Oyarekua, 2010; Muhimbula et al., 2011) pigeon peas (Asma et al., 2006) common bean and bambara nuts (Muhimbula et al., 2011; Owino et al., 2007) among others. 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).

An observational study showed that school going children in Kenya consuming diets high in animal source foods grew better (Grillenberger et al., 2006). However, studies on complementary foods development with animal foods as ingredients are fewer or have not been widely reported. Existing work have been based on the use of fish (Mosha and Bennink, 2005; Haug et al., 2010). Bwibo and Neumann (2003) observed underutilization of animal foods in complementary foods and recommended the need to increase animal source foods among

Kenyan children. Foods made from some animal food sources are better sources of minerals such as calcium, iron and zinc, vitamins and lipids. There has been an awakening on utilization of edible insects to fight food and nutrition security in Kenya (Ayieko et al., 2010b) and therefore their utilization in complementary feeding may be of interest.

2.11 Entomophagy

Entomophagy, i.e., the consumption of insects, is traditionally practiced in many parts of the world. Generally, insects were found to be highly nutritious and to represent good sources of proteins, fat, minerals, vitamins, and energy. For example, a 100 g of caterpillars (larvae of moth or butterfly) was found to provide with 76% of the daily required amount of proteins and with nearly 100% of the daily recommended amount of vitamins for humans. The energy content of insects is on average comparable to meat (on a fresh weight basis) except for pork because of its particularly high fat content. Taking into consideration that insects have a high fecundity, can be multivoltine, have a high feed conversion efficiency, low space requirement, and are omnivorous in addition to their nutritive value, edible insects can contribute to world food security and represent an interesting food and feed alternative, especially to meat Products and fish meal.

2.11.1 Merits of entomophagy

Powerful arguments in support of entomophagy include nutritional benefits, poverty reduction through food security and the potential for income generation. Incentives for pesticide avoidance and conservation of bio- and cultural diversity are also frequently cited as motives to promote this practice (DeFoliart, 2005). The most compelling argument in favour of insects as food is their nutritional value and thus the potential to bolster food security and a balanced diet for better health. Insects are often eaten as fresh snacks on an opportunistic basis or as a stopgap during famine, especially in semi-arid environments where food choices are limited and emergencies are recurrent events. One-third of the population of Africa alone is chronically malnourished (Sene 2000). However, insects are rarely considered staples in the diet, but more likely sought as condiments, food additives, and delicacies or for rendered fat. Whenever their supply exceeds short-term needs, they can function as reserves for periods of dearth, or provide income through barter and trade. Food caterpillars and forest bees in particular are important for generating income, especially in Africa where their value often exceeds that of common agricultural crops (Balinga *et al.* 2004; Vantomme *et al.* 2004; Munthali and Mughogho 1992).

Where certain Orthoptera (or other edible insects) can generate higher income than agricultural crops, such as in Africa, the Philippines, Thailand, Mexico and the Republic of Korea, powerful arguments can be made for their conservation and against expensive and environmentally dubious pesticide applications in forests and on crops (Yeld, 1986). About 30 years ago, American Paiute Indians actually succeeded in stopping the USDA Forest Service from spraying insecticides against Pandora moth caterpillars (*Coloradia pandora*), a Saturniid defoliator of pine and a traditional food for these people (DeFoliart 1991c).

The scientific merit of entomophagy has by now been well-established by numerous papers documenting the undisputed nutritional value of many edible insects (Paoletti 2005). Their nutrient profiles are often very favourable from the point of view of dietary reference values (DRVs) and daily requirements for normal human growth and health. In general, insects tend to be a rich source of essential proteins and fatty acids, as well as dietary minerals and vitamins, and thus, play important roles in traditional diets (Bukkens 2005; Ramos- Elorduy 2005; DeFoliart 1989). However, now that the poorer segments of society in many developing countries no longer benefit from such traditional diets, protein deficiencies (kwashiorkor) in particular are more common, especially in Africa.

Adequate daily protein requirements for adults, as established by biennial FAO/WHO/UNU expert consultations, are listed at around 0.72-0.75 grams/ kilograms/day, or about 10 percent of daily energy uptake, slightly less for women than for men. Plant proteins are generally considered to be of poorer quality than animal proteins, but in combination provide a better balance of certain essential amino acids than one alone. Insect proteins tend to be low in methionine and cysteine, but high in others, especially lysine and threonine (DeFoliart 1992). Eight of the 20 standard amino acids, isoleucine, leucine, lysine, methionine, phenylalamine, threonine, tryptophan and valine are not synthesized by humans themselves and thus must be obtained from food, as they are considered essential for normal growth and health. In Mexico and Central Africa, crude protein content of numerous edible insects on a dry weight basis exceed 50 percent and range as high as 82 percent, with digestible protein as high as 64 percent (Ramos-Elorduy 2005; DeFoliart 1989).

In addition to Carbon, Hydrogen, Nitrogen and Oxygen, certain dietary minerals, including macrominerals (Ca, Cl, Mg, P, K, Na, S and NaCl) as well as trace minerals (Co, Cu, F, I, Fe, Mn, Mo, Ni, Se, V and Zn) are required for normal growth and health. Caterpillars tend to provide many of these minerals in abundance (Paoletti 2005; Balinga *et al.*, 2004) and the majority of edible insects have a very high proportion of K, Ca, Fe and Mg (Ramos-Elorduy 2005). Interestingly, geophagy, the eating of earthy substances such as soil from termitaria and sphecid nests during pregnancy, religious rituals or as medicine, as reported from Africa and parts of the United States, purportedly augments mineral-deficient diets (Van Huis 2005).

Vitamins A, B 1-12, C, D, E, and K are biochemical substances needed in tiny amounts for normal growth and health. Caterpillars are especially rich in B1, B2 and B6 (Ramos-Elorduy 2005; Balinga *et al.* 2004). Bee brood (pupae) is rich in vitamins A and D (Hocking and Matsumara 1960). Daily human requirements for calories, as obtained from fat, protein, carbohydrates and alcohol range from about 1000 for children to 3 900 for adult males. Variance is not only based on age and gender, but also on body size and activity levels. Calories obtainable from insects run as high as 776.9 kcal/100 grams of insects, often exceeding those from soybeans, maize and beef, but not pork (Ramos-Elorduy 2005; DeFoliart 1999). Under favourable circumstances, collecting edible insects can also be highly labour-efficient. According to one study in Utah, USA, the collecting of locusts (*Melanoplus sanguinipes*) yielded an average return of 273 000 calories per hour of effort invested by one collector (Anon 1989).

2.11.2 Ethnic Preferences and Prohibitions In Entomophagy

Barreteau (1999) and Lévi- Luxereau (1980) give a detailed account of the Latin names of locusts that are eaten. Both deal with locusts from the Sahelian region, the first in the extreme north of Cameroun and the second from the Maradi area in Niger. It appears that the different ethnic groups have different preferences, for example the Mofu-Gudur in Cameroun eat a number of grasshopper species (*Acorypha picta, A. glaucopsis*, *Acrida bicolor, Oedaleus senegalensis, Pyrgomorpha cognata, Truxalis johnstoni*), which are not eaten by the Haussa in Niger, and vice versa (*Humbe tenuicornis*).

There are also prohibitions to eat insects, for example the Pygmies eat the larvae and the nymphs of the Goliath beetle, but not the adult which is considered sacred and used in fetishes (Bergier, 1941). Members of termite clans in Malawi, Tanzania, Mozambique, Zambia and

Zimbabwe have certain termite species as totems, and members are forbidden to eat winged termites (Silow, 1983). The Logo-Avokaya in northcentral Africa have rules determining the consumption of termites, which take into account the state of being married or pregnant, as well as the swarming behaviour of the termites (Costermans, 1955). Members of the Ire clan of the Yoruba tribe in Nigeria (predominantly blacksmiths) do not eat crickets, because the worshipped Iron God Ogun does not accept animals that have no blood (Fasoranti and Ajiboye, 1993). Any woman of the grasshopper clan of the Baganda in Uganda may catch and cook the edible grasshopper *R. differens* for her husband, though she is not allowed to eat any herself (Roscoe, 1965). The Bahaya in Tanzania also seem to have a number of rules related to the eating of this particular insect (Mors, 1958). Pagezy (1975) mentions for the Oto and Twa in the D.R. Congo quite a number of prohibitory rules relating to eating beetle larvae, termites and caterpillars depending on the person's sex, age, physiological status (e.g. pregnancy), and/or being a twin.

2.11.3 Edible and Medicinal Termites

Since ancient times, complex interactions among humans and other animals have been recorded, including harmonic and conflicting relations (Alves *et al.,* 2009; Alves, 2012). Termites illustrate this situation, as they can cause significant economic damage in urban and rural areas. At the same time, people from different parts of the world use them as food (for humans and livestock), and as a source material for popular medicine. The importance of insects as a food source for humans is not surprising, since this is the group with the highest number of species in nature, thereby representing a significant biomass (Meyer-Rochow & Chakravorty, 2013). Considered as important natural resources, insects are, in many ways, a basic component of the diets of humans and other animals (Raubenheimer & Rothman, 2012) and have played an important role as a source of medicinal resources (Dossey, 2010; Alves & Alves, 2011; Costa-Neto, 2005). Entomophagy, as the practice of using insects as a part of the human diet is called (Meyer-Rochow 2010), has played an important role in the history of human nutrition in Africa, Asia and Latin America (Srivastava, 2009). Another important use of insects by humans is medicinal use, featuring a practice known as entomotherapy (Costa-Neto, 2005; 2002).

Termites are commonly used insects in traditional popular medicine (Alves & Alves, 2011; Solavan *et al*., 2006; Coutinho *et al*., 2009). They are used in the treatment of various diseases that affect humans, such as influenza, asthma, bronchitis, whooping cough, sinusitis, tonsillitis and hoarseness (Alves, 2009)

Additionally, these animals have historically been an important source of food that may contribute to improving human diet, particularly for people who suffer from malnutrition due to a deficit of protein, as they are considered a nonconventional food with great economic and social importance. They have been consumed for generations in many regions of the world, a practice that has increased in popularity in recent years

A total of 45 termite species belonging to four families were recorded as being used by human populations, with 43 species used in the human diet or for livestock feeding and nine species used as a therapeutic resource (Figueirêdo *et al.,* 2015*)*. Among the species used in human and/or livestock diet, the species *Macrotermes bellicosus* stood out, with a record of usage in several countries, especially in Africa (Figueirêdo *et al.,* 2015*).*

In addition, four species are used both in the human diet and for livestock feeding. Furthermore, five species had only one registered use, including *Macrotermes herus* (Sjoestedt, 1914), *Macrotermes lilljeborgi* (Sjoestedt, 1896) and *Macrotermes muelleri* (Sjoestedt, 1898), which are used only for livestock feeding. For medicinal purposes, the use of ten species of termites was recorded. These species are used as an alternative treatment for physiological and spiritual problems. The species *Nasutitermes macrocephalus* was the most frequently recorded, and it is widely used in Brazil as a therapeutic resource for the treatment of asthma, hoarseness and sinusitis, among other diseases (Figueirêdo *et al.,* 2015*)*

Another example is *Macrotermes nigeriensis*, which is used in Nigeria in the treatment of wounds, sickness of pregnant women and as a charm for spiritual protection. The use of termites was registered in 29 countries over three continents. Africa is the continent with the highest number of records (19 countries), followed by America (5 countries) and Asia (5 countries). Congo recorded the highest number of species used as food by humans and other animals (7 species recorded in the compiled studies), followed by China (6 spp.), Venezuela (6 spp.) and Zambia (5 spp.). Among species with a therapeutic use, there was a predominance of records in Brazil, suggesting that the use of termites in Brazilian popular medicine is relatively common. This kind of use was also recorded in other countries such as India (2 spp.), Zambia (1 sp.) and Nigeria (1 sp.) (Figueirêdo *et al.*, 2015*)*

These animals are among the most commonly consumed insects on the planet, second only to grasshoppers (Anankware *et al*., 2015). In a survey on the consumption of termites held in Côte d'Ivoire, from 500 people surveyed, 97% consumed or had consumed termites, demonstrating that such use is part of the reality of rural and urban populations in that country, showing a consumption driven by the nutritional value, flavour and aroma of these insects, as well as by the curiosity of the people who consume them (Niaba *et al*., 2012). It is noticeable that some species are widely consumed in some countries, suggesting a preference for them, especially those belonging to the genus Macrotermes.

Ramos-Elorduy (Mitsuhashi, 2010) identified the Americas and Africa as the areas with the highest number of insect species consumed as food. Complementing this information, in Asia, there is a large number of insects used as food by people (Jongema, 2014). One should also point out that most ethnobiological studies that reported the use of termites by humans were carried out in countries of Africa and the Americas. Currently, more attention is given to alternative food resources traditionally used by humans, which, if used or exploited, are likely to be a more sustainable solution to nutrient deficiency among human populations (Kinyuru *et al*., 2013) In addition, as we can see from literature, termites can provide an important contribution in this regard.

2.11.4 Collection of winged termites

Winged termites are collected in various ways. In urban areas, they are attracted to electric light and are trapped in a receptacle with water which is placed under or near the light source. In rural areas, they are often caught at the termite mound itself. When emerging they are attracted by the light of a grass torch and then swept with a broom into a dug-out hole. In parts of the DRC a basket is put upside down over an emergence hole and the termites which cling to the bottom of the basket are then detached every few minutes by shaking the basket (Bergier, 1941).

Another method is to build a dome-shaped framework of sticks or elephant grass covered with banana, Maranta leaves or a blanket to cover part of the emergence holes (Bergier, 1941; Osmaston, 1951; Roulon-Doko, 1998). Other emergence holes outside the structure are closed, forcing the termites to emerge from the holes within the tent structure. The tent structure has an opening at one side to which the flying termites are attracted by light (from the sun, moon, torch or fire) and near this opening there is a receptacle into which they are collected (Bergier, 1941;

Harris, 1940; Ogutu, 1986). Osmaston (1951) mentions from Uganda a complicated structure of clay pipes constructed over the emergence holes which leads them to the receptacle. Ogutu (1986), Owen (1973), Roulon-Doko (1998) and several reports confirmed that continuous beating and drumming on the ground around the hill triggers certain termite species to emerge.

Soldiers from the larger termite species are also eaten (D.R. Congo: Bequaert, 1921; Bergier, 1941; Owen, 1973. Central African Republic: Roulon-Doko, 1998, Zimbabwe: Chavanduka, 1976). To extract them from the mounds, women or children lower saliva wetted grass blades (Uganda), often of *Imperata cylindrica* (Roulon-Doko, 1998), or parts of tree pods or barks (Takeda, 1990) into the shafts of termite mounds opened by machete. The Ngandu from the D.R. Congo also blow smoke from charcoal from certain trees into the opening (Takeda, 1990). In defence, the soldiers bite into the grass blades, which are then pulled out and the soldiers stripped into a container. The inside of the basket may be lined with the slippery leaves from the *Sarcophrynium shweinfurthianum* tree to prevent the termites from climbing out (Takeda, 1990). As the nest of the harvester termite *H. mossambicus* is undetectable, the San women in Botswana dash out in the direction of the swarm, to identify the site of the nest (Nonaka, 1996).

2.11.5 Preparation and Preservation of Edible Insects

Many insects are only seasonally available; they are frequently conserved for later consumption. This is often done by drying insects in the sun, over ashes or in the oven. Preservation with salt after boiling is also common. Some insects are not prepared at all and are eaten alive. For example, in most parts of Africa a small quantity of termites is eaten alive as a relish during collection. In South Africa, the heteropteran insect *N. delegorguei* is also eaten alive.

2.11.6 Preparation and Preservation of Winged termites

The termites are killed by boiling or roasting for a few minutes the morning after the swarming, and then they are sun-dried or smoke-dried, or both, depending on the weather (Silow, 1983). Sometimes they are then crushed to a mush with a pestle and motar and eaten with honey (Ogutu, 1986). Termites are fried in their own fat. This fat can also be used to fry meat (Bequaert, 1921), as by the Azande and pygmies in the D.R. Congo (Bergier, 1941). The pygmies also use the oil to treat their body and hair. The oil is also obtained by pressing dried termites in a tube (Costermans, 1955). Termites can also be steamed or smoked in banana leaves (Uganda).

In many East African towns and villages, sun-dried termites can be bought at the local market at the right season of the year (Osmaston, 1951; Owen, 1973). Fried or dried termites contain 32–36% protein (Nkouka, 1987; Tihon, 1946) and in most parts of Africa they are considered a delicacy. *Macrotermes bellicosus*, collected in Nigeria, is probably also valuable in complementing maize protein, while others, like *Macrotermes subhyalinus* from Angola were not (Bukkens, 1997) The queen in particular is considered an exquisite dish and often reserved for special occasions (Owen, 1973). Queens are often fed to children (Uganda and Zambia)

2.11.7 The Nutritive Value of Edible Insects

Protein and amino acids of edible insects

Protein is the basis of all organism activity and constitutes many important materials such as enzymes, hormones and haemoglobin. Protein is an important component of antibodies as it bolsters the immunity function of the body. It is the only material to produce nitrogen for maintaining acid and alkali balance, transforming genetic information and transporting important materials in the human body. As a nutritive element that produces heat, it can supply energy.

Insect bodies are rich in protein. In nearly 100 analysed edible insects (Chen and Feng 1999; Yang 1998; Hu 1996; DeFoliart 1992; Mitsuhashi 1992; Comby 1990; Ramos-Elorduy and Pino 1989), at egg, larva, pupa or adult stages, the raw protein content is generally 20-70 percent. Raw protein content is 66.26 percent in Ephemeroptera larvae, 40-65 percent in Odonata larvae, 40-57 percent in Homoptera larvae and eggs, 42-73 percent in Hemiptera larvae, 23-66 percent in Coleoptera larvae and 20-70 percent in Lepidoptera larvae. Protein content of Apidae, Vespidae and Formicidae in the Hymenoptera Order is also high (38-76 percent). According to analysed data, the protein content of insects is higher than most plants; the protein content of some insects (e.g. larvae of *Ephemerella jianghongensis* [66.26 percent], *Sphaerodema rustica* Fabricius [73.52 percent]) is higher than that of commercial meat, fowl and eggs.

Protein is composed of more than 20 types of amino acid that benefit the human body; among them eight are necessary for human nutrition as they cannot be synthesized in the human body (Jin 1987). Analysis of nearly 100 types of edible insects has shown that necessary amino acid content is 10-30 percent, covering 35-50 percent of all types of amino acids, which is close to the amino acid model proposed by the World Health Organization and FAO.

Insects can fulfil some human nutritional requirements and most of them can be grouped among the high value protein sources since their essential amino acid score (the essential amino acids requirement expressed as percentage in an ideal protein) ranges from 46% to 96% (Ramos-Elorduy and others 1997), although the majority of insects has been found to have limited levels of either tryptophan or lysine (Ramos-Elorduy and others 1997; Bukkens 2005).

Insect proteins are highly digestible (between 77% and 98%) (Ramos-Elorduy and others 1997), although insect forms with an exoskeleton have lower values, due to the presence of chitin.

Chitin removal increases the quality of insect protein to a level comparable to that of products from vertebrate animals (DeFoliart 2002). Furthermore, humans are still able to digest some chitin, as 2 catalytically active chitinases have been discovered, AMCase and chitotriosidase, both belonging to the family of 18 glycosylhydrolases (van Aalten and others 2001; Synstad and others 2004). AMCase is more active at acidic pH (Boot and others 2001; Chou and others 2006), whereas chitotriosidase (Boot and others 1995; Renkema and others 1995) has a different pH optimum than AMCase. AMCase has the potential to moderately digest chitin in the human stomach (Paoletti and others 2007; Muzzarelli and others 2012).

In countries in Africa where maize is a staple food – such as Angola, Kenya, Nigeria and Zimbabwe – there are occasionally widespread tryptophan and lysine deficiencies; supplementing diets with termite species like *Macrotermes bellicosus* (Angola) should be a relatively easy step, as they already form accepted parts of traditional diets. Not all termite species are suitable, however: *Macrotermes subhyalinus*, for example, is not rich in these amino acids (Sogbesan and Ugwumba, 2008).

Fat in edible insects

Fat is an important component of the human body, storing and supplying energy as well as supporting and protecting different organs. Fat can also help in the absorption of vitamins. Phosphate, carbohydrate and cholesterol are components of many tissues and cells; combined with protein, they can form fat protein and cell membranes. Recent studies show that phosphatide is good for the brain and liver, reduces blood fat, produces clean cholesterol, helps cells and skin to grow and postpones senility (Jin, 1987). Fatty acids can be separated into saturated fatty acid and unsaturated fatty acid. Unsaturated fatty acid can help human growth, protect the skin and reduce the formation of thrombi and clotting of blood platelets.

According to reports and analysis (Feng *et al.* 2001 a,b,c; 2000 a,b; 1999; Chen and Feng 1999; He *et al*. 1999; Lu 1992; DeFoliart 1991), many edible insects are rich in fat. During edible larvae and pupae stages, their fat content is higher; during the adult stage, the fat content is relatively lower. The fat content of edible insects is between 10 and 50 percent; the fat content of *Oxya chinensis* (Thunberg) of Orthoptera only reaches 2.2 percent; some larvae and pupae of Lepidoptera have higher fat content, such as *Pectinophora gossypeilla* Saunders (49.48 percent) and *Ostrinia furnacalis* Gunnee (46.08 percent). The fatty acid of edible insects is different from animal fat; it has higher fatty acid that the human body needs, such as that found in the larvae and pupae of *Dendrolimus houi* Lajonquiere, larvae of *Musca domestica* Linnaeus, *Chilo Fuscidentalis* Hampson and some ants. Therefore, the fat of edible insects has good nutritive value. Edible insects have similar fat materials, such as phosphatide, which has health benefits.

Edible insects are a considerable source of fat. Womeni *et al.* (2009) investigated the content and composition of oils extracted from several insects. Their oils are rich in polyunsaturated fatty acids and frequently contain the essential linoleic and α-linolenic acids. The nutritional importance of these two essential fatty acids is well recognized, mainly for the healthy development of children and infants (Michaelsen *et al.*, 2009). Greater attention has been paid to the potential deficient intake of these omega-3 and omega-6 fatty acids in recent times, and insects could play an important role, in particular in landlocked developing countries with lower access to fish food sources, by supplying these essential fatty acids to local diets (N. Roos, personal communication, 2012). The fatty acid composition of insects appears to be influenced by the plants on which they feed (Bukkens, 2005). The presence of unsaturated fatty acids will also give rise to rapid oxidation of insect food products during processing, causing them to go rancid quickly.

Insects vary widely in fat content and thus energy. The fat content of insects ranges from 7 to 77 g/100 g dry weight and the caloric value of insects varies between 293 and 762 kcal/100 g dry weight (Ramos-Elorduy and others 1997). These values depend on insect diet and insect species. For instance, caterpillars and termites are known to contain more fat (Bukkens 2005) and, according to DeFoliart (1992), some insects contain more essential fatty acids, such as linoleic and/or linolenic acids, compared with meat.

In order to be considered beneficial for human health, the balance of fatty acid groups has to cover an appropriate range (Dietary Reference Levels for the Italian population – LARN – 2012). The best composition of fat intake occurs when the ratio of fat sources is mammalian meat: plant: fish = 4: 5: 1, with an ideal ratio among saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA) of SFA: MUFA: PUFA = 3: 4: 3.

Carbohydrates of edible insects

Carbohydrates are important nutritive elements in the human body. They are the main heat source, can reduce consumption of protein and help detoxification. They are also important constituent materials of the human body. They can combine with protein and fat and their compounds have important physiological functions (Jin 1987). Edible insects have rich protein and fat, but less carbohydrate. Types of edible insects differ and their carbohydrate contents also vary (1-10 percent). An unusual source is insect tea, the excrement of insects, which has higher carbohydrate content (16.27 percent). Recent research has revealed that insects have considerable amounts of polysaccharide that can enhance the immunity function of the human body (Sun *et al*. 2007).

Chitin is a macromolecular compound that has high nutritive and health food value. Chitin can be made into a health food that has medicinal value for it can stop bleeding, prevent thrombus and help wounds to heal; it can be made into a medicinal film and can also be used in making cosmetics. The body and skin of edible insects are rich in chitin; different forms of edible insects have different chitin content (5-15 percent), such as *Bombyx mori* L. dried pupa (3.73 percent), defatted pupa (5.55 percent) and *Dendrolimus houi* Lajonquiere pupa (7.47 percent) and adult 17.83 (percent) (Chen and Feng 1999; He *et al*. 1999). The scientific study of the chitin of insect bodies is just beginning, with potential for many human uses.

Inorganic salts and trace elements in edible insects

Inorganic salts and trace elements are important components of the human body. They are necessary materials to maintain normal physiological functions (Jin 1987). According to analysed results, edible insects have rich trace elements such as potassium (K), sodium (Na), calcium (Ca), copper (Cu), iron (Fe), zinc (Zn), manganese (Mn) and phosphorus (P). Many edible insects are also high in calcium, zinc and iron. Therefore, edible insects can supply necessary nutritive elements for human body functions.

Micronutrient deficiencies, which are commonplace in many developing countries, can have major adverse health consequences, contributing to impairments in growth, immune function, mental and physical development and reproductive outcomes that cannot always be reversed by nutrition interventions (FAO, 2011c). Consumption of the entire insect body generally elevates nutritional content. A study on small fish, for example, suggested that consuming the whole organism including all tissues is a better source of minerals and vitamins than the consumption of fish fillets. In much the same way, consuming the entire insect is expected to provide higher micronutrient content than eating individual insect parts (N. Roos, personal communication, 2012).

The recommended dietary allowance (RDA) and adequate intake are generally used to quantify suggested daily intake of minerals. Most edible insects boast equal or higher iron contents than beef (Bukkens, 2005). Beef has an iron content of 6 mg per 100 g of dry weight, while the iron content of the mopane capterpillar, for example, is 31–77 mg per 100 g. The iron content of locusts (*Locusta migratoria*) varies between 8 and 20 mg per 100 g of dry weight, depending on their diet (Oonincx *et al.*, 2010).

Edible insects are undeniably rich sources of iron and their inclusion in the daily diet could improve iron status and help prevent anaemia in developing countries. WHO has flagged iron deficiency as the world’s most common and widespread nutritional disorder in developing countries, one in two pregnant women and about 40 percent of preschool children are believed to be anaemic. Health consequences include poor pregnancy outcomes, impaired physical and cognitive development, and increased risk of morbidity in children and reduced work productivity in adults. Anaemia is a preventable deficiency but contributes to 20 percent of all maternal deaths. Given the high iron content of several insect species, further evaluation of more edible insect species is warranted (FAO/WHO, 2001b).

Zinc deficiency is another core public health problem, especially for child and maternal health. Zinc deficiencies can lead to growth retardation, delayed sexual and bone maturation, skin lesions, diarrhea, alopecia, impaired appetite and increased susceptibility to infections mediated via defects in the immune system (FAO/WHO, 2001b). In general, most insects are believed to be good sources of zinc. Beef averages 12.5 mg per 100 g of dry weight, while the palm weevil larvae (*Rhynchophorus phoenicis*), for example, contains 26.5 mg per 100 g (Bukkens, 2005).

Vitamin content of edible insects

Vitamins are one group of organic compounds that are necessary for metabolism in human bodies. As vitamins cannot be synthesized in the human body, they must be supplied constantly by food. Studies on vitamins in edible insects are insufficient. But according to analysed results (Feng *et al.* 2001 a,b,c; 2000 a,b; 1999; Chen and Feng 1999; He *et al*. 1999; Lu 1992; DeFoliart 1991), edible insects have vitamin A, carotene, vitamins B1, B2, B6, D, E, K, C, etc. For example, the vitamin A content of *Macrotermes annandalei* Silvestri reaches 2 500 IU/100 gram, vitamin D reaches 8 540 IU/100 gram, vitamin E reaches 1 116.5 mg/100 gram and the vitamin C content of insect tea reaches 15.04 mg/100 gram. Edible insects are rich in vitamins for human health and nutrition.

Vitamins essential for stimulating metabolic processes and enhancing immune system functions are present in most edible insects. Bukkens (2005) showed for a whole range of insects that thiamine (also known as vitamin B1, an essential vitamin that acts principally as a co-enzyme to metabolize carbohydrate into energy) ranged from 0.1 mg to 4 mg per 100 g of dry matter. Riboflavin (also known as vitamin B2, whose principle function is metabolism) ranged from 0.11 to 8.9 mg per 100 mg. By comparison, wholemeal bread provides 0.16 mg and 0.19 mg per 100 g of B1 and B2, respectively. Vitamin B12 occurs only in food of animal origin and is well represented in mealworm larvae, *Tenebrio molitor* (0.47 µg per 100 g) and house crickets, *Acheta domesticus* (5.4 µg per 100 g in adults and 8.7 µg per 100 g in nymphs). Nevertheless, many species have very low levels of vitamin B12, which is why more research is needed to identify edible insects rich in B vitamins (Bukkens, 2005; Finke, 2002)

Retinol and β-carotene (vitamin A) have been detected in some caterpillars, including *Imbrasia* (*=Nudaurelia*) *oyemensis, I. truncata* and *I. epimethea*; values ranged from 32 µg to 48 µg per 100 g and 6.8 µg to 8.2 µg per 100 g of dry matter for retinol and β-carotene, respectively. The levels of these vitamins were less than 20 µg per 100 g and less than 100 µg per 100 g in yellow mealworm larvae, superworms and house crickets (Finke, 2002; Bukkens, 2005; Oonincx and Poel, 2011). Generally, insects are not the best source of vitamin A (D. Oonincx, personal communication, 2012). Vitamin E featured in the palm weevil larvae, for example, which boasts 35 mg and 9 mg per 100 g of α-tocopherol and β+γ tocopherol, respectively; the daily recommended intake is 15 mg (Bukkens, 2005). The vitamin E content in ground and freeze-dried silkworm powder (*Bombyx mori*) is also relatively high, at 9.65 mg per 100 g (Tong, Yiu and Liu, 2011).

2.11.8 Toxicity of Edible Insects

Although some insects are considered toxic, they are still eaten. For instance *Zonocerus variegatus* in Cameroun (Barreteau, 1999) and Nigeria. The larvae have to be prepared specially, such as by repeated cooking. The tessaratomid *Natalicola delagorguei* in Zimbabwe and South Africa excretes a pungent fluid (Bodenheimer, 1951; Faure, 1944) which can cause severe pain and even temporary blindness if it comes in contact with the eyes (Scholtz, 1984). Therefore, the fluid is removed by squeezing the thorax and by diluting the 'poison' by putting the insect in hot water. The consumption of caterpillars with hairs containing toxic substances can be very dangerous and these have to be burned off by shaking them in a recipient with glowing coals (Tango Muyay, 1981).

There have also been reports of seasonal ataxic syndrome after people consumed the silkworm *Anaphe venata* in southwest Nigeria (Adamolekun, 1993). This may occur in poorly nourished people who are marginally thiamine deficient as a result of a mainly carbohydrate diet containing thiamine-binding cyanogenetic glycosides, and who experience seasonal exacerbation of their thiamine deficiency from thiaminases in seasonal foods. The silkworm contains such thiaminases.

Bouvier (1945) observed in D.R. Congo that when grasshoppers and locusts are consumed without removing the legs, intestinal constipation may occur, caused by the large spines on the tibia. Surgical removal of locust legs is then often the only remedy. Autopsy of dead monkeys during locust invasions also proved that the consumption of locusts proved to be fatal for the same reason. Another problem is the pesticide applications against locusts and grasshoppers, which can cause problems because of their toxic residues.

2.12 Vitamin and Mineral Requirements In Human Nutrition

2.12.1 Vitamin A

Vitamin A (retinol) is an essential nutrient needed in small amounts by humans for the normal functioning of the visual system; growth and development; and maintenance of epithelial cellular integrity, immune function, and reproduction. These dietary needs for vitamin A are normally provided for as preformed retinol (mainly as retinyl ester) and provitamin A carotenoids.

Biochemical mechanisms for vitamin A functions

Vitamin A functions at two levels in the body: the first is in the visual cycle in the retina of the eye; the second is in all body tissues where it systemically maintains the growth and soundness of cells. In the visual system, carrier bound retinol is transported to ocular tissue and to the retina by intracellular binding and transport proteins. Rhodopsin, the visual pigment critical to dim-light vision, is formed in rod cells after conversion of all-*trans*-retinol to retinaldehyde, isomerization to the 11-*cis*-form, and binding to opsin. Alteration of rhodopsin through a cascade of photochemical reactions results in the ability to see objects in dim light (Rando, 1994) . The speed at which rhodopsin is regenerated is related to the availability of retinol. Night blindness is usually an indicator of inadequate available retinol, but it can also be due to a deficit of other nutrients that are critical to the regeneration of rhodopsin, such as protein and zinc, and to some inherited diseases, such as retinitis pigmentosa.

The growth and differentiation of epithelial cells throughout the body are especially affected by vitamin A deficiency (VAD). In addition, goblet cell numbers are reduced in epithelial tissues and as a consequence, mucous secretions (with their antimicrobial components) diminish. Cells lining protective tissue surfaces fail to regenerate and differentiate; hence they flatten and accumulate keratin. Both factors the decline in mucous secretions and loss of cellular integrity reduce the body’s ability to resist invasion from potentially pathogenic organisms. Pathogens can also compromise the immune system by directly interfering with the production of some types of protective secretions and cells (Ross & Stephensen, 1996). Classical symptoms of xerosis (drying or non-wetability) and desquamation of dead surface cells as seen in ocular tissue (i.e. xerophthalmia) are the external evidence of the changes also occurring to various degrees in internal epithelial tissues.

Populations at risk for, and consequences of, vitamin A deficiency

Definition of vitamin A deficiency

VAD is not easily defined. WHO defines it as tissue concentrations of vitamin A low enough to have adverse health consequences even if there is no evidence of clinical xerophthalmia (WHO, 1996) In addition to the specific signs and symptoms of xerophthalmia and the risk of irreversible blindness, nonspecific symptoms include increased morbidity and mortality, poor reproductive health, increased risk of anaemia, and contributions to slowed growth and development. However, these nonspecific adverse effects may be caused by other nutrient deficits as well, making it difficult to attribute non-ocular symptoms specifically to VAD in the absence of biochemical measurements reflective of vitamin A status.

Risk factors of vitamin A deficiency

VAD is most common in populations consuming most of their vitamin A needs from provitamin carotenoid sources and where minimal dietary fat is available (Mele et al., 1991). About 90% of ingested preformed vitamin A is absorbed, whereas the absorption efficiency of provitamin A carotenoids varies widely, depending on the type of plant source and the fat content of the accompanying meal (Erdman, 1988). Where possible, an increased intake of dietary fat is likely to improve the absorption of vitamin A in the body.

In areas with endemic VAD, fluctuations in the incidence of VAD throughout the year reflect the balance between intake and need. Periods of general food shortage (and specific shortages in vitamin A-rich foods) coincide with peak incidence of VAD and common childhood infectious diseases (e.g. diarrhoea, respiratory infections, and measles). Seasonal food availability influences VAD prevalence directly by influencing access to provitamin A sources; for example, the scarcity of mangoes in hot arid months followed by the glutting of the market with mangoes during harvest seasons (Marsh *et al*., 1995). Seasonal growth spurts in children, which frequently follow seasonal post-harvest increases in energy and macronutrient intakes, can also affect the balance. These increases are usually obtained from staple grains (e.g. rice) and tubers (e.g. light coloured yams) that are not, however, good sources of some micronutrients (e.g. vitamin A) to support the growth spurt (Sinha & Bang, 1973).

Food habits and taboos often restrict consumption of potentially good food sources of vitamin A (e.g. mangoes and green leafy vegetables). Culture specific factors for feeding children, adolescents, and pregnant and lactating women are common ((Mele et al., 1991; Johns et al., 1992; Tarwotjo et al., 1982 and Zeitlan *et al.*,1992). Illness and childbirth-related proscriptions of the use of specific foods pervade many traditional cultures (Mahadevan, 1961). Such influences alter short- and long-term food distribution within families. However, some cultural practices can be protective of vitamin A status and they need to be identified and reinforced.

Units of expression

In blood, tissues, and human milk, vitamin A levels are conventionally expressed in mg/dl or mmol/l of all-*trans*-retinol. Except for postprandial conditions, most of the circulating vitamin A is retinol whereas in most tissues (such as the liver), secretions (such as human milk), and other animal food sources, it exists mainly as retinyl esters, which are frequently hydrolysed before analytical detection.

To express the vitamin A activity of carotenoids in diets on a common basis, a Joint FAO/WHO Expert Group ((WHO, 1996) in 1967 introduced the concept of the retinol equivalent (RE) and established the following relationships among food sources of vitamin A:

1 mg retinol = 1 RE

1 mg b-carotene = 0.167 mg RE

1 mg other provitamin A = 0.084 mg RE.

carotenoids

Infants and children Vitamin A requirement

Vitamin A requirements for infants are calculated from the vitamin A provided in human milk. During at least the first 6 months of life, exclusive breastfeeding can provide sufficient vitamin A to maintain health, permit normal growth, and maintain sufficient stores in the liver (WHO, 1998). Reported retinol concentrations in human milk vary widely from country to country (0.70–2.45mmol/l). In some developing countries, the vitamin A intake of breast-fed infants who grow well and do not show signs of deficiency ranges from 120 to 170mg RE/day (Newman, 1994 and WHO, 1998). Such intakes are considered adequate to cover infant requirements if the infant’s weight is assumed to be at least at the 10th percentile according to WHO standards (FAO/WHO, 1988). However, this intake is unlikely to build adequate body stores, given that xerophthalmia is common in preschool-age children in the same communities with somewhat lower intakes. Because of the need for vitamin A to support the growth rate of infancy, which can vary considerably, a requirement estimate of 180mg RE/day seems appropriate. The safe level for infants up to 6 months of age is based on observations of breast-fed infants in communities in which good nutrition is the norm. Average consumption of human milk by such infants is about 750ml/day during the first 6 months (WHO, 1998). Assuming an average concentration of vitamin A in human milk of about 1.75mmol/l, the mean daily intake would be about 375mg RE, which is therefore the recommended safe level. From 7–12 months, human milk intake averages 650ml/day, which would provide 325mg of vitamin A daily. Because breast-fed infants in endemic vitamin A-deficient populations are at increased risk of death from 6 months onward, the requirement and recommended safe intake levels are increased to 190mg RE/day and 400mg RE/day, respectively.

The requirement (with allowance for variability) and the recommended safe intake for older children may be estimated from those derived for late infancy (i.e. 20 and 39mg RE/kg body weight/day) (FAO/WHO, 1988). On this basis, and including allowances for storage requirements and variability, requirements for preschool-age children would be in the range of 200–400mg RE daily. In poor communities where children 1–6 years old are reported to have intakes of about 100–200mg RE/day, signs of VAD do occur; in southern India these signs were relieved and risk of mortality was reduced when the equivalent of 350–400mg RE/day was given to children weekly (Rahmathullah, 1990).

2.12.2 Role of Vitamin D in Human Metabolic Processes

Vitamin D is required to maintain normal blood levels of calcium and phosphate, which are in turn needed for the normal mineralization of bone, muscle contraction, nerve conduction, and general cellular function in all cells of the body. Vitamin D achieves this after its conversion to the active form 1,25- dihydroxyvitamin D [1,25-(OH)2D], or calcitriol. This active form regulates the transcription of a number of vitamin D-dependent genes which code for calcium-transporting proteins and bone matrix proteins.

Overview of vitamin D metabolism

Vitamin D, a seco-steroid, can either be made in the skin from a cholesterollike precursor (7-dehydrocholesterol) by exposure to sunlight or can be provided pre-formed in the diet (Feldman et al., 1997). The version made in the skin is referred to as vitamin D3 whereas the dietary form can be vitamin D3 or a closely-related molecule of plant origin known as vitamin D_2. Because vitamin D can be made in the skin, it should not strictly be called a vitamin, and some nutritional texts refer to the substance as a prohormone and to the two forms as colecalciferol (D3) and ergocalciferol (D2).

From a nutritional perspective, the two forms are metabolized similarly in humans, are equal in potency, and can be considered equivalent. It is now firmly established that vitamin D3 is metabolized first in the liver to 25-hydroxyvitamin D (calcidiol) (Blunt et al., 1968) and subsequently in the kidneys to 1, 25-(OH) 2D (calcitriol) (Fraser & Kodicek, 1970) to produce a biologically active hormone. The 1, 25-(OH) 2D compound, like all vitamin D metabolites, is present in the blood complexed to the vitamin D-binding protein, a specific a-globulin. Calcitriol is believed to act on target cells in a similar way to a steroid hormone.

Free hormone crosses the plasma membrane and interacts with a specific nuclear receptor known as the vitamin D receptor, a DNA-binding, zinc finger protein with a relative molecular mass of 55 000 (Haussler, 1986). This ligand-receptor complex binds to a specific vitamin D-responsive element and, with associated transcription factors (e.g. retinoid X receptor), enhances transcription of mRNAs which code for calcium-transporting proteins, bone matrix proteins, or cell cycle-regulating proteins (DeLuca, 1998) . As a result of these processes, 1, 25-(OH) 2D stimulates intestinal absorption of calcium and phosphate and mobilizes calcium and phosphate by stimulating bone resorption (DeLuca, 1998). These functions serve the common purpose of restoring blood levels of calcium and phosphate to normal when concentrations of the two ions are low.

2.12.3 Role of Vitamin E in Human Metabolic Processes

A large body of scientific evidence indicates that reactive free radicals are involved in many diseases, including heart disease and cancers (Diplock, 1994). Cells contain many potentially oxidizable substrates such as polyunsaturated fatty acids (PUFAs), proteins, and DNA. Therefore, a complex antioxidant defence system normally protects cells from the injurious effects of endogenously produced free radicals as well as from species of exogenous origin such as cigarette smoke and pollutants. Should our exposure to free radicals exceed the protective capacity of the antioxidant defence system, a phenomenon often referred to as oxidative stress (Sies, 1993), then damage to biological molecules may occur. There is considerable evidence that disease causes an increase in oxidative stress; therefore, consumption of foods rich in antioxidants, which are potentially able to quench or neutralize excess radicals, may play an important role in modifying the development of disease.

For dietary purposes, vitamin E activity is expressed as a-tocopherol equivalents (α-TEs). One a-TE is the activity of 1mg *RRR*-α-tocopherol (*d*-α-tocopherol). To estimate the a-TE of a mixed diet containing natural forms of vitamin E, the number of milligrams of b-tocopherol should be multiplied by 0.5, γ-tocopherol by 0.1, and α-tocotrienol by 0.3. Any of the synthetic all-*rac*- a-tocopherols (*dl*-a-tocopherol) should be multiplied by 0.74. One milligram of the latter compound in the acetate form is equivalent to 1IU of vitamin E. Vitamin E is an example of a phenolic antioxidant. Such molecules readily donate the hydrogen from the hydroxyl (-OH) group on the ring structure to free radicals, making them unreactive. On donating the hydrogen, the phenolic compound itself becomes a relatively unreactive free radical because the unpaired electron on the oxygen atom is usually delocalized into the aromatic ring structure thereby increasing its stability (Scott, 1997).

The major biological role of vitamin E is to protect PUFAs and other components of cell membranes and low-density lipoprotein (LDL) from oxidation by free radicals. Vitamin E is located primarily within the phospholipid bilayer of cell membranes. It is particularly effective in preventing lipid peroxidation- a series of chemical reactions involving the oxidative deterioration of PUFA. Elevated levels of lipid peroxidation products are associated with numerous diseases and clinical conditions (Duthie, 1993). Although vitamin E is primarily located in cell and organelle membranes where it can exert its maximum protective effect, its concentration may only be one molecule for every 2000 phospholipid molecules. This suggests that after its reaction with free radicals it is rapidly regenerated, possibly by other antioxidants (Kagan, 1998).

Populations at risk for vitamin E deficiency

There are many signs of vitamin E deficiency in animals, most of which are related to damage to cell membranes and leakage of cell contents to external fluids. Disorders provoked by traces of peroxidized PUFAs in the diets of animals with low vitamin E status include cardiac or skeletal myopathies, neuropathies, and liver necrosis (McLaren, 1993). Muscle and neurological problems are also a consequence of human vitamin E deficiency (Sokol, 1988). Early diagnostic signs of deficiency include leakage of muscle enzymes such as creatine kinase and pyruvate kinase into plasma, increased levels of lipid peroxidation products in plasma, and increased erythrocyte haemolysis. The assessment of the vitamin E requirement for humans is confounded by the very rare occurrence of clinical signs of deficiency because these usually only develop in infants and adults with fat-malabsorption syndromes or liver disease, in individuals with genetic anomalies in transport or binding proteins, and possibly in premature infants (McLaren *et al*, 1993; Traber,1990). This suggests that diets contain sufficient vitamin E to satisfy nutritional needs.

Evidence used for estimating recommended intakes of Vitamin E

There was insufficient evidence to enable a recommended nutrient intake (RNI) to be based on the additional health benefits obtainable from nutrient intakes above those usually found in the diet. At present, data are not sufficient to formulate recommendations for vitamin E intake for different age groups except for infancy. There is some indication that newborn infants, particularly if born prematurely, are vulnerable to oxidative stress because of low body stores of vitamin E, impaired absorption, and reduced transport capacity resulting from low concentrations of circulating low-density lipoproteins at birth (Lloyd, 1990). However, term infants nearly achieve adult plasma vitamin E concentrations in the first week (Kelly *et al*., 1990) and although the concentration of vitamin E in early human milk can be variable, after 12 days it remains fairly constant at 0.32mg α-TE/100ml milk (Jansson *et al.*, 1981). Thus a human-milk-fed infant consuming 850ml would have an intake of 2.7mg α-TE. It seems reasonable that formula milk should not contain less than 0.3mg α-TE/100ml of reconstituted feed and not less than 0.4mg α-TE/g PUFA.

2.12.4 Biological Significance of Ascorbic Acid (Vitamin C) In Human Health

Vitamin C (Ascorbic Acid) is a water-soluble antioxidant. It was first isolated in 1928, by the Hungarian biochemist and Nobel Prize winner Szent-GyorGyi. It is an unstable, easily oxidized acid and can be destroyed by oxygen, alkali and high temperature. Unlike animals humans cannot synthesize vitamin C, rendering its ingestion from exogenous supplement or diet necessary. It has been proposed that the cause of human inability to synthesize ascorbic acid is the absence of the active enzyme, l-gulonolactone oxidase from the liver (Burns, 1959). Body requires vitamin C for normal physiological functions. It helps in the metabolism of tyrosine, folic acid and tryptophan. It helps to lower blood cholesterol and contributes to the synthesis of the amino acids carnitine and catecholamine that regulate nervous system. It is needed for tissue growth and wound healing. It helps in the formation of neurotransmitters and increases the absorption of iron in the gut. Being an antioxidant, it protects the body from the harmful effects of free radicals and pollutants. Mega doses of vitamin C is used in the treatment and prevention of large number of disorders like diabetes, cataracts, glaucoma, macular degeneration, atherosclerosis, stroke, heart diseases and cancer.

Deficiency of this vitamin can lead to anemia, scurvy, infections, bleeding gums, muscle degeneration, poor wound healing, atherosclerotic plaques, capillary hemorrhaging and neurotic disturbances. Toxicity normally does not occur. Infections deplete the body stores of vitamin C, thus making the body immune system weak. For strong immunity, body requires vitamin C. Optimal tissue stores maintain resistance to infections. Vitamin C therapy is beneficial in the treatment of different infections and infectious diseases, for example hepatitis, HIV, H. pylori infection, common cold, flu and influenza etc.

Dietary sources of vitamin C

Ascorbic acid, the accepted name for vitamin C, is available in reduced form (L-ascorbic acid) and oxidized form (Ldehydroascorbic acid). It is found in citrus fruits, green peppers, red peppers, strawberries, tomatoes, broccoli, brussels sprouts, turnip and other leafy vegetables. Fish and milk also contain small amounts of vitamin C. There is a gradual decline in the amount of vitamin C as foods age (Platt *et al*., 1963).

Biological functions of vitamin C

Vitamin C helps in the metabolism of tyrosine, folic acid and tryptophan. It also helps in the metabolism of cholesterol, increasing its elimination and thereby assisting lower blood cholesterol (Rath, 1993). Vitamin C contributes to the synthesis of the amino acid carnitine and the catecholamines that regulate the nervous system. It also helps the body to absorb iron and to break down histamine, the inflammatory component of many allergic reactions (Gaby and Singh, 1991).

Absorption of iron, especially the non-heme variety found in plants and drinking water is enhanced by Vitamin C. It has been shown to facilitate iron absorption by its ability to reduce ferric iron to the ferrous form (Sayers, Lynch and Jocobs, 1973.). Ordinarily our absorption of iron is quite poor, putting us at risk of iron deficiency anaemia. One milligram of ascorbic acid is approximately equivalent in enhancing power to 1 g of cooked MFP (iron present in meat, fish and poultry) or 1.3 g of raw MFP (Monsen, 1978). It is also necessary for the conversion of tryptophan to 5-hydroxy tryptophan and the neurotransmitter serotonin and the formation of the neurotransmitter, nor epinephrine, from dopamine.

Anti-Carcinogenic Effects of Vitamin C

Since the second half of ‘90s, a growing body of literature aimed at demonstrating that vitamin C may reduce the incidence of most malignancies in humans (Block, 1991). Indeed, high-dose of intravenous vitamin C has been found to increase the average survival of advanced cancer patients and for a small group of responders, survival was increased to up to 20 times longer than that of controls (Cameron and Pauling, 1976; Cameron and Campbell, 1991). Other researchers reported benefits consisting of increased survival, improved well-being and reduced pain (Murata *et al*., 1982 and Campbell *et al*., 1991). The anti-inflammatory action of ascorbic acid in cellular ambient is evident in a number of cytoprotective functions under physiological conditions, including prevention of DNA mutation induced by oxidation (Izzi, *et al*., 2012 and Marzocchella *et al*., 2011). Since DNA mutation is likely a major contributor to the age-related development of cancer, attenuation of oxidation-induced mutations by vitamin C may be considered as a potential anti-cancer mechanism (Palumbo *et al*., 2008). Plasma vitamin C at normal to high physiological concentrations (60–100 µmol/L) neutralizes potentially mutagenic ROS thus decreasing oxidative stress-induced DNA damage (Murata *et al*., 1982 and Campbell *et al*., 1991). Moreover, in vivo studies confirmed that consumption of vitamin C-rich foods is inversely related to the level of oxidative DNA damage (Rehman *et al*., 1999 and Thompson et al., 1999).

Vitamin C may also function as cancer cells killer due to its pro-oxidant capacity (Stich et al., 1976). The tumor cell-killing action is dependent upon ascorbate incubation time and extracellular ascorbate concentration (Chen *et al*., 2005). The effective concentration of vitamin C required to mediate cancer killing can be easier achieved by intravenous injection than by per os ingestion (Chen *et al*., 2005 and Padayatty *et al*., 2004). Regarding the modality of cytotoxicity to cancer cells, it remains an unsolved issue. Among the possible mechanisms, stimulatory effects on apoptotic pathways (Masuelli *et al*., 2012 and Battisti *et al*., 2012) accelerated pro-oxidant damage that cannot be repaired by tumour cells, and increased oxidation of ascorbate to the unstable metabolite DHA, which in turn can be toxic, have been hypothesized. The killing of cancer cells is dependent on extracellular H_2O_2 formation with the ascorbate radical as an intermediate. The H_2O_2 formed from pharmacological ascorbate concentrations diffuses into cells (Antunes and Cadenas, 2000) and tumour cells are killed by exposure to H2O2 in less than minutes (Ahmad *et al.*, 2005). The H_2O_2 within the cells may cause breaks in DNA and mitochondria and the mitochondria in some cancer cells may have increased sensitivity to H_2O_2 (Comelli, *et al.*, 2003, Tomasello *et al.*, 2008 and Ahmad *et al.*, 2005).

Among other mechanisms of anti-cancer action of vitamin C, it has been earlier hypothesized a possible role of ascorbic acid in increasing collagen synthesis (McCormick, 1959) and inhibiting hyaluronidase (Cameron and Rotman, 1972). These mechanisms are supposed to prevent cancer spread by increasing extracellular matrix, thus walling in tumors (Bei *et al.*, 2012). In contrast with these results, other studies have reported no effects after using vitamine C as a therapeutic drug (Creagan *et al.*, 1979 and Moertel et al., 1985). Another randomized, placebo-controlled clinical study in which a high dose of vitamin C was given orally to advanced cancer patients led to inconsistent results, ultimately casting doubt over the effectiveness of vitamin C in treating cancer (Moertel *et al.*, 1985). Due to the controversy of results on the vitamin C-cancer correlation and lack of validated mechanistic basis for its therapeutic action, further research is needed to determine the feasibility of using vitamin C in clinical treatment or prevention of cancer.

2.12.5 Thiamine, Riboflavin, Niacin, and Vitamin B6

Rice and wheat are the staples for many populations of the world. Excessive refining and polishing of cereals removes considerable proportions of B vitamins contained in these cereals. Clinical manifestations of deficiency of some B vitamins—such as beriberi (cardiac and dry), peripheral neuropathies, pellagra, and oral and genital lesions (related to riboflavin deficiency) were once major public health problems in some parts of the world. These manifestations have now declined, the decline being brought about not through programmes which distribute synthetic vitamins but through changes in the patterns of food availability and consequent changes in dietary practices.

2.12.5.1 Thiamine deficiency

Thiamine (vitamin B1, aneurin) deficiency results in the disease called beriberi, which has been classically considered to exist in dry (paralytic) and wet (oede-matous) forms

(McCormick, 1988, McCormick, 1997*)*. Beriberi occurs in human-milk-fed infants whose nursing mothers are deficient. It also occurs in adults with high carbohydrate intakes (mainly from milled rice) and with intakes of anti-thiamine factors, such as the bacterial thiaminases that are in certain ingested raw fish ((McCormick, 1988).

Beriberi is still endemic in Asia. In relatively industrialized nations, the neurologic manifestations of Wernicke-Korsakoff syndrome are frequently associated with chronic alcoholism in conjunction with limited food consumption (McCormick et al., 1994). Some cases of thiamine deficiency have been observed with patients who are hypermetabolic, are on parenteral nutrition, are undergoing chronic renal dialysis, or have undergone a gastrectomy.

Thiamine deficiency has also been observed in Nigerians who ate silk worms, Russian schoolchildren (Moscow), Thai rural elderly, Cubans, Japanese elderly, Brazilian Xavante Indians, French Guyanese, south-east Asian schoolchildren who were infected with hookworm, Malaysian detention inmates, and people with chronic alcoholism.

Role in human metabolic processes

Thiamine functions as the coenzyme thiamine pyrophosphate (TPP) in the metabolism of carbohydrates and branched-chain amino acids. Specifically the Mg2+-coordinated TPP participates in the formation of a-ketols (e.g.among hexose and pentose phosphates) as catalysed by transketolase and in the oxidation of α-keto acids (e.g. pyruvate, a-ketoglutarate, and branchedchain a-keto acids) by dehydrogenase complexes (McCormick, 1996; McCormick et al., 1997). Hence, when there is insufficient thiamine, the overall decrease in carbohydrate metabolism and its interconnection with amino acid metabolism (via α-keto acids) has severeconsequences, such as a decrease in the formation of acetylcholine for neural function.

Evidence used to derive recommended intakes

Recommendations for infants are based on adequate food intake. Mean thiamine content of human milk is 0.21 mg/l (0.62mmol/l) (Forbes et al., 1985), which corresponds to 0.16 mg (0.49mmol) thiamine per 0.75 l of secreted milk per day. The blood concentration for total thiamine averages 210 ± 53nmol/l for infants up to 6 months but decreases over the first 12–18 months of life (Wyatt *et al*., 199). A study of 13–14-year-old children related dietary intake of thiamine to several indicators of thiamine status (Bailey et al., 1994). Sauberlich et al. (1979) concluded from a carefully controlled depletion–repletion study of seven healthy young men that 0.3mg thiamine per 4184kJ met their requirements. Intakes below this amount lead to irritability and other symptoms and signs of deficiency (Wood et al., 1980). Anderson et al. (1985) reported thiamine intakes of 1.0 and 1.2mg/day as minimal for women and men, respectively. Hoorn *et al*. (1975) reported that 23% of 153 patients aged 65–93 years were deemed deficient based on a transketolase activation coefficient greater than 1.27, which was normalized after thiamine administration. Nichols and Basu (1994) found that only 57% of 60 adults aged 65–74 years had TPP effects of less than 14% and suggested that ageing may increase thiamine requirements. An average total energy cost of 230 MJ has been estimated for pregnancy (FNB, 1990). With an intake of 0.4mg thiamine/4184kJ, this amounts to a total of 22 mg thiamine needed during pregnancy, or 0.12 mg/day when the additional thiamine need for the second and third trimesters (180 days) is included. Taking into account the increased need for thiamine because of an increased growth in maternal and fetal compartments and a small increase in energy utilization, an overall additional requirement of 0.3mg/day is considered adequate (FNB, 1998). It is estimated that lactating women transfer 0.2 mg thiamine to their infants through their milk each day. Therefore, an additional 0.1 mg is estimated as the need for the increased energy cost of about 2092 kJ/day associated with lactation (FNB, 1998).

Table 2.1: Recommended nutrient intakes (RNIs) for thiamine, by group

Group	RNI mg/day
Infants and children	
0-6months	0.2
7-12months	0.3
1-3years	0.5
4-6years	0.6
7-9years	0.9

2.12.5.2 Riboflavin

Riboflavin (vitamin B2) deficiency results in the condition of hypo- or ariboflavinosis, with sore throat; hyperaemia; oedema of the pharyngeal and oral mucous membranes; cheilosis; angular stomatitis; glossitis; seborrheic dermatitis; and normochromic, normocytic anaemia associated with pure red cell cytoplasia of the bone marrow (McCormick,1997). As riboflavin deficiency almost invariably occurs in combination with a deficiency of other B-complex vitamins, some of the symptoms (e.g. glossitis and dermatitis) may result from other complicating deficiencies. The major cause of hyporiboflavinosis is inadequate dietary intake as a result of limited food supply, which is sometimes exacerbated by poor food storage or processing. Children in developing countries will commonly demonstrate clinical signs of riboflavin deficiency during periods of the year when gastrointestinal infections are prevalent.

Role in human metabolic processes

Conversion of riboflavin to flavin mononucleotide (FMN) and then to the predominant flavin, flavin adenine dinucleotide (FAD), occurs before these flavins form complexes with numerous flavoprotein dehydrogenases and oxidases. The flavo-coenzymes (FMN and FASD) participate in oxidation– reduction reactions in metabolic pathways and in energy production via the respiratory chain (McCormick, 1997).

Diet Recommendations

The estimated average requirement and RDA for riboflavin that cover men and women between the ages of 19 and 70 y are 0.9–1.1 and 1.1–1.3 mg/d, respectively (FNB, 2003). These values are based on observing clinical evidence of deficiency with intakes of, 0.6 mg/d and measuring normal erythrocyte glutathione reductase activation coefficient values with dietary intakes of ;1 mg/d. RDAs for riboflavin in children ages 1–18 y are based on extrapolated data from adult values after compensating for growth and metabolic differences. Accordingly, RDAs for riboflavin in children 1–9 y and adolescents 10–18 y range from 0.5 to 0.6 mg/d and from 0.9 to 1.3 mg/d, respectively. Adequate Intakes of riboflavin for infants 0–12 mo of age are based on mean volume (0.78 L/d) of milk consumed. Thus, Adequate Intake for infants 0–12 mo is 0.3–0.4 mg/d.

2.13 Inhibition of Mineral Bioavailability by Phytates

Plant-based complementary foods often contain high levels of phytate, a potent inhibitor of iron, zinc, and calcium bioavailability. One of the common methods of predicting mineral bioavailability is phytate:mineral molar ratio Gibson et al., (2010) reported that 62% of indigenous and 37% of processed complementary foods in low income countries have phytate:mineral molar ratios that exceed suggested desirable levels for mineral bioavailability (i.e., phytate:iron <1, phytate:zinc <15, phytate:calcium <0.17). Mineral bioavailability from these products is typically low due to the presence of the phytic acid (Cook et al., 1997; Egli et al., 2002; Davidsson, 2003). Several recent *in vivo* isotope studies in adults (Mendoza et al., 1998; Egli et al., 2004; Hambige et al 2004) and infants (Davidsson et al., 2004) have reported improvements in absorption of iron, zinc, and calcium in cereal-based foods prepared with a reduced phytate content. Dietary phytates inhibit iron absorption whereas ascorbic acid and meats enhance it (Miller, 1996). Phytic acid is a major inhibitor of zinc absorption, especially when the content of animal protein is low. Zinc and phytic acid form insoluble complexes and the negative effect of such complexes on zinc absorption can be predicted by phytate-to–zinc molar ratios (Oberleas and Harland, 1981). High amounts of calcium exacerbate the inhibitory effect of phytate on zinc absorption by forming a calcium– zinc–phytate complex in the intestine that is even less soluble than phytate complexes formed by either ion alone (Davidsson *et al*. 1994; Cook, 1997; Egli *et al*. 2002; Davidsson, 2003).

2.13.1 Indigenous strategies for reducing phytic acid in plant foods

Germination

Germination has been an indigenous technique practiced in developing countries. Though the locals attribute the practice to solely improved taste of beverages made for germinated grain, the practice also improves the nutritional value of the foods. Germination increases endogenous phytase activity in cereals through *de novo* synthesis, activation of intrinsic phytase, or both. Tropical cereals such as maize and sorghum have a lower endogenous phytase activity than do rye, wheat, triticale, buckwheat, and barley (Egli et al., 2002). The rate of phytate hydrolysis varies with the species and variety as well as the stage of germination, pH, moisture content, temperature (optimal range 45–57$_{o}$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 has been reported to increase 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 (e.g., *Vicia faba*) and red sorghum may also be reduced during germination as a result of the formation of polyphenol complexes with proteins and the gradual degradation of oligosaccharides (Camacho et al., 1992). The Amylase activity is also increased during germination of cereals, especially sorghum and millet. This enzyme hydrolyzes amylase and amylopectin to dextrins and maltose, thus reducing the viscosity of thick cereal porridges without dilution with water. This simultaneously enhances the energy and nutrient densities of the porridge (Gibson et al, 1998b).

Soaking

Soaking has been an indigenous technique practiced in western Nigeria especially for cereal grains before cooking. It said to soften the grain and therefore cooks faster. The practice has other advantages. Soaking cereal 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. Some polyphenols and oxalates that inhibit iron and calcium absorption, respectively, may also be lost by soaking (Erdman and Pneros-Schneier, 1994).

Fermentation

Fermentation is an age old practice in Nigeria and the world over. 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; Hurrel, 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 90% or more of phytate can be removed by fermentation of maize, soy beans, sorghum, cassava, cocoyam, cowpeas, and lima beans. In cereals with a high tannin content (e.g., bulrush millet and red sorghum), phytase activity is inhibited, making fermentation a less-effective phytate-reducing method for these cereal varieties (Sandberg and Svanberg, 1991).

Mechanical processing

Household pounding of cereal grains has been practiced in Nigeria for a long time. 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., rice, sorghum, and wheat) or in the germ (i.e., maize) (Nout and Ngoddy, 1997). Hence, bioavailability of minerals may be enhanced, although the content of minerals and some vitamins of these pounded cereals is simultaneously reduced. Household mechanical processing may only be possible for large seeds such as maize but may be cumbersome for small ones like amaranth grains or millet thus making it less viable in some settings.

Addition of animal foods to improve mineral bioavailability

The bioavailability of iron is generally higher from animal foods than from plant foods (Lartey et al., 1999; Allen, 2003). Addition of even a small amount of animalsource foods is one of the strategy to improve the content and bioavailability of micronutrients in plant-based diets in resource-poor settings. 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). Inclusion of red meat has been found to significantly increase non-heme iron bioavailability (Engelm-ann et al., 1998; Davidsson, 2003; Sorensen et al., 2007a) in plant based foods. There have been negative associations between the avoidance of animal source foods and mineral deficiency in young children from developed countries (Black, 2002; Black et al., 2002; Dagnelie et al., 1994). According to Kinyuru et al. (2009) and Ayieko et al., (2010b), it is possible to develop commercial products from edible insects in Kenya. Wheat based products developed with incorporation of edible insects have been observed to have high nutrient content and to be highly acceptable among consumers.

2.14 Consumer Acceptability of Complementary Foods

Many factors affect acceptance of a complementary foods both by mothers and infants including culture and food properties like taste, color and consistency (Codex 1991). Traditional foods are locally accepted and therefore pose no challenge to consumers. If a food is to be cooked to gruel, consistency has been found to affect acceptability (Owino et al., 2007). Some studies have found that complementary food formulations with addition of sugar were found to be more tasty and appealing than those without sugar by mothers and infants (Martin et al., 2010; Muhimbula et al., 2011).

CHAPTER THREE

MATERIAL AND METHODS

3.1 Study location

This research work was carried out in Oyo and Ekiti State. Winged termites (*M. bellicosus)* samples were collected between May and August of every year from 2011 through 2015 within the two States.

3.2 Study design

The study employed both experimental design involving laboratory analyses of parameters and use of animal model and cross-sectional study of respondents who consumed the insect.

3.3 Materials

Winged termites (*M. bellicosus),* Maize (*Zea maize*), Sorghum (*Sorghum bicolour*), Rice (Oryza sativa) and White yam (*Discorea Rotundata*). All the materials except Winged termites and Albino rats were purchased from Bodija market. Albino rats were purchased from the Department of Human Physiology, University of Ibadan.

3.4 Consumption Survey of Edible Winged Termites

The study was descriptive cross sectional in design. The consumption survey was carried out in five Local Government Areas (LGAs) in Ekiti State among respondents who were aged 12 years and above. Sampling procedure was as follows: sample size was calculated based on 50% consumption rate, 5% precision, 95% Confidence Interval. Sample size was estimated at 384 and rounded to 693 after various adjustments. A three-stage sampling procedure was used to select those who participated in the consumption survey –Ekiti state has a total population of 2, 384,212 with 16 Local Government Areas (LGAs). Five LGAs were selected by balloting and two wards from each of the LGAs were selected through a simple random sampling technique. Houses in the wards were assigned numbers and systematic random sampling procedure was used to select the households that participated in the study. Exclusion criteria included individuals unable to provide the requested information or written consent to participate in the study.

3.4.1 Sample Size Determination;

Taking a precision of 0.05 at 95% confidence interval (z). The minimum sample size (n) calculated by single proportion formula based on 50% consumption rate was calculated thus.

$$n = \frac{Z^2pq}{D^2}$$

Where Z^2= confidence level at 95 n= minimum sample size D= desired precision =0.05

P= assumed consumption rate of winged termites in Ekiti state = 50%

$$q=1-p= 0.5$$

$$n= \frac{1.96\times0.5\times0.5}{0.05^2} =384$$

Adjust for the size of Ekiti state population;

n/(1+(n-1)/N)

n=384; N=2, 384, 212

Therefore n= 384/(1+(384-1)/2,384,212)

n = 384

Adjust for estimated sample effect = 1.3

n = 384 ×1.3=499

Adjust for the expected response rate = 0.80

n= 499÷0.80 = 624

Adjust for the expected proportion eligible = 0.90

n= 624 ÷ 0.90 = 693

The final sample size of 693 ~700 were used for the study

3.4.2 Survey procedure

The interviewers were first trained prior to the commencement of the survey in order to standardize all the information collected. The data were collected in the participants' respective houses during a personal visit.

3.4.3 Data collection

The sampling instruments included a semi-structured questionnaire which was validated and pre-tested in another setting that was not selected for the main study. These people were within the age (12 years and above) group qualified for the study. The questionnaire was designed to elicit information on demographic and socio-economic characteristics (age, sex, ethnic group, religion, monthly allowance, highest education, occupation etc), consumption pattern and frequency of consumption, availability and accessibility, knowledge on nutrient content and benefits of consumption to health, limitation and restriction to consumption.

3.5 Sample Collection and Preparation

Winged termites (*M. bellicosus)* were collected during their swarming at night. Maize (*Zea maize)*, Sorghum (*Sorghum bicolour*), Rice (*Oryza sativa*), White yam (*Discorea spp*.) were purchased from Bodija market in Ibadan, Oyo state, Nigeria. Albino rats used for the study were purchased from the Department of Human Physiology, University of Ibadan Oyo State.

3.5.1 Preparation of *M. bellicosus*

Collected samples were pooled together, small portion of fresh *M. Bellicosus* was analysed for its nutrient composition while the rest was oven-dried, de-winged,kept inside plastic containers and refrigerated at – 4^0C till when needed. *Marcrotermes bellicosus* was ground to pasty powder (mush) and sieved through small-mesh size sieve whenever needed.

3.5.2 Preparation of maize flour

The maize grains were cleaned and washed thoroughly to remove adhering dirt and dust. The grain was soaked for about 72 hours (to allow fermentation and stimulate domestic practice in Nigeria in preparation of ‘Ogi’ pap), thoroughly washed, wet milled, sieved, and allowed to settle for 3 hours, drained, and oven dried at 60^oC for 12 hours. The oven dried sample was grinded to fine powder, packaged using foil paperand kept until when needed (Inyang&Idoko, 2006).

3.5.3 Preparation of sorghum flour

The Sorghum grains were cleaned and washed thoroughly to remove adhering dirt and dust. The grain was soaked for about 72 hours to allow fermentation and stimulate domestic practice in Nigeria in preparation of sorghum pap, which is a popular weaning food, thoroughly washed, wet milled, sieved, and allowed to settle for 3 hours, drained, and hot air dried dried at 60^{o}C for 12 hours. The oven dried sample was ground to fine powder, packaged using foil paper and kept until when needed (Inyang & Idoko, 2006).

3.5.4 Preparation of yam and rice flour

About 1.5kg each of White yam (*Disscorea Rotundata*) and polished rice (*Oryza sativa*) were purchased from Bodija Market, Ibadan. The yam was washed, truncated at the extreme ends, (peeled, sliced and boiled for 30 minutes. It was then drained and mashed. The drained samples were oven-dried for 12 hours at 60^{0}C. Sample of raw polished rice seed were obtained, extraneous matter such as unhealthy or infected seeds, stones and chaff were removed from the samples. The rice was boiled for 30 minutes at 100°C and mashed to a homogenous form and oven-dried for 12 hours at 60^{0}C. The dried yam and rice were separately milled into flour with malex blender and packed in cellophane nylon and kept till when needed.

3.6 Complementary Food formulation

Locally formulated complementary foods were prepared from mixture of the basal ingredients and *M. bellicosus* mush at various inclusion levels using standardized methods of preparation. Complementary foods flours from maize, sorghum, boiled rice and boiled yam at 10%, 15% and 20% level of inclusion of *M. bellicosus* mush were prepared and analysed for proximate, minerals, vitamins and anti-nutritional factors.

Ratio of Mixing Food Samples with *Marcrotermes bellicosus*

Sample BR = 100 g Boiled rice
Sample BR_1 = 90 g Boiled rice + 10 g M. *bellicosus*
Sample BR_2 = 85 g Boiled rice + 15 g M. *bellicosus*
Sample BR_3 = 80 g Boiled rice + 20 g M. *bellicosus*

Sample BY = 100 g Boiled yam
Sample BY_1 = 90 g Boiled yam + 10 g M. *bellicosus*
Sample BY_2 = 85 g Boiled yam + 15 g M. *bellicosus*
Sample BY_3 = 80 g Boiled yam + 20 g M. *bellicosus*

Sample MF = 100 g Fermented Maize flour
Sample MF_1 = 90 g Fermented Maize flour + 10 g M. *bellicosus*
Sample MF_2 = 85 g Fermented Maize flour + 15 g M. *bellicosus*
Sample MF_3 = 80 g Fermented Maize flour + 20 g M. *bellicosus*

Sample SF = 100 g Fermented Sorghum flour
Sample SF_1 = 90 g Fermented Sorghum flour + 10 g M. *bellicosus*
Sample SF_2 = 85 g Fermented Sorghum flour + 15 g M. *bellicosus*
Sample SF_3 = 80 g Fermented Sorghum flour + 20 g M. *bellicosus*

BY = Boiled yam, BR = Boiled rice, MF = Fermented Maize flour, SF = Fermented Sorghum flour

A total of twelve complementary foods were prepared using different samples at varying level of inclusion of winged termites (*M. bellicosus*)

3.7 Proximate analysis of food samples

Samples from *M. bellicosus*, fermented maize flour, fermented sorghum flour, boiled yam, and boiled rice, M.bellicosus+maize flours at (10%, 15% &20% inclusion), M.bellicosus+sorghum flours at (10%, 15% &20%inclusion), M. bellicosus+boiled yam/dry matter basis at (10%, 15%, 20%), M. bellicosus+boiled rice/dry matter basis at (10%, 15% &20%), M.bellicosus+Maize pap/wet matter basis, M.bellicosus+sorghum pap/wet matter basis, M. bellicosus+boiled yam/wet matter basis, and bellicosus+boiled rice/wet matter basis were analyzed for moisture content, ash, crude protein, crude fat, and total carbohydrate following the Association of Official Analytical Chemists (AOAC) procedure of 2005

3.7.1 Moisture

Moisture content was determined by oven drying method (AOAC, 2005).

Procedure

1. The aluminium dishes were thoroughly washed and dried in a moisture oven. They were put inside the desicator to cool. Each dish was weighed in triplicates and weights recorded
2. Each sample was carefully prepare and mixed thoroughly. Five grams of macerated Sample was accurately weighed into the dish. The weight of each dish plus the weighed sample was taken in triplicates.
3. The sample was dried in the moisture oven at 70-80^{o}C for 20 hours and at 100-135^{o}C (usually 105^{o}C) for the next 4 hours until weight is constant.
4. Sample was cooled in the desiccators. Dry weight of sample+dish were taken and recorded.
5. Moisture content of each sample was calculated as indicated below.

Calculation

Percentage of moisture in the sample was calculated using the following formula:

$$\% \text{ Moisture} = \frac{\text{weight of moisture}}{\text{weight of wet sample}} \times 100$$

3.7.2 Ash

The determination of inorganic substance as residue after ignition of food samples at specific temperatures.

Method (AOAC, 2005)

1. One gramme of finely ground dry sample of each sample was weighed into a crucible
2. Each sample was charred on an electric heaterinside a fume cupboard to drive off most of the smoke
3. Each sample was transfer into a preheated muffle furnance at 600°C and heated at this temperature for 2 hours
4. The crucible was transferred to a desiccators to cool
5. The white ash obtained was weighed.

Calculation

$$\textbf{\% Ash} = \frac{\text{Ash weight (gm)}}{\text{sample weight (gm)}} \times 100$$

3.7.3 Crude Protein Determination (AOAC, 2005)

The crude protein in the sample was determined by the routine semi-micro Kjeldahl, procedure/technique. This consists of three techniques of analysis namely Digestion, Distillation and Titration.

Apparatus: Analytical Balance, Digestion tubes, Digestion Block Heaters, 50ml Burette, 5ml Pipette, 10ml Pipette, 10ml Measuring Cylinder, 100ml Beakers, Fume Cupboard.

Reagents: ConC.H_2SO_4, 0.01NHCl, 40% (W/V) NaOH, 2% Boric Acid Solution, Methyl Red – Bromocresol green mixed indicator, Kjeldahl Catalyst tablet.

Digestion

0.5g of each finely ground dried sample was weighed carefully into the Kjeldahl digestion tubes to ensure that all sample materials got to the bottom of the tubes. 1 Kjeldahl catalyst tablet and 10ml of Conc. H_2SO_4 were added. These were set in the appropriate hole of the Digestion Block Heaters in a fume cupboard. The digest was left on for 4 hours, after which a clear colourless solution was obtained in the tube. The digest was allowed to cool down and carefully transfer into 100ml volumetric flask, thoroughly rinsing the digestion tube with distilled water and the flask was made up to mark with distilled water.

Distillation

The distillation was performed with Markham Distillation Apparatus which allows volatile substances such as ammonia to be steam distilled with complete collection of the distillate. The apparatus was steamed out for about ten minutes. The steam generator was then removed from the heat source to the entire developing vacuum to remove condensed water. The steam generator was placed on the heat source (i.e. heating mantle) and each component of the apparatus was fixed up appropriately.

Determination: 5ml portion of the digest above was pipetted into the body of the apparatus via the small funnel aperture. To this 5ml of 40% (W/V) NaOH was added through the same opening with the 5ml pipette. The mixture was steam-distilled for 2 minutes into a 50ml conical flask containing 10ml of 2% Boric Acid plus mixed indicator solution placed at the receiving tip of the condenser. The Boric Acid plus indicator solution were observed for colour changes from red to yellow.

Titration

The colour solution obtained above was then titrated against 0.01N HCl contained in a 50ml Burette. At the end point or equivalent point, the green colour turns to wine colour which indicates that all the Nitrogen trapped as Ammonium Borate [$(NH_4)_2BO_3$] have been removed as Ammonium chloride (NH_4Cl).The percentage nitrogen in this analysis was calculated using the formula:

% N = Titre value x Atomic mass of Nitrogen x Molarity of HCl used x 4

Or % N = Titre value x Molarity of HCl used x Atomic mass of N x Volume of flask containing the digest x $\frac{100}{1}$

Weight of sample digested in milligram x Vol. of digest for steam distillation. The crude protein content was determined by multiplying percentage Nitrogen by a constant factor of 6.25 i.e. % CP = % N x 6.25.

3.7.4 Crude Fat or Ether Extract Determination

Apparatus: Soxhlet apparatus and accessories, oven, desicator and analytical balance.

Reagents: Petroleum spirit or Ether (40° – 60°C b.pt).

Determination: 1gm of each dried sample was weighed into fat free extraction thimble and pug lightly with cotton wool. The thimble was placed in the extractor and fitted up with reflux condenser and a 250ml soxhlet flask which has been previously dried in the oven, cooled in the desicator and weighed. The soxhlet flask was filled to $^3/_4$ of its volume with petroleum ether (b.pt. 40º – 60ºC), and the soxhlet flask. Extractor plus condenser set were placed on the heater. The heater was put on for six hours with constant running water from the tap for condensation of ether vapour. The set up was constantly watched for ether leaks and the heat source was adjusted appropriately for the ether to boil gently. The Ether was allowed to siphon over several times say over at least 10 – 12 times until it is short of siphoning. Ether content of the extractor was drained carefully into the ether stock bottle. The thimble containing sample was removed and dry on a clock glass on the bench top. The extractor, flask and condenser were replaced and the distillation continues until the flask is practically dry. The flask which now contains the fat or oil is detached, its exterior cleaned and dried to a constant weight in the oven. If the initial weight of dry soxhlet flask was Wo and the final weight of oven dried flask + oil/fat is W_1, percentage fat/oil is obtained by the formula:

$$\frac{W_1 - Wo}{\text{Wt. of Sample taken}} \times \frac{100}{1}$$

3.7.5 Fibre Determination (AOAC official method, 2005)

Apparatus: Heating mantle, crucibles, furnace, sieve cloth, fibre flask, funnel, analytical weighing balance, a dessicator.

Reagents: 0.255N H_2SO_4, 0.313N NaOH and Acetone.

Determination: 2.0gm of the sample was weighed accurately into the fibre flask and 100ml of 0.255N H_2SO_4 added. The mixture was heated under reflux for 1 hour with the heating mantle. The hot mixture was filtered through a fibre sieve cloth. The filterate obtained was thrown off and the residue returned to the fibre flask to which 100ml of (0.313N NaOH) was added and heated under reflux for another 1 hour. The mixture was filtered through a fibre sieve cloth and 10ml of acetone added to dissolve any organic constituent. The residue was washed with about 50ml hot water on the sieve cloth before it was finally transferred into the crucible. The crucible and the residue were oven-dried at 105ºC overnight to drive off moisture. The oven-dried crucible containing the residue was cooled in a dessicator and later weighed to obtain the weight W_1. The crucible with weight W_1 was transferred to the muffle furnace for Ashing at 550°C for 4 hours. Crucible containing white or grey ash (free of carbonaceous material) was cooled in the dessicator and weighed to obtain W_2. The difference $W_1 - W_2$ gives the weight of fibre. The percentage fibre was obtained by the formula:

$$\% \text{ Fibre} = \frac{W_1 - W_2}{\text{Wt. of sample}} \times \frac{100}{}$$

3.7.6 Nitrogen-Free Extract (NFE) Determination

The NFE (carbohydrates was) determined by difference. This was done by subtracting sum of (Moisture % + % Crude Protein + % Ether Extract + % Crude Fibre + % Ash) from 100 i.e. CHO = 100 – (% M + % CP + % EE + % CF + % Ash).

3.8 Gross Energy Determination (AOAC, 2005)

Apparatus: Gallenkamp Ballistic Bomb Calorimeter.

Reagents: Benzoic Acid.

Determination: 0.25gm of each sample depending on bulkiness was weighed into the steel capsule. A 10cm cotton thread was attached to the thermocouple to touch the capsule. The Bomb was closed and charged in with oxygen up to 30 atm. The Bomb was fixed up by depressing the ignite switch to burn the sample in an excess of oxygen. The maximum temperature rise in the bomb was measured with the thermocouple and galvanometer system. The rise in temperature was compared with that obtained for 0.25gm of Benzoic acid value.Each sample was determined by the following stepwise calculations:

Calculations:

Mass of Benzoic Acid $= W_1$gm

Calorific value of 1gm Benzoic Acid = 6.32 Kcal/g

Heat released from Benzoic Acid = 6.32 x W_1 Kcal

Galvonometer deflection without sample = T_1

Galvonometer deflection of Benzoic Acid = $T_2 - T_1$

Calibration constant $= \frac{6.32 \times W1}{T_2 - T_1} = y$

The standardizing is repeated five times and average value calculated for y.

Mass of sample = 0.25gm

Galvonometer deflection with sample = T_3

Galvonometer deflection of sample = $T_3 - T_1$

Heat released from sample = $(T_3 - T_1)$ y Kcal

Calorific value of sample = $\underline{(T_3 - T_1)\ y}$ Kcal/g 0.25

Pauzenga Equation For Metabolizable Energy

M.E(Kcal/kg) = 37 x %CP + 81.8 x %Fat + 35 x %NFE (Pauzenga, 1985).

3.9 Determination of Minerals

3.9.1 Calcium Potassium and Sodium

Apparatus: Heating mantle, Crucible, Glass rod, Flame photometer, 100ml volumetric flask, Whatman No. 1 Filter paper, Wash bottle, 10ml pipette, funnel.

Reagents: 2 MHCl.

Determination: The ash of each sample obtained was digested by adding 5ml of 2 MHCl to the ash in the crucible and heat to dryness on a heating mantle. 5ml of 2 MHCl was added again, heat to boil, and filtered through a Whatman No. 1 filter paper into a 100ml volumetric flask. The filterate was made up to mark with distilled water stopped and made ready for reading of concentration of Calcium, Potassium and Sodium on the Jenway Digital Flame Photometer(PFP7 Model) using the filter corresponding to each mineral element.

The concentration of each of the element was calculated using the formula:

$$\%Ca \text{ or } \%K \text{ or } \%Na = \frac{\text{Meter Reading(MR)} \times \text{Slope} \times \text{Dilution factor}}{10000}$$

3.9.2 Phosphorus Determination (Spectrophotometric method)

Phosphorus was determined by the routine spectrophotometric method.

Apparatus: Spectrophotometer, 50ml volumetric flask, 10ml pipette, filter paper, funnel, wash bottle, glass rod, heating mantle, crucibles.

Reagents: Vanadate – Molybdate yellow solution, 2MHCl

Determination: The ash of each sample obtained was treated with 2MHCl solutions as described for calcium determination above. 10ml of the filterate solution was pipetted into 50ml standard flask and 10ml of vanadate yellow solution was added and the flask made up to mark with distilled water, stoppered and left for 10 minutes for full yellow development. The concentration of phosphorus was obtained by taking the optical density (OD) or absorbance of the solution on a Spectronic20 spectrophotometer at a wavelength of 470nm. The percentage phosphorus was calculated using the formula:

$$\% \text{ Phosphorus} = \frac{\text{Absorbance} \times \text{Slope} \times \text{Dilution factor}}{10000}$$

3.9.3 Determination of Magnesium, Iron, Copper and Zinc

The digest of the ash of each sample above as obtained with deionised or distilled water and made up to mark. These diluents were aspirated into the Buck 200 Atomic Absorption Spectrophotometer (AAS) through the suction tube. Each of the trace mineral elements was read at their respective wavelengths with their respective hollow cathode lamps using appropriate fuel and oxidant combination.

3.10 Determination of Fatty Acid Profile

The dried powdered specimens were extracted with chloroform: methanol (2:1, v/v) as described by (Kim et al., 1997) and the solid, non-lipid material was removed by filtration. The total extracted lipid material was recovered after solvent removal in a stream of nitrogen. The samples were then re-dissolved in chloroform: methanol (2:1, v/v) and centrifuged. Transmethylation was performed using 14% (w/v) BF3 in methanol (Morrison and Smith, 1964). Fifty nanograms of heptadecanoic acid (internal standard) and a 1mL aliquot of each sample were transferred to a screw-cap tube. After removal of solvent, the sample was mixed with 2mL of BF3 reagent, placed in a warm bath (Thermolyne, USA) at 100 ^{o}C for 30 min and cooled. After the addition of 2mL of saline solution, the transmethylated fatty acids were extracted into hexane. Aliquots of the hexane phase were analyzed by gas chromatography. Fatty acids were separated and quantified using a Hewlett–Packard gas chromatograph (5890 Series II). One to two microliter aliquots of the hexane phase were injected in split-mode onto a fused-silica capillary column (Omegawax; 30m x 0.32mm ID, Supelco, Bellefonte, PA). The injector temperature was set at 200 ^{o}C, detector at 230 ^{o}C, and oven at 120^{o}C initially, then 120–205 ^{o}C at 41C per min, 205^{o}C for 18 min .The carrier gas was helium and the constant flow rate was approximate 50 cm/s. The internal standard (heptadecanoic acid, 17:0) and calibration standards GLC-85 (Nu-chek, Elysian, MN) were used for quantitation of fatty acids in the various lipid extracts. The data reported represent the average of three determinations.

3.11 Vitamins Determination

3.11.1 Vitamin A determination (AOAC Method 960.5 & 974.29, 2005)

Vitamin A was determined through ultraviolet absorption measurement at 328 nm after extraction with chloroform. Calibration curve of vitamin A acetate was made and sample vitamin A concentration estimated as microgram (µg) of vitamin A acetate.

3.11.2 Tocopherol (Vitamin E) determination (AOAC Method 992.03, 2005)

One gramme of sample was weighed into a 250ml conical flask fitted with a reflux condenser wrapped in aluminium foil, and refluxed with 10ml of absolute ethanol and 20ml of 1M ethanolic sulphuric acid for 45 minutes. The resultant solution was cooled for 5 minutes, followed by addition of 50ml of distilled water and then transferred into a separating funnel covered with aluminium foils. The unsaponifiable matter in the mixture was extracted with 5 x 50 ml diethyl ether. The combined extract was washed free of acid and dried over anhydrous sodium sulphate. The extract was later evaporated at a low temperature and the residue obtained was immediately dissolved in 10ml absolute alcohol. Aliquots of solutions of the sample and standard were transferred to a 20ml volumetric flask. 5ml absolute ethanol was added, followed by a careful addition of 1ml conc. HNO_3 and placed on a water bath at 900^C for exactly 30 minutes from the time the ethanol begins to boil. Rapid cooling under running water follows. The absorbance of sample solution was read at 470 nm.

3.11.3 Thiamine (Vitamin B_1) Determination (Woollard & Indyk, 2002)

Thiamine content of the sample was determined by weighing 1g of it into 100ml volumetric flask and adding 50ml of 0.1M H_2SO_4 and boiled in a boiling water bath with frequent shaking for 30 minutes. 5ml of 2.5M sodium acetate solution was added and flask set in cold water to cool contents below 50^0C. The flask was stoppered and kept at 45-50^0C for 2 hours and thereafter made up to 100ml mark. The mixture was filtered through a No. 42 Whatman filter paper, discarding the first 10ml. 10ml was pipetted from remaining filterate into a 50ml volumetric flask and 5ml of acid potassium chloride solution was added with thorough shaking. Standard thiamine solutions were prepared and treated same way. The absorbance of the sample as well as that of the standards was read on a fluorescent UV Spectrophotometer (Cecil A20 Model) at a wavelength of 285nm.

3.11.4 Riboflavin (Vitamin B_2) Determination (AOAC Official Method 981.15, 1995)

1g of each sample was weighed into a 250ml volumetric flask, 5ml of 1M HCl was added, followed by the addition of 5ml of dichloroethene. The mixture was shaken and 90ml of de-ionized water was added. The whole mixture was thoroughly shaken and was heated on a steam bath for 30 minutes to extract all the riboflavin. The mixture was then cooled and made up to volume with de-ionized water. It was then filtered, discarding the first 20ml of the aliquot. 2ml of the filterate obtained was pipetted into another 250ml volumetric flask and made up to mark with de-ionized water. Sample was read on the fluorescent spectrophotometer at a wavelength of 460nm. Standard solutions of riboflavin were prepared and readings taken at 460nm, and the sample riboflavin obtained through calculation.

3.11.5 Niacin (Vitamin B_3) Determination (AOAC Methods 944.13, 985.34, 2000)

5g of sample was extracted with 100ml of distilled water and 5ml of this solution was drawn into 100ml volumetric flask and make up to mark with distilled water. Standard solutions of niacin were prepared and absorbance of sample and standard solutions were measured at a wavelength of 385nm on a spectrophotometer and niacin concentration of the sample estimated.

3.11.6 Ascorbic acid determination (AOAC official Method 967.21, 2005)

Ascorbic acid in the sample was determined by titrating its aqueous extract with solution of 2, 6-dichlorophenol-indophenol dye to a faint pink end point.

3.12 Antinutritional Factors Determination

3.12.1 Oxalate Determination. (Massey, 2007)

2g of sample was boiled in 40ml of water for 30 minutes in a reflux condenser. About 10ml of 20% Na_2CO_3 was boil for another 30 minutes. The liquid extract was filtered and washed with hot water until the wash water was not showing any alkaline reaction. The combined wash water was concentrated and filter to a small volume and cool. With constant stirring, HCl was added at (1:1) drop wise until the final acid concentration after neutralization was about 4% at which stage a heavy precipitate appears (which was allowed to flocculate). The extract was carefully filtered into a 250ml flask and made up to mark. It was kept overnight; the supernatant liquid was filtered through a dry filter paper in a dry beaker. An aliquot of the filterate was taken into a 400ml beaker, dilute with water to 200ml and make just ammoniacal, and re-acidify with Lactic Acid. 10ml was added in cold medium of a 10% calcium chloride solution and stirred well to allow calcium oxalate precipitate to appear, and allowed to settled overnight. The clean supernatant liquid was carefully decanted off through Whatman No. 42 filter paper, without disturbing the precipitate. The precipitate was dissolved in HCl (1:1). Oxalic acid was precipitate by adjusting the pH with ammonium hydroxide solution. The contents was boiled and allowed to settle overnight. Oxalic acid was determined by titrating against 0.05N $KMnO_4$ solution.

Calculation 1ml of 0.05N $KMNO_4$ = 0.00225 anhydrous Oxalic Acid

$$\% \text{ Oxalic Acid} = \underline{\text{Titre value x } 0.00225} \text{ x } \frac{100}{1}$$

$$= \text{T.V x } 0.1125$$

3.12.2 Phytate Determination (Engelen et al.,1994)

Determination.2g of each sample was weighed into 250ml conical flask. 100ml of 2% Hydrochloric Acid was added to soak each sample in the conical flask for 3 hours. This was filtered through a double layer of hardened filter paper. 50ml of each filterate was placed in 0.50ml conical flask and 107ml distilled water was added in each case to give proper acidity. 10ml of 0.3% Ammonium thiocyanate (NH_4SCN) solution was added to each solution as indicated. This was titrated with standard iron (III) chloride solution which contained 0.00195g Iron per ml. The end point which was brownish-yellow persisted for 5 minutes.

The % phytic acid was calculated using the formula:

$$\% \text{ Phytic Acid} = \frac{\text{Titre value x } 0.00195 \text{ x } 1.19 \text{ x } 100 \text{ x } 3.55}{\text{Wt. of Sample}}$$

3.12.3 Tannin Determination (Folin Denis Method; Makkar *et al*. 1993)

The reaction is based on the fact that phosphotungstomolybdic acid is reduced by tannin-like compounds in alkaline solution, producing a highly coloured blue solution which is measured at 760nm. 0.01g of sample was weighed. 20ml cold (4^0C) methanol was added and vorteexed. It was thereafter centrifuge at 3,000rpm for 20minutes. The absorbance was measured at 760nm. Percent tannin from standard curve was calculated as follows:

$$\%\text{Tannin} = \frac{(A-1) \times V \times 100 \times D}{B \times W \times 10^6}$$

Where A= Absorbance of sample; I= Intercept; V= Total volume of extract; B= Slope of standard curve; W= Weight of sample.

3.12.4 Trypsin Inhibitor (The Casein Digestion Method, Kakade et al., 1974)

Procedure: 0.2g of defatted ground sample was weighed into a centrifuge tube. 10ml of 0.1M Phosphate buffer was added and shake on a shaker at room temperature for 1hr.

The suspension was centrifuged at 5000rpm in a centrifuge for 5min.The content was later filtered through a Whatman no 42 filter paper into a 250ml conical flask. 0.2,0.4,0.6,0.8,and 1.0ml of the filtrate was pipetted into a set of triplicate set of test-tubes(one set for each level of extract).The final volume was adjusted to 2ml by the addition of 0.1M phosphate buffer. These test-tubes was arranged into a water bath maintained at 37^0C. A blank was prepared by adding 6ml of 5% TCA solution to one set of triplicate tubes. 2ml of 2% casein solution was added to all the tubes which were previously kept at 37^0C to incubate for 20min.The reaction of casein was stopped by the addition of 6ml of 5% TCA solution and this was allowed to proceed for 1hr at room temperature. The mixture was later filtered at room temperature through a Whatman No 42 filter paper into 100ml conical flask.0.2, 0.4, 0.6, 0.8, and 1.0ml of stock trypsin solution was also pipetted into a triplicate set of test-tubes (one set for each level of trypsin) as above and treated similarly as sample to the point of filteration.

The absorbances of the filterates of both samples and standard trypsin solution was read on a Spectrophotometer at a wavelength of 280nm.The actual absorbance of sample was the difference between absorbance of stock trypsin filterate and that of sample filterate. The absorbance of blank was read. One trypsin inhibitor unit (TIU) is arbitrarily defined as an increase of 0.01absorbance units at 280nm in 20min per 10ml of the reaction mixture under the conditions mentioned herein.

Trypsin Inhibitor Unit for each sample was calculated using the formula:

$$\frac{\text{Change in absorbance of sample extract}}{0.01 \times \text{mg protein in sample}}$$

3.13 ANIMAL AND DIETS

3.13.1. Protein and micronutrients bioavailability determination in *Marcrotermes bellicosus*

Three iso-caloric diets, of which two sets were iso-nitrogenous, comprising a basal diet (0% protein), an experimental diet (15% protein), and a lactalbumin diet (control) (15% protein) were prepared as shown in table 3.1. Eighteen twenty-one day old weanling albino rats of Wister strain were purchased from the Physiology Department, University of Ibadan. These rats were housed individually in metabolic cages all through the feeding experiment in a room maintained at a 12 h light–dark cycle and a constant temperature of 20±3^{o}C and relative humidity of 65±15% at the animal house of the Department of Animal science University of Ibadan, Ibadan, Nigeria. The rats were allowed to acclimatise in the cages for seven days and fed *ad libitum* with commercial rat pellets and clean tap water before randomisation into diet groups. At the end of acclimatisation, the rats were weighed and then randomly distributed to three diets groups (i.e., basal, experimental, and control) of six rats each based on their weight. An amount of 10 g of prepared diet was supplied to each rat on daily basis and water was changed every other day for 28 days. The left-over of the diets were collected and weighed on a daily basis. The rats were weighed on a weekly basis throughout the duration of the experiment. (Itam, Eka & Ifon, 1986) The rats were allowed to fast overnight on the twentieth day and were anaesthetised with sodium pentobarbital (50 mg/kg body weight), blood samples were collected from the inferior vena cava using heparinised syringes. The collected blood was centrifuged at 9500 g for 3 min to collect plasma, which was stored at -40^{o}C for analysis of nutrient bioavailability. Serum retinol was determined using the method of the Association of Official Analytical Chemists (AOAC) (2005), whilst serum ferritin and zinc were determined using AOAC International (2006) methods 983.24 and 991.11. All experiments were performed in accordance with the Guidelines of the Committee for Animal Experimentation

Table 3.1 *M. bellicosus* Nutrient Bioavailability: Rats group diet Composition (g/1000 g diet)

Feed component	**Basal**	***M. bellicosus***	**Control**
Starch	820.0	391.4	605.7
Cellulose	50.0	50.0	50.0
Vegetable oil	80.0	80.0	80.0
Minerals mix	40.0	40.0	40.0
Vitamins mix	10.0	10.0	10.0
M.bellicosus	-	428.6	-
Lactalbumin	-	-	214.3
Total	1000	1000	1000
Gross energy (kcal/ g)	2.89	3.11	3.02
Nitrogen (%)	0.12	2.62	2.51

3.13.2 Protein and micro nutrient bioavailability in the *Marcrotermes bellicosus*-enriched diets

Six iso-caloric diets, of which five sets, were iso-nitrogenous, comprising a basal diet (0% protein), experimental diets (maize, sorghum, yam, and rice) (20% MB protein), and casein diet (control) (20% protein) were prepared as shown in Table 3.2. Thirty weanling albino rats of Wister strain brand were purchased from the Physiology Department, University of Ibadan, and were housed These rats were housed individually in metabolic cages in the animal house of the Department of Animal Science, University of Ibadan, Ibadan, Nigeria. The animal house was maintained at a 12 h light–dark cycle, constant temperature of 20±3^{o}C and relative humidity of 65±15%. The rats were allowed to acclimatise for one week, fed *ad libitum* with commercial rat pellets, and clean tap water. At the end of seven days, they were weighed and then randomly distributed to six diet groups (i.e., basal, maize, sorghum, yam, rice and control) of six rats each based on their weight. An amount of 10 g of prepared diets was supplied to each rat on daily basis and water was changed every other day for 28 days. The left-over of the diets were collected and weighed on daily basis. The rats were weighed on a weekly basis throughout the duration of the experiment. (Itam *et al*, 1986)

Table 3.2 Composition of formulated diets for feeding trial (g/1000 g diet)

Nutrient	Basal diet	Maize diet	Sorghum diet	Yam diet	Rice diet	Control diet
Starch	1033.2	530.8	549	499.9	506.8	778.2
Cellulose	63	63	63	63	63	63
Vegetable oil	100.8	100.8	100.8	100.8	100.8	100.8
Minerals	50.4	50.4	50.4	50.4	50.4	50.4
Vitamins	12.6	12.6	12.6	12.6	12.6	12.6
MB + maize (MF_3)	-	502.4	-	-	-	-
MB + sorghum (SF_3)	-	-	484.2	-	-	-
MB +yam (BY_3)	-	-	-	533.3	-	-
MB+ rice (BR_3)	-	-	-	-	526.4	-
Casein	-	-	-	-	-	280.0
TOTAL	1260	1260	1260	1260	1260	1260

WT = winged termite

3.14 Measurement of Nutritional Indices

The nutritional indices determined in the study were calculated according to equations described as follow:

3.14.1. Consumption Index (CI), Growth Rate (GR) and Efficiency of Conversion Ingested Food to The Body Tissues (ECI) Waldbauer (1968)

$$C1 = \frac{C}{TA.}; \quad GR = \frac{G}{TA}; \quad ECI = \frac{G}{C} \times 100$$

Where:

C = fresh weight of feed consumed.

T = duration of feeding period.

A = mean fresh weight of the rat during the feeding period.   G = fresh weight gain of the rat

3.14.2. Determination of Protein Efficiency Ratio

Protein efficiency ratio (PER) estimates protein nutritional quality in an in vivo assay by measuring rat growth as weight gain per gram of protein fed.

The weight of each rat was measured at the beginning of the assay and then the weight of each rat was taken every 7 days (weekly) and at the end of 28days.The food intake of each animal was recorded for 28 days.

The PER was calculated using the average total weight gain and average total protein intake for each diet group at day 28.

$$\text{PER} = \frac{\text{Total Weight Gain in Test Group (g)}}{\text{Total Protein Consumed}}$$

The PER value was normalized for the test protein (i.e. comparing the quality of the test protein to that of casein) by assigning casein a PER of 2.5.

$$\text{Adjusted or Corrected PER} = \frac{\text{PER of test protein}}{\text{PER of casein control}}$$

Mean weight gain: This was determined as the difference in the mean final weight and the mean initial weight of rats in each diet group.

3.14.3. Feed Efficiency Ratio (FER)

$$\text{FER} = \frac{\text{Gain in body weight (g)}}{\text{Food intake (g)}}$$

3.15 Serum Analysis

After the feeding trial which lasted for 28 days, blood sample of each animal was taken.

Blood Collection from the Orbital Sinus (ocular bleeding method) *(*Janet Hoff, 2000)

Rats were anesthetized using diethyl ether. The skin was pulled away from above and below the eye, so that the eyeball is protruding out of the socket as much as possible taking care not to occlude the trachea with the thumb. The tip of a fine-walled Pasteur pipette (1-2 mm) was inserted into the corner of the eye socket underneath the eyeball, directing the tip at a 45-degree angle toward the middle of the eye socket. The pipette was rotated between fingers and gentle downward pressure was applied and then released until the vein was broken and blood was visualized entering the pipette and allowed to flow freely into ETDA bottles with anticoagulant. When a small amount of blood filled the pipette, it was withdraw slightly and the pipette was allowed to fill. Same process was repeated for all the rats.

3.15.1 Determination of Serum Zinc, Calcium and Ferritin (AOAC, 2005)

One millilitre (1ml) of the blood sample was pipetted using a 3ml micropipette into 30ml digestion tube, 5ml of concentrated HNO_3 (Optima grade), 2ml of concentrated H_2O_2(Hydrogen peroxide), and 13ml of deionized water were added to the digestion tube. The peroxides permitted higher digestion temperatures by reducing the nitric acid vapours as well as removing complex matrix and blood biohazards. The digestion tube was placed in the appropriate hole of the Digestion Block Heater (TECATOR BD20) and allowed to digest to a clear colourless solution. The clear colourless solution after cooling down was carefully quantitatively transferred to a 50ml volumetric flask and made up to the mark with deoinised water. This diluent was used to read for metals such as Fe, Ca, Zn and others on a BUCK 211 VGP Atomic Absorption Spectrophotometer (AAS) at the respective wavelength of each metal using each metal respective hollow cathode lamp to atomise.

3.15.2 Determination of Serum Retinol in Blood (AOAC, 2005)

A well homogenized sample of 0.5ml was weighed into a 250ml Quartz round flask (QRF). 25ml of Methanol and 10ml of 50% KOH were added for stability.The mixture above was placed in a water bath set at 100^oC connected to a condenser (cold finger type) for 30 min to reflux.The QRF mixture was then cooled down in ice and kept in the dark for 1 hour. The whole mixture in QRF was transferred to a 250ml volumetric flask and washed with 3:1 methanol/H_2O mixture and made up to 250ml mark. The flask was rotated up and down to ensure uniform mixing. The volumetric flask was put in the dark overnight. 20ml supernatant of the above was pipetted into a centrifuge tube and 20ml petroleum ether added and shakes for 1min. This mixture was centrifuge for 30min in a Gallenkamp centrifuge. 2ml of the supernatant from the centrifuge tube was pipetted into 20ml tube and 1ml of chloroform added. 10ml of carr-price reagent (20% antimony chloride dissolved in chloroform with acetone). USP reference standard solution of transretinyl acetate which is equivalent to 30mg retinol was used as stock and working standard of range 0-5 ug/ml was prepared from the stock. The working standard was treated like sample above.The absorbance of standard as well as sample was read on a cecil 2483 spectrophotometer at a wavelength of 430nm. Vitamin A unit/100ml as retinol is calculated using the formula:

$$\frac{\text{Absorbance of standard}}{\text{Absorbance of sample}} \times \frac{\text{Conc. of standard}}{1}$$

3.16 Histological Preparation of Tissues

The liver, intestine, spleen and kidney, were immediately fixed in 10% formalin. The tissues were then cut in slabs of about 0.5 cm transversely and the tissues were dehydrated by passing through different grades of alcohol: 70% alcohol for 2 hours, 95% alcohol for 2 hours, 100% alcohol for 2 hours, 100% alcohol for 2 hours and finally 100% alcohol for 2 hours. The tissues were then cleared to remove the alcohol; the clearing was done for 6 hours using xylene. The tissues were then infiltrated in molten paraffin wax for 2 hours in an oven at 57^{O}C, thereafter the tissues were embedded. Serial sections were cut using rotary microtone at 5 microns (5µm). The satisfactory ribbons were picked up from a water bath (50-55 ^{O}C) with microscope slides that had been coated on one side with egg albumin as an adhesive and the slides were dried in an oven. Each section was deparaffinized in xylene for 1 minute before being immersed in absolute alcohol for 1 minute and later in descending grades of alcohol for about 30 seconds each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solution of hematoxylin for about 18 minutes. The slides were rinsed in water, then differentiated in 1% acid alcohol and then put inside a running tap water to blue and then counterstained in alcoholic eosin for 30 seconds and rinsed in water for a few seconds, before being immersed in 70%, 90% and twice in absolute alcohol for 30 seconds each to dehydrate the preparations. The preparations were cleared of alcohol by dipping them in xylene for 1 minute. Each slide was then cleaned, blotted and mounted with DPX and cover slip, and examined under the microscope. Photomicrographs were taken at x40, x100 and x400 magnifications.

3.17 Consumer Acceptability of the Complementary Foods

3.17.1 Sensory evaluation of the formulated CFs using nursing mothers

The formulated complementary food samples were reconstituted into light gruels; about 300g each of Maize and Sorghum flour were thoroughly mixed separately in 500ml of cold water to a fine consistency with no lumps. The mixture were allowed to boil on cooking gas while stirring with wooden stirrer was done continuously until maize and sorghum paps were formed. Maize pap and sorghum pap were mixed separately with 80g of powdered winged termites each. About 240g each of boiled '*Aroso*' rice and white yam were prepared as outline in section 3.5.4. The samples were mixed separately with 60g of winged termites. All the samples were then allowed to cool to 45^{o}C and maintain in a water bath at that temperature. Sensory evaluation of the reconstituted complementary foods in relation to taste, aroma, appearance, texture and overall consumer acceptability were carried out by 60 untrained nursing mothers.

3.17.2 Consumer Acceptance by mothers

The acceptability study was done in Ado-Ekiti, the capital of Ekiti State, Nigeria. The acceptability study was done among nursing mothers who come with their infants (6-24 months old) to the Comprehensive Health Care Centre Okeyinmi Ado-Ekiti. About 60 untrained nursing mothers were recruited into the study. The inclusion criteria were; mothers must have infants 6-24 months of age as authenticated by the birth records. Although the infants were not involved directly in the acceptability study, some of the mothers introduced the foods to their infants if they deem the formulated foods fit for their infants' consumption. The purpose of the study was explained to the mothers and written consent obtained (Appendix 4). The prepared foods were coded and presented to the mothers by the study team. Each mother tasted all the four porridges. The samples were rated on colour, taste, texture, odour and overall acceptability of the foods were assessed based on a a 7-point hedonic scale as follows: 7– Like Very Much, 6 – Like Moderately, 5 – Like Slightly, 4 – Neither Like Nor Dislike, 3 – Dislike Slightly, 2 – Dislike Moderately, 1 – Dislike Very Much.

3.18 Statistical analysis

The means and standard deviations were calculated for all values. Comparison between the control and experimental groups was done using one-way analysis of variance (ANOVA) with least significant difference (LSD). Independent sample T-test was used to compare mean between two independent groups. Differences were considered statistically significant at $p<0.05$.

CHAPTER FOUR

RESULTS

Table 4.1 Socio demographic characteristics of respondents

Variable	N	%
Gender		
Male	298	43
Female	395	57
Population distribution		
Teenager	324	46.7
Young adult	293	42.3
Adult	76	11
Educational attainment		
No formal education	67	9.6
Primary	90	13.1
Secondary	330	47.6
Tertiary	206	29.7
Local Government Areas		
Ijero	138	19.9
Irepodun/ Ifelodun	137	19.8
Ikere	146	21.1
Ado	148	21.3
Ido Osi	123	17.8

4.1: Socio demographic characteristics of respondents

The 693 participants interviewed consisted of 43.0% of male and 57.0% of female with 46.7% of teenagers, 42.3% young adult and 11.6% of adults. Those who did not have any formal education were 9.6%. Participants who had primary, secondary and tertiary education were; 13.1%, 47.6%, and 29.7% respectively. Five different local government areas enumerated during the survey include; Ijero (19.9%), Irepodun/Ifelodun (19.8 %), Ikere (21.1%), Ado (21.3%), and Ido Osi (17.8%).

Consumption survey of *M. bellicosus* has revealed that more female consumed the insect than male. Looking across the population distribution of those who eat *M. bellicosus*, more teenager follow by young adult, while the adult recorded the least number of participants who consumed the insect. Some adults held the belief that *M.bellicosus* is children's and young adults' snacks. This belief was responsible for the least percentage recorded among the adult population who consumed *M.bellicosus*. Education levels or status one has attained in the society does not affect consumption of the insect. The insect was being consumed by all age group, both illiterate and literate. The insect was well known in all the five local Government areas surveyed.

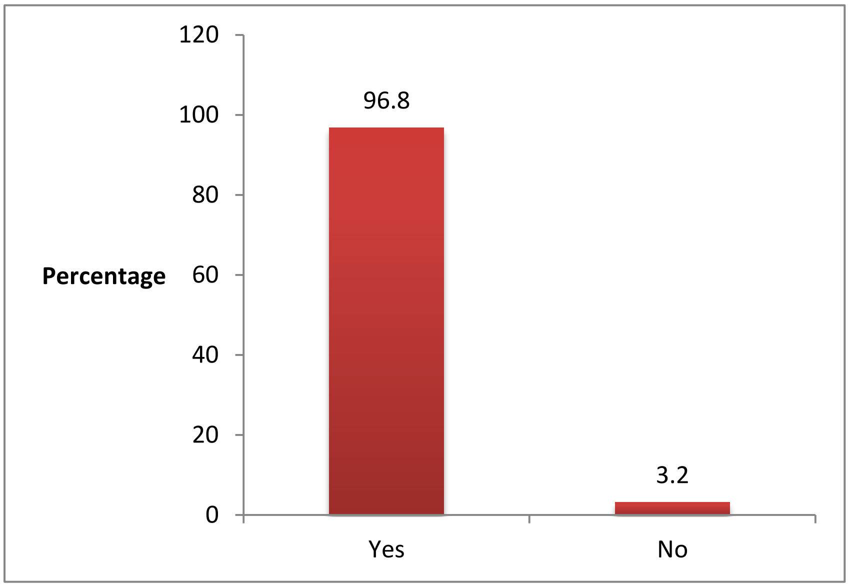

Figure 4.1.1: Knowledge of Winged termite

4.1.2 Knowledge of winged termite

The study revealed that 96.8% of the participants had perfect knowledge of Winged Termites. Only 3.2% of the respondents reported that they do not know the insect very well, these set of people were non-indigenes of Ekiti State.

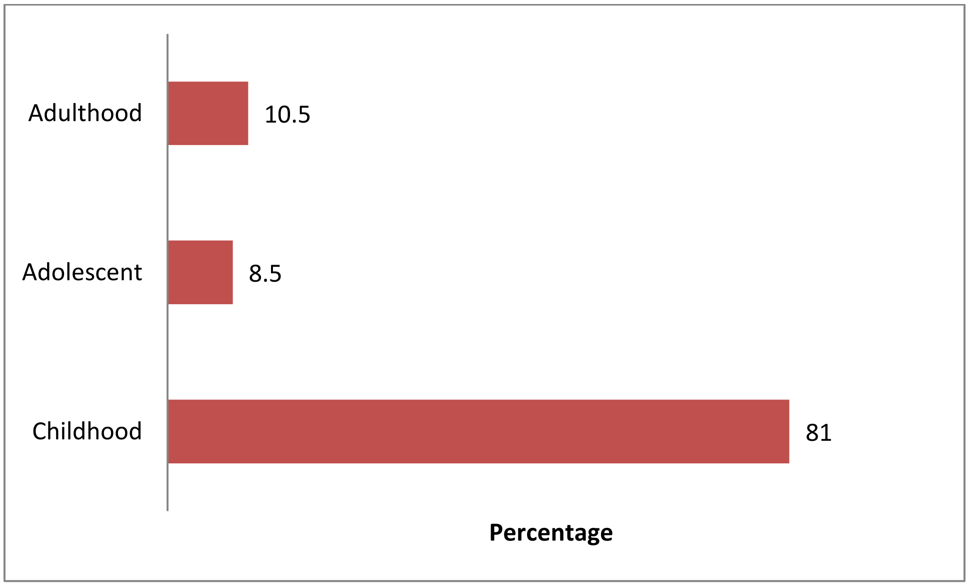

Figure 4.1.2: Population distribution by age range of Winged termite consumption

4.1.3: Population distribution by age range of termite consumption

Among the study participants, Consumption of Winged termites was more common among the children (81.0%), and few among Adolescents (8.5%) and 10.5% among adults (Figure 4.1.2). Consumption of *M. bellicosus* was not restricted to particular age category of participants among the study population.

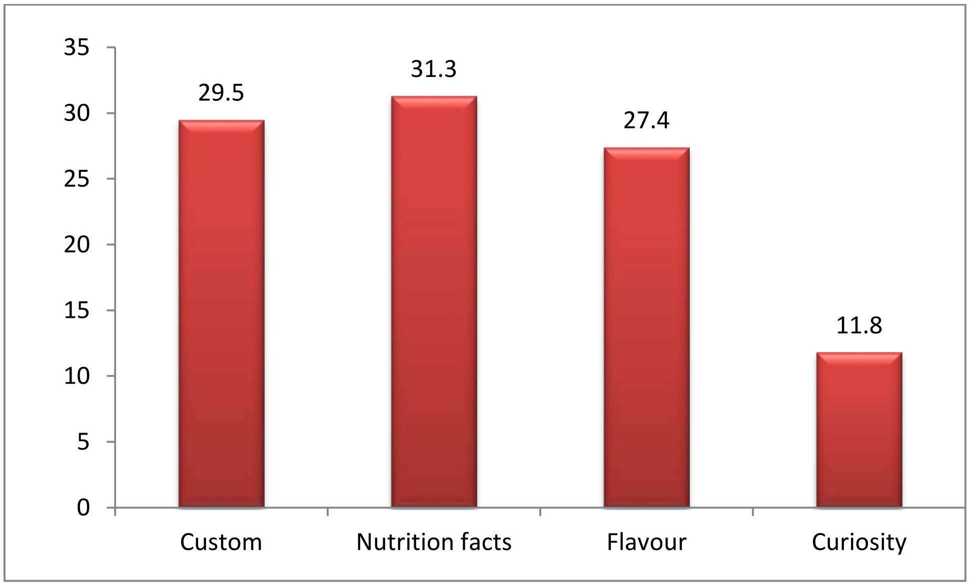

Figure 4.1.3: Source of motivation for Winged Termite’s consumption

4.1.4: Source of motivation for Winged Termite’s consumption

About one-quarter (24.9%) of respondents reportedly consumed Termites by tradition (custom), 31.3% reported that termites are nutritious while 27.4% consume them for their flavours. Furthermore, 11.8% consumed Winged Termites by curiosity (Figure 4.1.3).

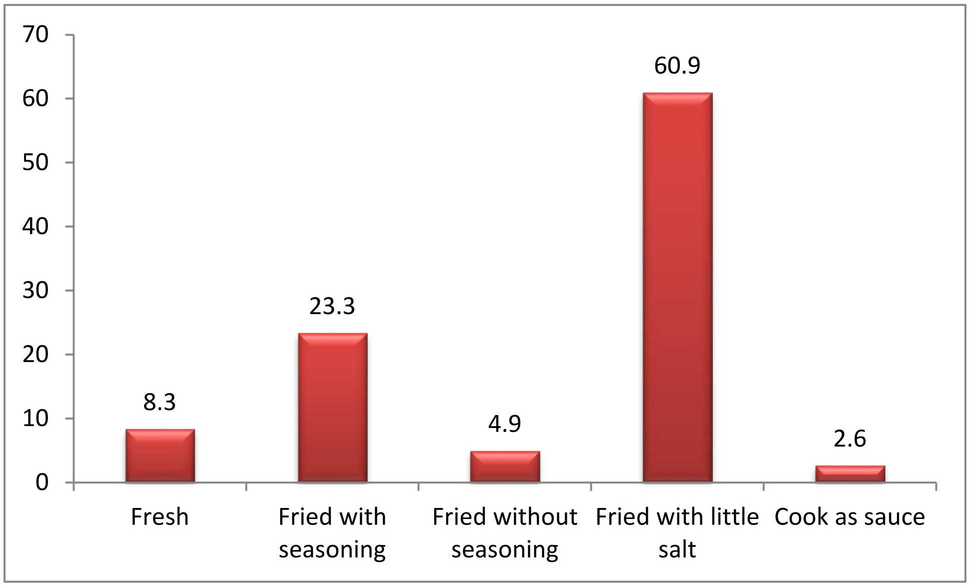

Figure 4.1.5: Methods of preparation of winged termites

4.1.5: Pattern of Winged termites' consumption and methods of preparation

The consumption of termites varies depending on their availability. Reproduction and swarming of winged termites depend on seasonal fluctuations. Termites are abundant during the rainy season. During this season, 75.3% of the participants consumed termites as much as it is available to them while 24.7% consumed termites occasionally. Termites are eaten fresh or dried and can be prepared in different ways, this result revealed that 23.4% participants preferred fried termites seasoned with spices while 4.9% consume them fried without seasoning, 60.9% prefer frying the termites with little salt, 2.6% cooked it as sauce and 8.3% eat them freshly to fully benefit from their nutrients (Figure 4.1.5).

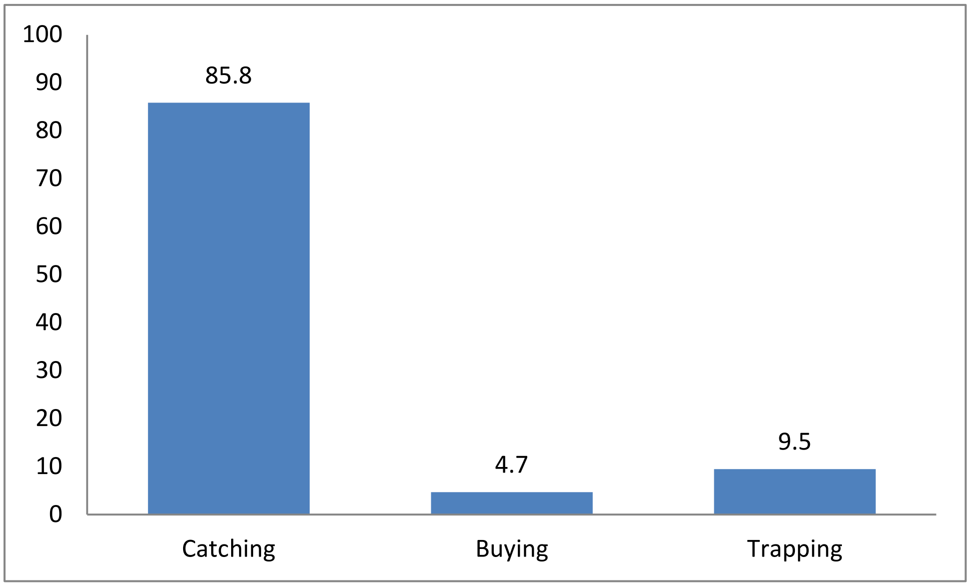

Figure 4.1.6: Mode of supply of Winged termites

4.1.6 Mode of supply of Winged termites

Majority (85.8%) of participants captured Winged termites by attracting them with light, 4.7% prefer buying dried or fried ones at the market and 9.5% trapped termites at the entrance of termite mounds (Figure 4.1.6).

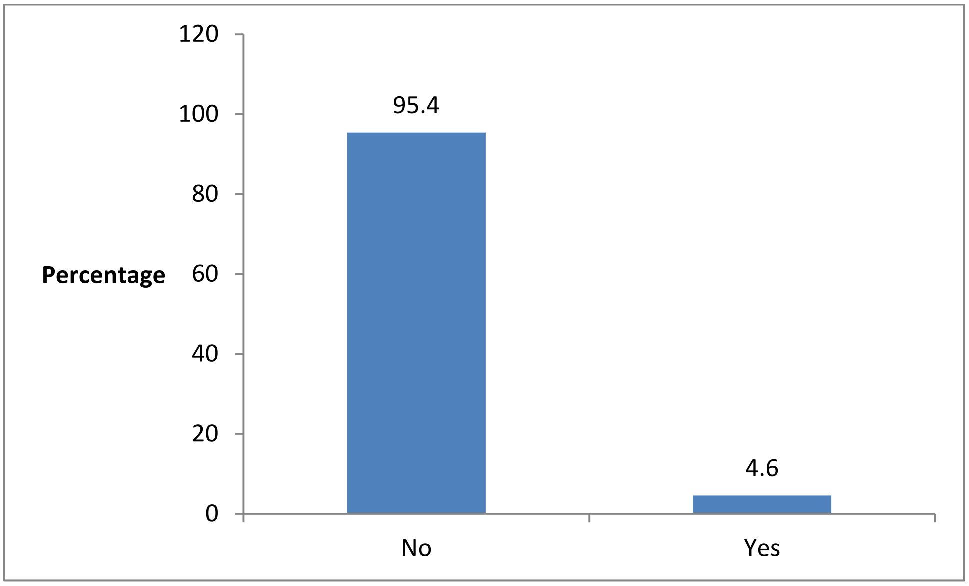

Figure 4.1.7: Possible Side effects on consumption of winged termites

4.1.7: Possible Side effects on consumption of winged termites

Participants who reported that they have not experience any negative side effect like stomach ache, skin rash, vomiting etc upon short time consumption of the insect were 95.4%. Only 4.6% reported that they have experience side effects such as vomiting and stomach ache.

Table 4.2: Proximate composition of roasted *M. bellicosus* (g/100g)

Sample	Mean±SD
Moisture	4.0±0.04
Crude protein	31.8±0.10
Crude fat	16.4±0.03
Ash	3.8±0.03
Total Carbohydrate	43.0±0.10
Gross Energy (Kcal)	450.7±0.00

Values are means ± SD of triplicate determinations.

4.2 Proximate composition of roasted winged termites (*Macrotermes bellicosus*)

The proximate composition of Macrotermes *bellicosus* (winged termites) is presented in Table 4 .2 *M. bellicosus* is very high in protein, fat and ash. The gross energy value of 450.7 ± 0.00 Kcal/100g is also very high. The high protein, fat and gross energy content of the insect makes it a suitable source of dietary animal protein and energy in boosting the nutritional quality of complementary foods for infants and young children from local foodstuffs.

Table 4.3: Mineral composition of roasted *M. bellicosus* (mg/100g dry matter)

Sample	Mean±SD
Sodium	98.40±0.20
Potassium	164.13±0.31
Calcium	227.50±0.20
Magnesium	23.47±0.25
Phosphorus	361.30±0.20
Iron	2.07±0.0.25
Zinc	15.03±0.31
Manganese	2.35±0.25
Copper	8.40±5.54
Selenium (µg/100g)	0.07±0.02

Values are means ± SD of triplicate determinations.

4.3 Mineral composition of roasted *M. bellicosus*

The mineral composition of *Macrotermes bellicosus* is shown in Table 4.3 *M. bellicosus* is rich in potassium, calcium, phosphorus, zinc, and copper, moderate in sodium, iron and manganese, but low in magnesium .Consumption of 100g roasted *M. bellicosus* would provide 500%, 84 .3%, 131.4% and 18.8% of the Adequate Intake (AI*) and the Recommended Dietary Allowances (RDA) for zinc, calcium, phosphorus and iron respectively. The sodium and potassium levels in the roasted *M. bellicosus* give sodium to potassium ratio of approximately 0.6.

Table 4.4: Fatty Acid Profile of *M. bellicosus*

Fatty acid	(%)
Caprylic acid (8:0)	ND
Capric acid (10:0)	ND
Lauric acid (12: 0)	ND
Myristic acid (14:0)	0.22
Palmitic acid (16:0)	11.81
Margeric acid (17:0)	ND
Stearic acid (18:0)	ND
Arachidic acid (20:0)	0.02
Behenic acid (22:0)	0.42
Lignoceric acid (24:0)	ND
Oleic acid (C18:1)	5.97
Euricic acid (C22:1)	1.56
Linoleic acid (C18:2)	24.91
α-Linolenic acid (C18:3)	52.30

ND = Not detected

Total Saturated Fatty Acids (SFA)	12.47%
Total Monounsaturated Fatty Acids (MUFA)	7.53%
Total Polyunsaturated Fatty Acids (PUFA)	77.21%

P/S ratio = 6.2

4.4: Fatty acid composition of the roasted *M. belicosus*

Table 4.4 shows the fatty acid profile of *M. bellicosus*. Saturated fatty acids account for 12.47% of the fat. However, the level of these saturated fatty acids was low in the *M. bellicosus*. Polyunsaturated fatty acid (n-3, 52.30%) and (n-2, 24.91%) were the predominant lipid found in the insect while 7.5% were monounsaturates.

Table 4.5: Selected Vitamins composition of roasted *M. bellicosus* (mg/100g)

Parameters	Mean±SD
Vitamin A (RE)*	330.42±0.12
Vitamin B1	0.09±0.01
Vitamin B2	0.01±0.02
Vitamin B3	0.85±0.01
Vitamin B6	0.27±0.02
Ascorbic acid	0.97±0.05
Vitamin D (μg/100g)	6.74±0.02
Vitamin E (μg/100g)	9.00±0.02

Values are means ± SD of triplicate determinations. *RAE = 1 Retinol activity equivalent= 1 μg/100g retinol

4.5: Vitamins composition of roasted *M.* bellicosus

Vitamin composition of roasted *M. bellicosus* is shown in table 4.5. The insect is rich in fat soluble vitamins (A, D, and E), while the level of all the water soluble vitamin in the insect was very low. Therefore, the insect is not a good sources water soluble vitamin and may have to supplement with other sources.

Table 4.6: Antinutrient composition of roasted *Macrotermes bellicosus* (mg/100g)

Sample	mean±SD
Phytate	0.01±0.00
Oxalate	0.01±0.00
Tannin	0.01±0.00
Saponin	0.00±0.00
Trypsin inhibitor (Tiu/mg)	0.12±0.02

Trypsin inhibitor Unit (Tiu)

4.6: Antinutritional factors composition of roasted *Marcrotermes bellicosus*

The insect was very low in antinutritional factors studied (Table 4.6). The levels of phytate, oxalate, saponins and tannins were highly insignificantly low since they are majorly found in foods of plant origin. The level of trypsin inhibitor is very low and cannot constitute any malabsorption or bioavailability of proteins from other sources.

Table 4.7: Mean feed intake, weight change, and protein efficiency ratio of rats groups

Parameters	Experimental group	Control group	Basal group
Initial weight of rat (g)	47.83±3.71	47.50±11.72	53.33±12.14
Final weight of rat (g)	71.00±8.81	64.33±6.06	33.83±7.25
Mean weight gain (g)	+23.17±6.71	+16.83±6.88	-19.50±9.01
Feed intake (g)	9.50±0.24	9.01±0.51	5.99±0.41
CI	11.48^a	14.98^b	8.60^c
GR	6.41^a	8.03^b	3.64^c
E.C.I	7.47^a	7.14^b	5.64^c
PER	2.44	1.87	-

Means with different superscripts in a row are significantly different ($p<0.05$) .Consumption index (CI), Growth rate (GR), Efficiency of conversion of ingested feed (ECI)

4.7: Mean feed intake, weight change, and protein efficiency ratio of rat groups

The mean rat feed intake of prepared diets is shown in table 4.7. The mean rat feed intakes of the experimental and control groups were significantly higher than that of basal diet ($p<0.05$). There was also a significant difference between the rat feed intake of the experimental and control diet intake with the intake of the experimental diet being slightly higher than that of the control group. The rats feed intakes were significantly different with the basal group having consumed slightly above half of the daily supply of 10 g each, whilst the experimental group (insect-source protein diet) recorded the highest mean feed intake, which was slightly higher than that of the control group (casein diet).

The difference between the CI, GR, and ECI in all the diet were significant ($p<0.05$). The basal group had the least CI, GR, and ECI; the experimental diet was highest in ECI, while the control diet was the highest in feed CI and GR. Rats' weight change could be attributed to feed intake and protein content of the diet with the basal group losing weight while the experimental and control groups gained weight. Protein efficiency ratio (PER) of experimental diet was slightly greater than that of control diet, this means that both experimental and control diet will support rat growth adequately.

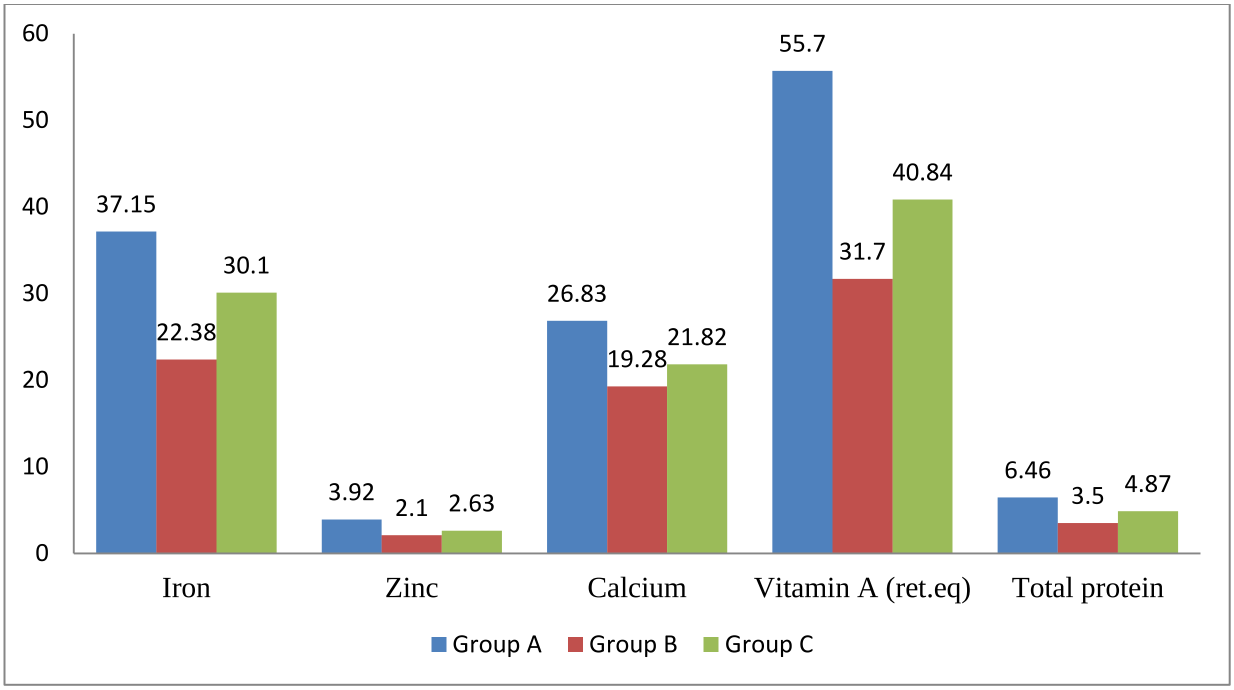

Figure 4.2.1: Mean (±SD) Bioavailability of selected minerals, vitamin and serum total protein of group A (experimental diet), group B (basal diet) and group C (control diets) (mg/100ml) ($p<0.05$)

4.2.7: Serum micronutrient bioavailability of rats

The serum micronutrient levels of the rat fed the basal diet were significantly lower in value than the experimental and control groups. The rats fed with experimental diets had the highest value as shown in figure 4.2.1. All the serum micronutrient levels of rats fed with experimental diets were significantly higher than that of the control group ($p<0.05$). The values obtained for serum total protein in rats group fed with the experimental diet were significantly higher ($p<0.05$) than the values obtained from the control group and basal group.

Table 4.8 proximate composition of roasted *M. bellicosus* and cereal flour (g/100g)

		Maize		Sorghum	
Sample	*M. bellicosus*	(Wet)	(Dry)	(Wet)	(Dry)
Moisture	4.0±0.04	65.0±0.12	11.7±0.03	64.1±0.50	10.6±0.05
Crude Protein	31.8±0.10	3.3±0.03	8.9±0.09	3.9±0.04	9.7±0.07
Crude Fat	16.4±0.03	1.2±0.01	3.1±0.03	1.1±0.01	2.8±0.02
Ash	3.8±0.03	0.8±0.00	2.0±0.02	0.8±0.00	2.1±0.01
Total Carbohydrates	43.0±0.10	29.7±0.05	74.3±0.11	30.1±0.05	74.8±1.84
Gross Energy (kcal/)	450.7±0.00	-	353.9±0.28	-	358.6±6.81

Values are means ± standard deviations of triplicate determinations. The table compare the proximate composition of cereal on wet and dry matter basis

4.8: Proximate Composition of Roasted *M. bellicosus*, Cereal Flours

The result of proximate composition of roasted *M. bellicosus* and fermented maize and sorghum flour is as shown in Table 4.8. Roasted *M. bellicosus* moisture content was very low while the crude protein, fat, carbohydrate and gross energy content were very high. The moisture and crude fat content of the two cereal flour were low; the crude protein value was moderately high while the ash and total carbohydrates content were very high. The moisture and crude fat values of the maize flour were not significantly different from that of sorghum ($p>0.05$), while sorghum was significantly higher in crude protein ($p<0.05$) but insignificantly higher in ash, total carbohydrates and gross energy ($p>0.05$).

Table 4.9: Proximate composition of enriched complementary foods (g/100)

Sample	Moisture	C. Protein	C. Fat	Ash	T. Carbohydrates	Gross E
MF	11.7 ± 0.03^a	8.9 ± 0.09^a	3.1 ± 0.03^a	2.0 ± 0.02^a	74.3 ± 0.11^a	353.91 ± 0.28^a
MF_1	11.4 ± 0.03^b	17.8 ± 0.10^b	5.1 ± 0.02^b	2.9 ± 0.01^b	61.7 ± 0.11^b	362.36 ± 0.64^b
MF_2	11.1 ± 0.04^c	18.6 ± 0.09^c	5.6 ± 0.03^c	3.2 ± 0.02^c	61.6 ± 0.11^b	364.85 ± 0.09^c
MF_3	10.7 ± 0.04^d	19.7 ± 0.10^d	5.8 ± 0.04^d	3.5 ± 0.02^b	60.5 ± 0.16^c	367.12 ± 0.12^d
SF	10.6 ± 0.05^e	9.7 ± 0.07^e	2.8 ± 0.02^e	2.1 ± 0.01^e	73.8 ± 1.84^d	358.64 ± 6.81^e
SF_1	10.3 ± 0.04^f	18.3 ± 0.07^f	4.3 ± 0.03^f	3.8 ± 0.02^f	61.5 ± 0.12^e	357.40 ± 0.22^f
SF_2	10.1 ± 0.05^g	19.7 ± 0.11^g	4.7 ± 0.12^g	4.1 ± 0.01^g	60.8 ± 1.84^f	364.25 ± 0.31^g
SF_3	9.8 ± 0.04^h	21.2 ± 0.12^h	5.3 ± 0.04^h	4.4 ± 0.03^h	56.1 ± 0.02^g	363.08 ± 0.18^h
*RV	<5	>15	10-25	<3	64	400-425

Values are means ± standard deviations of triplicate determinations. Means with different superscripts in a column are significantly different *($p<0.05$)* *RV = Recommended values (g/100g) *(CODEX CAC/GL 08. 1991): Codex alimentarius: Guidelines on formulated supplementary foods for older infants and young children. Sample MF = 100g Fermented Maize flour; Sample MF_1=90g Fermented Maize flour + 10g *M. bellicosus* Sample MF_2 = 85g Fermented Maize flour+15g *M. bellicosus;* Sample MF_3= 80g Fermented Maize flour+20g *M. bellicosus*. Sample SF = 100g Fermented Sorghum flour; Sample SF_1 = 90g Fermented Sorghum flour+10g *M. bellicosus;* Sample SF_2 = 85g Fermented Sorghum flour+15g *M. bellicosus;* Sample SF_3 = 80g Fermented Sorghum flour+20g *M. bellicosus*.

4.9 Proximate Composition of Complementary Foods

There was a significant reduction in moisture content of the formulated complementary foods, the level of reduction increasing with increasing level of inclusion of MB ($p<0.05$), Table 4.9. Significant differences also existed between the enriched complementary foods, the level of reduction in moisture content increasing with increase in inclusion level ($p<0.05$). There were significant increase in values of crude protein, fat, ash, total carbohydrates and gross energy content of the enriched maize and sorghum complementary foods ($p<0.05$), the values increasing with increasing level of inclusion of *M. bellicosus*.

Table 4.10: Proximate composition of roasted *M. bellicosus, boiled rice* (BR) and boiled yam (BY) (g/100g)

		Boiled rice (BR)	**Boiled yam (BY)**
Sample	*M. bellicosus*	(Dry)	(Dry)
Moisture	4.0±0.04	5.52±0.04	7.17±0.02
Crude Protein	31.8±0.10	3.78±0.11	5.90±0.09
Crude Fat	16.4±0.03	2.66±0.61	2.40±0.03
Ash	3.8±0.03	2.70±0.02	2.57±0.02
Total Carbohydrates	43.0±0.10	85.38±0.70	81.98±0.09
Gross Energy (kcal/)	450.7±0.00	380.50±0.00	373.12±0.00

Values are means ± standard deviations of triplicate determinations. The table compare the proximate composition of Macrotermes bellicosus, boiled rice, and boiled yam on dry matter basis

4.10: Proximate Composition of Roasted *M. bellicosus*, boiled yam and rice

The result of proximate composition of roasted *M. bellicosus,* boiled yam and boiled rice is as shown in Table 4.10. Moisture content of Roasted *M. bellicosus* was very low while the crude protein, fat, carbohydrate and gross energy content were very high. The moisture, crude protein and crude fat content of both boiled yam and rice were low, while the ash and total carbohydrates content were very high. The moisture and crude protein values of boiled yam were significantly higher than boiled rice ($p<0.05$), there were no significant differences in crude fat and ash values of the two samples. Boiled rice was significantly higher in total carbohydrate and gross energy ($p<0.05$) than boiled yam.

Table 4.11: Proximate composition of enriched rice and yam complementary foods (g/100)

Sample	Moisture	C. Protein	C. Fat	Ash	T. Carbohydrates	Gross .E
BR	5.52 ± 0.04^{a}	3.78 ± 0.11^{c}	2.66 ± 0.61^{l}	2.70 ± 0.02^{h}	85.34 ± 0.70^{c}	380.42 ± 0.00^{r}
BR$_1$	5.40 ± 0.02^{c}	11.57 ± 0.11^{z}	3.57 ± 0.03^{n}	3.33 ± 0.02^{a}	76.12 ± 0.02^{p}	382.89 ± 0.01^{r}
BR$_2$	4.58 ± 0.02^{b}	13.78 ± 0.10^{y}	3.86 ± 0.03^{c}	3.69 ± 0.02^{s}	74.09 ± 0.10^{q}	386.22 ± 0.01^{c}
BR$_3$	4.01 ± 0.03^{k}	15.31 ± 0.05^{j}	4.08 ± 0.03^{s}	4.16 ± 0.03^{d}	72.52 ± 0.22^{f}	388.04 ± 0.00^{c}
BY	7.17 ± 0.02^{j}	5.90 ± 0.09^{l}	2.40 ± 0.03^{e}	2.57 ± 0.02^{c}	81.98 ± 0.09^{k}	373.12 ± 0.00^{e}
BY$_1$	7.02 ± 0.04^{d}	7.88 ± 0.10^{k}	2.89 ± 0.03^{e}	4.06 ± 0.03^{r}	75.99 ± 0.12^{h}	361.48 ± 0.00^{s}
BY$_2$	4.03 ± 0.04^{e}	11.44 ± 0.11^{b}	5.28 ± 0.03^{m}	4.45 ± 0.03^{h}	74.80 ± 0.15^{n}	392.48 ± 0.00^{k}
BY$_3$	4.02 ± 0.02^{a}	15.26 ± 0.08^{c}	5.02 ± 0.03^{m}	4.20 ± 0.03^{h}	71.48 ± 0.10^{c}	392.14 ± 0.00^{h}
*RV	<5	>15	10-25	<3	64	400-425

Values are means ± standard deviations of triplicate determinations. Means with different superscripts in a column are significantly different *($p<0.05$)* *RV = Recommended values (g/100g) *(CODEX CAC/GL 08. 1991): Codex alimentarius: Guidelines on formulated supplementary foods for older infants and young children. Sample BR = 100g Boiled rice; Sample BR$_1$ = 90g Boiled rice + 10g *M. bellicosus* Sample BR$_2$ = 85g Boiled rice +15g *M. bellicosus;* Sample BR$_3$ = 80g Boiled rice +20g *M. bellicosus* Sample BY = 100g Boiled yam; Sample BY$_1$ = 90g Boiled yam+10g *M. bellicosus;* Sample BY$_2$ = 85g Boiled yam+15g *M. bellicosus;* Sample BY$_3$ = 80g Boiled yam+20g *M. bellicosus*.

4.11 Proximate Composition of Roasted *M. bellicosus* and Complementary Foods

There was a significant reduction in moisture content of the formulated complementary foods, the level of reduction increased with increasing level of inclusion of MB ($p<0.05$), Table 4.10. Significant differences also existed between the enriched complementary foods, the level of reduction in moisture content increasing with increase in inclusion level ($p<0.05$). There were significant increase in values of crude protein, fat, ash, gross energy and decrease in total carbohydrate of the enriched rice and yam complementary foods ($p<0.05$), the values increased with increasing level of inclusion of *M. bellicosus*.

Table 4.12: Mineral composition of roasted *M. bellicosus* and cereal flour (mg/100)

Parameter	*M. bellicosus*	MF	SF
Potassium	164.13±0.31	211.83±0.17	205.33±0.25
Sodium	98.40±0.20	52.83±0.25	48.50±0.20
Calcium	227.50±0.20	6.67±0.26	26.60±0.30
Magnesium	24.33±0.15	14.00±0.36	14.73±0.15
Phosphorus	361.30±0.20	295.50±0.30	325.43±0.25
Iron	2.07±0.25	2.61±0.30	7.61±0.03
Zinc	15.03±0.31	3.19±0.04	2.41±0.03
Manganese	2.35±0.25	0.52±0.04	0.62±0.05
Copper	5.07±0.54	1.57±0.25	0.83±0.25

MF = 100g Fermented Maize flour; SF = 100g Fermented Sorghum flour

4.12: Mineral composition of roasted *M. bellicosus* and cereal flour (mg/100)

(Table 4.11) Maize and sorghum flour are high in potassium, phosphorus, and zinc, low in sodium, and very low in calcium, magnesium, and manganese content. Maize flour was significantly higher in potassium, sodium, zinc and copper than sorghum flour ($p<0.05$), while sorghum flour was significantly higher in calcium, magnesium, phosphorus, iron and manganese than maize flour ($p<0.05$).

Table 4.13a: Mineral composition of formulated maize complementary Foods (mg/100g)

	MF	MF_1	MF_2	MF_3	*RV
Potassium	211.83 ± 0.17^a	216.47 ± 0.25^b	219.50 ± 0.30^c	223.57 ± 0.35^d	516
Sodium	52.83 ± 0.25^a	55.60 ± 0.30^b	57.70 ± 0.17^c	59.47 ± 0.25^d	296
Calcium	6.67 ± 0.26^a	7.50 ± 0.20^b	9.27 ± 0.35^c	10.60 ± 0.17^d	500
Magnesium	14.00 ± 0.36^a	15.17 ± 0.25^b	16.73 ± 0.15^c	18.20 ± 0.36^d	76
Phosphorus	295.50 ± 0.30^a	297.30 ± 0.15^b	299.30 ± 0.15^c	301.43 ± 0.15^d	456
Iron	2.61 ± 0.30^a	2.81 ± 0.20^b	3.08 ± 0.03^c	4.13 ± 0.03^d	16
Zinc	3.19 ± 0.04^a	3.49 ± 0.04^b	3.82 ± 0.04^c	4.12 ± 0.03^d	3.2
Manganese	0.52 ± 0.04^a	0.57 ± 0.02^b	0.67 ± 0.02^c	0.82 ± 0.03^d	0.60**
Copper	1.57 ± 0.25^a	2.17 ± 0.35^b	2.40 ± 0.30^c	3.13 ± 0.25^d	0.34**

Values are mean ± standard deviation of triplicate determinations. Mean value with different superscripts in a row are significantly different *($p<0.05$)*. *RV = Recommended values (mg/100g) *(CODEX CAC/GL 08. 1991) / **RDA (Sareen, Jack & James, 2009). Sample MF = 100g Fermented Maize flour; Sample MF_1=90g Fermented Maize flour + 10g *M. bellicosus* Sample MF_2 = 85g Fermented Maize flour+15g *M. bellicosus;* Sample MF_3= 80g Fermented Maize flour+20g *M. bellicosus*.

Table 4.13b: Mineral composition of formulated sorghum complementary Foods (mg/100g)

	SF	SF_1	SF_2	SF_3	*RV
Potassium	205.33 ± 0.25^{e}	209.50 ± 0.25^{f}	211.77 ± 0.15^{g}	214.50 ± 0.20^{h}	516
Sodium	48.50 ± 0.20^{e}	49.40 ± 0.30^{f}	51.43 ± 0.2^{g}	54.06 ± 0.25^{h}	296
Calcium	26.60 ± 0.30^{e}	27.60 ± 0.30^{f}	29.07 ± 0.35^{g}	31.50 ± 0.03^{h}	500
Magnesium	14.73 ± 0.15^{e}	15.50 ± 0.20^{f}	16.50 ± 0.17^{g}	18.63 ± 0.15^{h}	76
Phosphorus	325.43 ± 0.25^{e}	327.70 ± 0.17^{f}	331.37 ± 0.21^{g}	334.43 ± 0.11^{h}	456
Iron	7.61 ± 0.03^{e}	7.91 ± 0.02^{f}	8.09 ± 0.02^{g}	9.22 ± 0.02^{h}	11
Zinc	2.41 ± 0.03^{e}	2.63 ± 0.05^{f}	3.31 ± 0.03^{g}	3.92 ± 0.04^{h}	3.2
Manganese	0.62 ± 0.05^{e}	0.66 ± 0.03^{f}	0.81 ± 0.03^{g}	0.92 ± 0.03^{h}	32
Copper	0.83 ± 0.25^{e}	1.20 ± 0.30^{f}	1.90 ± 0.25^{g}	2.53 ± 0.25^{h}	160

Values are means ± standard deviations of triplicate determinations. Mean value with different superscripts in a row are significantly different ($p<0.05$). Sample SF = 100g Fermented Sorghum flour; Sample SF_1 = 90g Fermented Sorghum flour+10g *M. bellicosus;* Sample SF_2 = 85g Fermented Sorghum flour+15g *M. bellicosus;* Sample SF_3 = 80g Fermented Sorghum flour+20g *M. bellicosus*.

4.13: Mineral composition of formulated maize and sorghum complementary Foods (mg/100g)

Addition of *M. bellicosus* to fermented maize and sorghum flour (Tables (4.13a) and (4.13b) resulted in significant increase in values of the minerals ($p<0.05$) in all the formulated complementary foods, the values increasing with increase in inclusion level of *M. bellicosus*. However, the mineral content of the formulated complementary foods were lower than the recommended values by FAO/WHO. The minerals values of enriched complementary foods were significantly different ($p<0.05$) from each other for both maize and sorghum, the 10% *M. bellicosus* incorporated flours having the lowest values while 20% *M. bellicosus* incorporated flours had the highest values.

Table 4.14: Mineral composition of roasted *M. bellicosus, Boiled rice* and Boiled yam (mg/100)

Parameter	*M. bellicosus*	Boiled rice (BR)	Boiled yam (BY)
Potassium	361.13±0.31	80.04±0.00	120. 00±0.00
Sodium	98.40±0.20	30.00±0.00	90.00±0.00
Calcium	227.50±0.20	70.03±0.00	120.00±0.00
Magnesium	24.33±0.15	140.02±0.00	160.03±0.00
Phosphorus	361.30±0.20	210.01±0.00	290.01±0.00
Iron	2.07±0.25	4.20±0.04	5.60±0.03
Zinc	15.03±0.31	1.18±0.02	1.53±0.02
Manganese	2.35±0.25	0.53±0.15	0.81±0.03
Copper	5.07±0.54	0.37±0.35	0.43±0.21

Values are mean ± standard deviation of triplicate determinations.

4. 14 Mineral Composition of Roasted M. bellicosus, boiled rice and yam

(Table 4.14). Boiled rice and yam are good sources of magnesium, phosphorus and calcium, moderate in potassium, sodium, iron and zinc respectively. Boiled yam was significantly higher in all the minerals than boiled rice ($p<0.05$).

Table 4.15a: Mineral composition of boiled rice and *M. bellicosus* enriched rice complementary Foods (mg/100g)

	BR	BR_1	BR_2	BR_3	*RV
Potassium	80.04 ± 0.00^{a}	158.70 ± 0.46^{f}	170.07 ± 0.51^{g}	176.63 ± 0.51^{h}	516
Sodium	30.00 ± 0.00^{a}	102.67 ± 0.25^{f}	109.10 ± 0.36^{g}	113.63 ± 0.45^{h}	296
Calcium	70.03 ± 0.00^{a}	242.23 ± 0.50^{f}	252.13 ± 0.29^{g}	258.06 ± 0.42^{h}	500
Magnesium	140.02 ± 0.00^{a}	24.10 ± 0.40^{f}	25.93 ± 0.35^{g}	27.13 ± 0.35^{h}	76
Phosphorus	210.01 ± 0.00^{a}	359.40 ± 1.57^{f}	372.40 ± 0.30^{g}	381.76 ± 0.57^{h}	456
Iron	4.20 ± 0.04^{a}	2.40 ± 0.20^{f}	2.80 ± 0.30^{g}	3.23 ± 0.35^{h}	16
Zinc	1.18 ± 0.02^{a}	2.40 ± 0.20^{f}	16.67 ± 0.40^{g}	$17.73^{z}\pm0.35^{h}$	3.2
Manganese	0.53 ± 0.15^{a}	25.03 ± 0.12^{f}	26.17 ± 0.42^{g}	28.27 ± 0.42^{h}	0.60**
Copper	0.37 ± 0.35^{a}	4.87 ± 0.60^{f}	5.73 ± 0.35^{g}	6.53 ± 0.40^{h}	0.34**

Values are mean ± standard deviation of triplicate determinations. Mean value with different superscripts in a row are significantly different ($p<0.05$). *RV = Recommended values mg/100g) *(CODEX CAC/GL 08. 1991) / **RDA (Sareen, Jack & James, 2009). Sample BR = 100g Boiled rice; Sample BR_1 = 90g Boiled rice + 10g *M. bellicosus* Sample BR_2 = 85g Boiled rice +15g *M. bellicosus;* Sample BR_3 = 80g Boiled rice +20g *M. bellicosus*

Table 4.15b: Mineral composition of formulated yam complementary Foods (mg/100g)

	BY	BY$_1$	BY$_2$	BY$_3$	*RV
Potassium	120. 00±0.00^{e}	162.20±0.36^{f}	174.10±0.45^{g}	181.50±0.30^{h}	516
Sodium	90.00±0.00^{e}	99.70±0.50^{f}	110.63±0.42^{g}	116.00±0.36^{h}	296
Calcium	120.00±0.00^{e}	232.90±15.68^{f}	253.87±0.35^{g}	264.50±0.30^{h}	500
Magnesium	160.03±0.00^{e}	25.87^{g}±0.25^{f}	28.17±0.32^{g}	31.47^{j}±0.25^{h}	76
Phosphorus	290.01±0.00^{e}	359.43^{s}±1.06^{f}	380.20±0.87^{g}	392.83±0.31^{h}	456
Iron	5.60±0.03^{e}	2.57±0.25^{f}	3.37^{l}±0.25^{g}	4.40±0.20^{h}	11
Zinc	1.53±0.02^{e}	16.10±0.20^{f}	17.57±0.25^{g}	19.80±0.30^{h}	3.2
Manganese	0.81±0.03^{e}	25.03±0.31^{f}	27.17±0.35^{g}	30.00±0.36^{h}	0.60**
Copper	0.43±0.21^{e}	5.23±0.12^{f}	6.50±0.20^{g}	7.40±0.20^{h}	0.34**

Values are means ± standard deviations of triplicate determinations. Mean value with different superscripts in a row are significantly different ($p<0.05$) *RV = Recommended values (mg/100g) *(CODEX CAC/GL 08. 1991) / **RDA (Sareen, Jack & James, 2009). Sample BY = 100g Boiled yam; Sample BY$_1$ = 90g Boiled yam+10g *M. bellicosus;* Sample BY$_2$ = 85g Boiled yam+15g *M. bellicosus;* Sample BY$_3$ = 80g Boiled yam+20g *M. bellicosus*.

4.15: Mineral composition of formulated boiled rice and yam Complementary Foods

Addition of *M. bellicosus* to boiled rice and boiled yam (Tables (4.15a) and (4.15b) resulted in significant increase in values of the minerals ($p<0.05$) in all the formulated complementary diets, the values increased with increase in inclusion level of M. *bellicosus*. However, the mineral content of the formulated complementary foods were lower than the recommended values by FAO/WHO. The values of the minerals of enriched complementary foods were significantly different ($p<0.05$) from one another for both rice and yam, the 10% M. *bellicosus* incorporated diets having the lowest values while 20% M. *bellicosus* incorporated foods sample had the highest values.

Table 4.16a: Vitamin composition of maize flour and *M. bellicosus* enriched complementary foods (mg/100 g)

	MF	MF_1	MF_2	MF_3	*RV
β-carotene (μg/)	216.23±0.40^a	223.77±0.40^b	225.50±0.30^c	227.60±0.30^d	400
Thiamine	0.35±0.02^a	0.31±0.02^b	0.26±0.02^c	0.21±0.03^d	0.5
Riboflavin	0.12±0.01^a	0.06±0.01^b	0.04±0.01^c	0.02±0.01^d	0.5
Niacin	2.09±0.25^a	2.16±0.03^b	2.24±0.03^c	2.35±0.02^d	6
Vitamin B_6	0.72±0.04^a	0.78±0.01^b	0.83±0.02^c	0.91±0.03^d	0.5
Vitamin B_{12} (μg/)	0.18±0.03^a	0.19±0.02^b	0.27±0.03^c	0.31±0.02^d	0.9
Vitamin C	2.83±0.06^a	2.40±0.03^b	2.22±0.04^c	2.00±0.05^d	30

Values are means ± standard deviations of triplicate determinations. Means with different superscripts in a row are significantly different ($p<0.05$). Sample MF = 100g Fermented Maize flour; Sample MF_1=90g Fermented Maize flour + 10g *M. bellicosus* Sample MF_2 = 85g Fermented Maize flour+15g *M. bellicosus;* Sample MF_3= 80g Fermented Maize flour+20g *M. bellicosus*.

Table 4.16b: Vitamin composition of sorghum flour and *M. bellicosus* enriched complementary foods (mg/100 g)

	SF	SF_1	SF_2	SF_3	*RV
β-carotene (µg/)	30.23 ± 0.50^{e}	34.80 ± 0.30^{f}	37.20 ± 0.46^{g}	39.10 ± 0.46^{h}	400
Thiamine	0.43 ± 0.02^{e}	0.37 ± 0.03^{f}	0.24 ± 0.01^{g}	0.18 ± 0.02^{h}	0.5
Riboflavin	0.14 ± 0.01^{e}	$0.08^{j}\pm0.01^{f}$	0.05 ± 0.01^{g}	0.18 ± 0.02^{h}	0.5
Niacin	4.11 ± 0.03^{e}	3.63 ± 0.02^{f}	3.71 ± 0.04^{g}	3.76 ± 0.02^{h}	6
Vitamin B_6	0.49 ± 0.02^{e}	0.52 ± 0.01^{f}	0.56 ± 0.02^{g}	0.64 ± 0.02^{h}	0.5
Vitamin B_{12} (µg/)	0.27 ± 0.02^{e}	0.31 ± 0.02^{f}	0.35 ± 0.03^{g}	0.64 ± 0.02^{h}	0.9
Vitamin C	3.84 ± 0.03^{e}	3.01 ± 0.04^{f}	2.85 ± 0.04^{g}	2.67 ± 0.04^{h}	30

Values are means ± standard deviations of triplicate determinations. Means with different superscripts in a row are significantly different ($p \leq 0.05$). Sample SF = 100g Fermented Sorghum flour; Sample SF_1 = 90g Fermented Sorghum flour+10g *M. bellicosus;* Sample SF_2 = 85g Fermented Sorghum flour+15g *M. bellicosus;* Sample SF_3 = 80g Fermented Sorghum flour+20g *M. bellicosus*.

4.17 Vitamin Composition of maize and sorghum Complementary Foods

Addition of *M. bellicosus* to maize and sorghum flour (Table 4.16a and 4.16b) resulted in significant increase in β-carotene, niacin,vitamin B6, and B12 content, with significant reduction in thiamine, riboflavin and ascorbic acid content of enriched complementary foods ($p<0.05$). For the vitamins with increased content, significant increase was observed as the level of inclusion increased, while there was also significant decrease with increasing level of inclusion for vitamins with decrease in values. Vitamin B_6 content of the formulated diets were higher in value compared with their FAO/WHO recommended value. The levels of thiamine, riboflavin and vitamin C content were lower than the value recommended by FAO/WHO.

Table 4.17a: Vitamin composition of boiled rice and *M. bellicosus* enriched complementary foods (mg/100 g)

	BR	BR_1	BR_2	BR_3	*RV
β-carotene (µg/)		50.13 ± 0.31^b	$58.97^b\pm0.42^c$	61.47 ± 0.25^d	400
Thiamine	0.40 ± 0.01^a	0.29 ± 0.02^b	0.39 ± 0.02^c	0.45 ± 0.02^d	0.5
Riboflavin	0.04 ± 0.02^a	0.01 ± 0.02^b	0.02 ± 0.01^c	0.03 ± 0.02^d	0.5
Niacin	0.88 ± 0.02^a	0.61 ± 0.03^b	0.69 ± 0.03^c	0.81 ± 0.03^d	6
Vitamin B_6	0.22 ± 0.02^a	1.07 ± 0.02^b	1.19 ± 0.02^c	1.26 ± 0.03^d	0.5
Vitamin C		0.00 ± 0.00^b	0.00 ± 0.00^c	0.00 ± 0.00^d	30

Values are means ± standard deviations of triplicate determinations. Means with different superscripts in a row are significantly different ($p<0.05$). Sample BR = 100g Boiled rice; Sample BR_1 = 90g Boiled rice + 10g *M. bellicosus* Sample BR_2 = 85g Boiled rice +15g *M. bellicosus;* Sample BR_3 = 80g Boiled rice +20g *M. bellicosus*

Table 4.17b: Vitamin composition of boiled yam and *M. bellicosus* enriched complementary foods (mg/100 g)

	BY	BY_1	BY_2	BY_3	*RV
β-carotene (µg/)		56.60 ± 0.30^f	61.33 ± 0.15^g	64.5 ± 0.20^h	400
Thiamine		0.33 ± 0.02^f	0.38 ± 0.03^g	0.51 ± 0.02^h	0.5
Riboflavin		0.11 ± 0.02^f	0.14 ± 0.02^g	0.17 ± 0.02^h	0.5
Niacin		0.61 ± 0.03^f	0.69 ± 0.02^g	0.82 ± 0.02^h	6
Vitamin B_6	0.38 ± 0.04^e	1.16 ± 0.02^f	1.26 ± 0.03^g	1.33 ± 0.03^h	0.5
Vitamin C		0.44 ± 0.02^f	0.37 ± 0.03^g	0.25 ± 0.04^h	30

Values are means ± standard deviations of triplicate determinations. Means with different superscripts in a row are significantly different ($p \leq 0.05$). Sample BY = 100g Boiled yam; Sample BY_1 = 90g Boiled yam+10g *M. bellicosus;* Sample BY_2 = 85g Boiled yam+15g *M. bellicosus;* Sample BY_3 = 80g Boiled yam+20g *M. bellicosus*.

4.17: Vitamin composition of boiled rice, and yam *M. bellicosus* enriched complementary foods

Vitamin composition of boiled rice and boiled yam and *M. bellicosus* enriched complementary foods is shown in Table 4.17a and 4.17b respectively. Addition of *M. bellicosus* brought about significant increment ($p<0.05$) in β-carotene content in boiled rice and boiled. Water soluble vitamins such as thiamine, riboflavin and niacin decreased in BR_1 and $BR_{2.}$ Vitamin B_6 content of the *M. bellicosus*-enriched rice complementary foods continue to increase at all levels of addition of the insect while the addition does not improve the vitamin C content of the complementary food. In boiled yam (Table 4.3.14) the reverse is the case, as the addition of *M. bellicosus* brought about progressive improvement in all the vitamins at all levels of inclusion of the insect.

Table 4.18: Antinutritional Factors of Maize, Sorghum and Enriched Complementary Foods (mg/100 g)

Parameters	Phytate	Oxalate	Tannin	Saponin	Trypsin Inhibitors
MF	0.063 ± 0.001^{c}	0.030 ± 0.002^{w}	0.022 ± 0.000^{a}	0.076 ± 0.002^{c}	ND
MF_1	0.051 ± 0.000^{b}	0.022 ± 0.001^{e}	0.013 ± 0.000^{h}	0.064 ± 0.002^{d}	ND
MF_2	0.041 ± 0.000^{z}	0.017 ± 0.002^{r}	0.001 ± 0.000^{f}	0.051 ± 0.001^{e}	ND
MF_3	0.029 ± 0.002^{m}	0.012 ± 0.001^{t}	0.007 ± 0.001^{g}	0.039 ± 0.003^{w}	ND
SF	0.23 ± 0.00^{a}	0.13 ± 0.00^{a}	0.09 ± 0.00^{a}	0.10 ± 0.00^{a}	ND
SF_1	0.22 ± 0.00^{b}	0.12 ± 0.00^{b}	0.08 ± 0.00^{b}	0.09 ± 0.00^{b}	ND
SF_2	0.20 ± 0.00^{c}	0.11 ± 0.00^{c}	0.07 ± 0.00^{c}	0.08 ± 0.00^{c}	ND
SF_3	0.19 ± 0.00^{d}	0.11 ± 0.00^{c}	0.05 ± 0.00^{d}	0.07 ± 0.00^{d}	ND

Mean value with different superscripts in a row are significantly different ($p<0.05$). T. I = Trypsin Inhibitors ND = Not detected at milligramme level

Sample MF = 100g Fermented Maize flour; Sample MF_1=90g Fermented Maize flour + 10g *M. bellicosus* Sample MF_2 = 85g Fermented Maize flour+15g *M. bellicosus;* Sample MF_3= 80g Fermented Maize flour+20g *M. bellicosus*. Sample SF = 100g Fermented Sorghum flour; Sample SF_1 = 90g Fermented Sorghum flour+10g *M. bellicosus;* Sample SF_2 = 85g Fermented Sorghum flour+15g *M. bellicosus;* Sample SF_3 = 80g Fermented Sorghum flour+20g *M. bellicosus*

4.18 Antinutritional Factors of Maize, Sorghum and Enriched Complementary Foods

Maize and sorghum flour were very low in phytate, oxalate, tannin and saponin content, with no detectable level of trypsin inhibitors (Tables 4.18). Significant reduction in values resulted on all antinutritional factors studied as the level of inclusion of *M. bellicosus* increased ($p<0.05$).

Table 4.19: Antinutritional factors in boiled rice and yam and *M. bellicosus* enriched complementary foods (mg/100 g)

Parameters	Phytate	Oxalate	Tannin	Saponin	T.I
BR	0.01 ± 0.01^{f}	0.01 ± 0.00^{f}	0.00 ± 0.00^{n}	0.04 ± 0.00^{f}	ND
BR$_1$	0.01 ± 0.00^{f}	0.01 ± 0.00^{f}	0.03 ± 0.00^{f}	0.01 ± 0.00^{s}	ND
BR$_2$	0.01 ± 0.00^{f}	0.00 ± 0.00^{b}	0.00 ± 0.00^{n}	0.01 ± 0.00^{s}	ND
BR$_3$	0.01 ± 0.00^{f}	$0.00^{b}\pm0.00$	0.00 ± 0.00^{n}	0.01 ± 0.00^{s}	ND
BY	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00	0.05 ± 0.00^{a}	ND
BY$_1$	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	0.00 ± 0.00^{b}	0.01 ± 0.00^{b}	ND
BY$_2$	0.20 ± 0.00^{c}	0.11 ± 0.00^{c}	0.00 ± 0.00^{b}	0.08 ± 0.00^{c}	ND
BY$_3$	0.01 ± 0.00^{d}	0.00 ± 0.00^{c}	0.00 ± 0.00^{b}	0.01 ± 0.00^{b}	ND

Mean value with different superscripts in a row are significantly different ($p<0.05$). T. I = Trypsin Inhibitors;ND = Not detected at milligramme level. Sample BR = 100g Boiled rice; Sample BR$_1$ = 90g Boiled rice + 10g *M. bellicosus* Sample BR$_2$ = 85g Boiled rice +15g *M. bellicosus;* Sample BR$_3$ = 80g Boiled rice +20g *M. bellicosus* Sample BY = 100g Boiled yam; Sample BY$_1$ = 90g Boiled yam+10g *M. bellicosus;* Sample BY$_2$ = 85g Boiled yam+15g *M. bellicosus;* Sample BY$_3$ = 80g Boiled yam+20g *M. bellicosus*.

4.19: Antinutritional Factors of boiled rice, boiled yam and Enriched Complementary Foods

Antinutritional factors in boiled rice, boiled yam and M. bellicosus enriched complementary foods are shown in Tables 4.19. Boiled rice and yam were very low in all the antinutritional factors studied. *M. bellicosus* inclusion does not bring about any remarkable change in the level of some antinutritional factors, while oxalate and tannin were not detectable at 15% and 20% inclusion (BR_2 and BR_3) level. Trypsin inhibitor was not detected at any level.

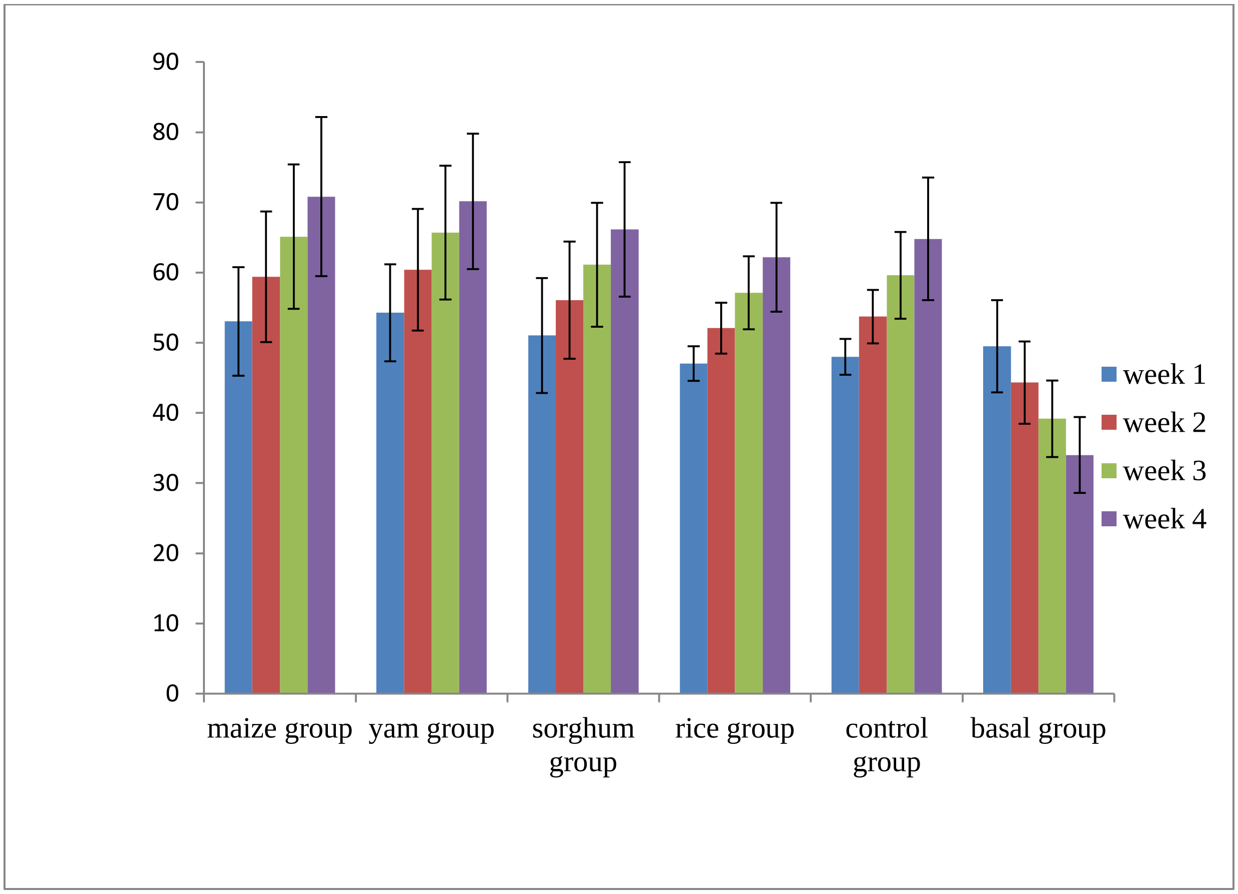

Figure 4.4.1: Growth performance of the experimental animals fed with formulated complementary foods, (M. *bellicosus* as protein source) control diet (casein as protein source) and basal diet (no source of protein) (mean values of 6 animals per group).

4.4.1: Growth performance of the experimental animals fed with formulated complementary foods.

The growth performance of the experimental animals fed with the four formulated complementary foods enriched at 20% inclusion of M. *bellicosus,* control and basal diets is shown in Figure 4.4.1. All the four complimentary diets and control diet supported rat's growth. Maize group (17.8g) and control group (16.8) recorded the highest mean weight gain. The mean weight gain in yam group, sorghum and rice groups were 15.9g, 15.2g and 15.1g respectively. Rat on basal diet continued to lose weight.

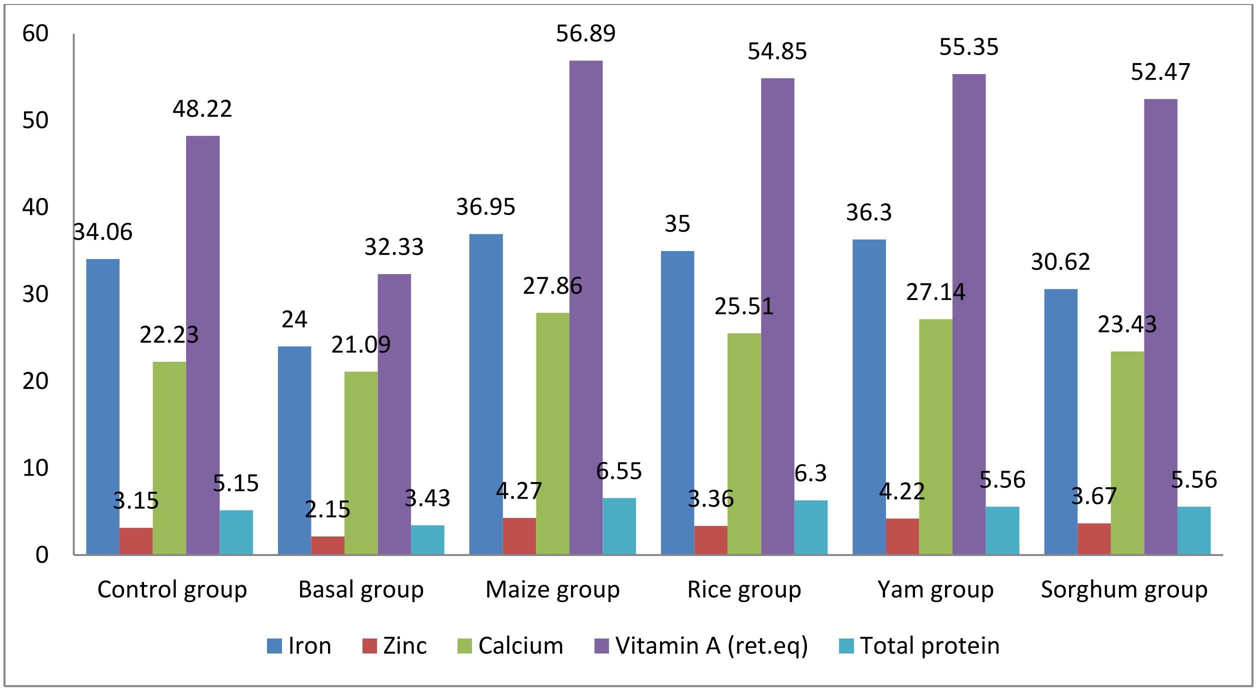

Figure 4.4.2: Mean (±SD) Bioavailability of selected minerals, vitamin and serum total protein (mg/100ml) of formulated complementary foods, group A (test diet), group B (basal diet) and group C (control diets) ($p<0.05$)

4.4.2: Bioavailability of selected minerals and vitamin in formulated complementary foods.

The serum ferritin of rats on maize, rice, and yam groups were significantly ($p<0.05$) higher than the control (Figure 4.4.2). Experimental animals in maize group (4.3mg/100ml) and yam group (4.2mg/100ml) had significantly higher serum zinc than the control, basal, rice and sorghum groups. The serum zinc values observed in the control, rice and sorghum group were not significantly different ($p>0.05$).Serum calcium was more bioavailable in maize and yam groups compared to other groups. The serum retinol level of rats fed with the formulated complementary foods was significantly higher ($p<0.05$) than those fed with control and basal diets. Total protein values observed in maize group (6.6%) and rice group (6.3%) was significantly higher ($p<0.05$) than other groups.

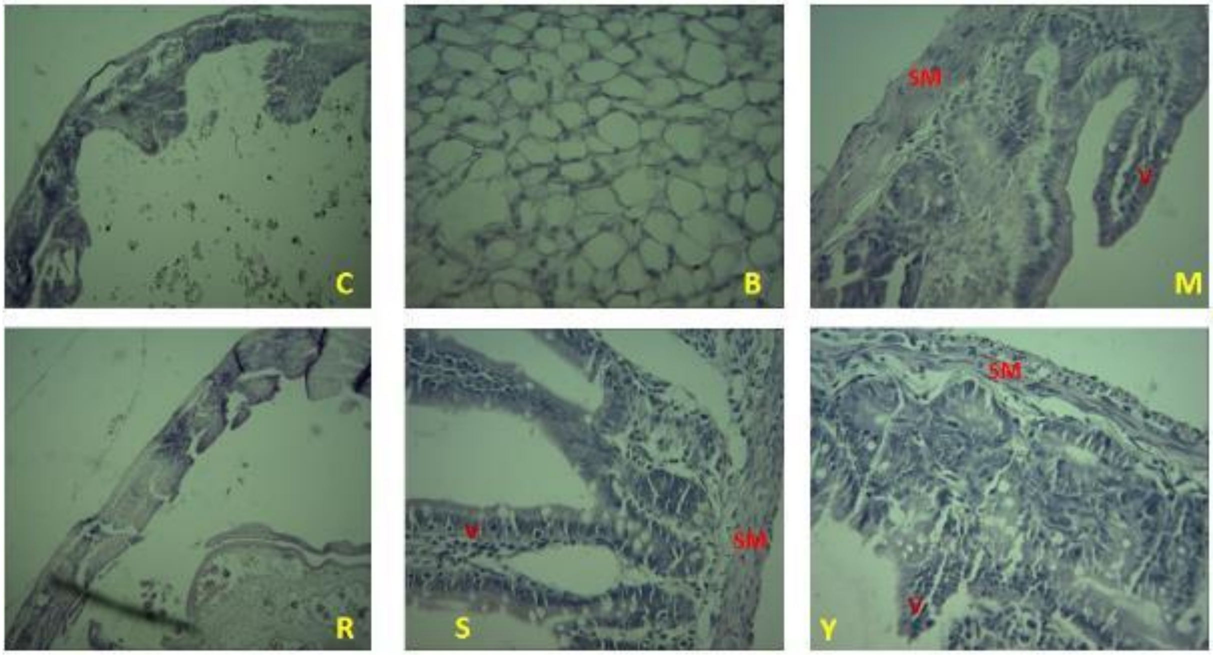

Plate 4.5.1: **Histopathology slide of the Small intestine using H&E stain.**

Control diet (C) shows normal morphology of the intestine with villus (V) and well arranges smooth muscle (SM) layer, Basal diet (B) degenerating intestine with vacuole present at the villi, maize diet (M) shows normal morphology of the intestine with villus (V) and well arranges smooth muscle (SM) layer, rice diet (R) not well processed intestine, sorghum diet (S)shows normal morphology of the intestine with villus (V) and well arranges smooth muscle (SM) layer. yam diet (Y) shows degenerating villi as the epithelium is degrading.

4.5.1: Histopathology results of the Small intestine of rats on various diets group

As shown in plate 4.5.1: The rats group on control diet demonstrated normal histopathological layer (mucosa, submucosa and musculosa) and no histopathological changes were observed. In basal diet (B) group, rats fed with diet without protein showed a degenerating intestine with vacuole present at the villi. After the feeding trial, maize (M) and sorghum (S) diets enriched with M. bellicosus 20% inclusion level showed no pathological lesion. The small intestines of rats in these diets groups show normal morphology with villus (V) and well arranged smooth muscle (SM) layer. The result for rats on rice (R) diet could not be interpreted because the intestine was poorly processed. Just like the basal group, rats fed with yam diet also show a degenerating intestine with vacuole present at the villi.

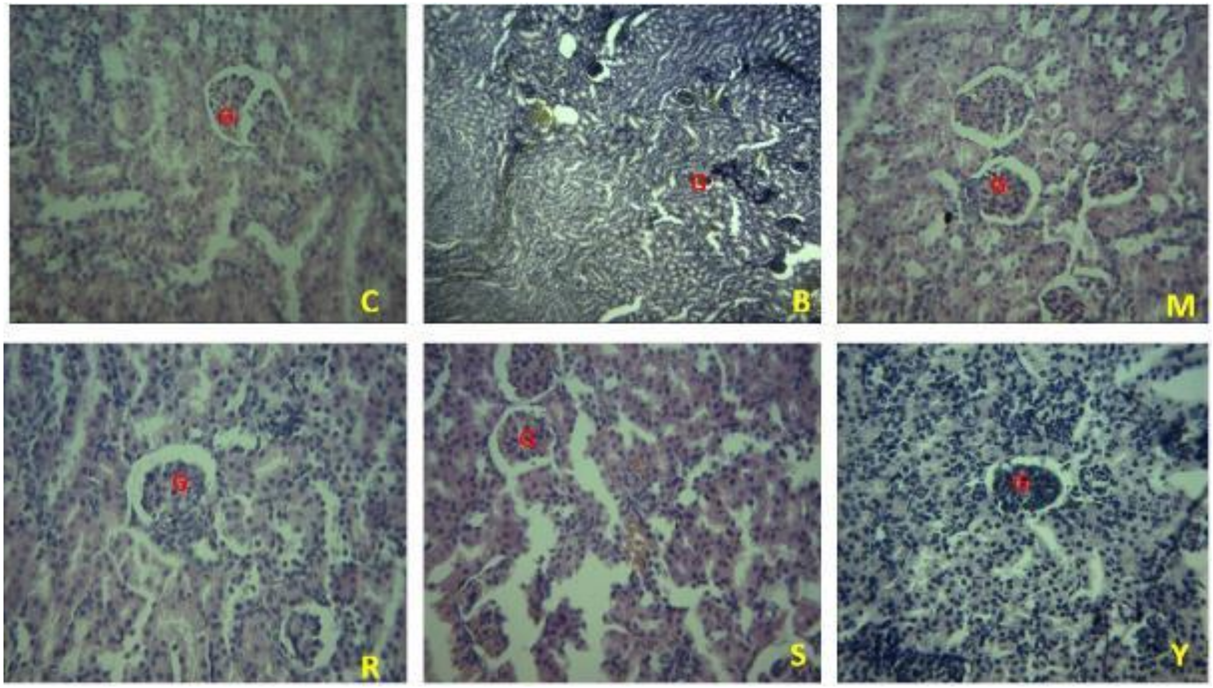

Plate 4.5. 2: **Histomorphology of the kidney of animals using H&E stain**

Control animals (C) show normal glomerular morphology (G) and tubular arrangement. B animals show slight degeneration with increase urinary space size. M animals show normal morphology like the control animals, R animals show normal morphology like the control animals while S and Y were not well processed.

4.5.2: Histomorphology of the kidney of animals

Histopathological examination of kidney sections from control group (C) (plate 4.5.2) after the feeding trial showed no histopathological changes and the normal histopathological structure of renal parenchyma. It also shows normal glomerular morphology (G) and tubular arrangement Photomicrograph of Kidney from rats of basal group indicates some histopathological changes, these included slight degeneration with increased urinary space size. Rats belonging to maize and rice group also showed normal glomerular morphology (G) and tubular arrangement just like the rats in the control group.

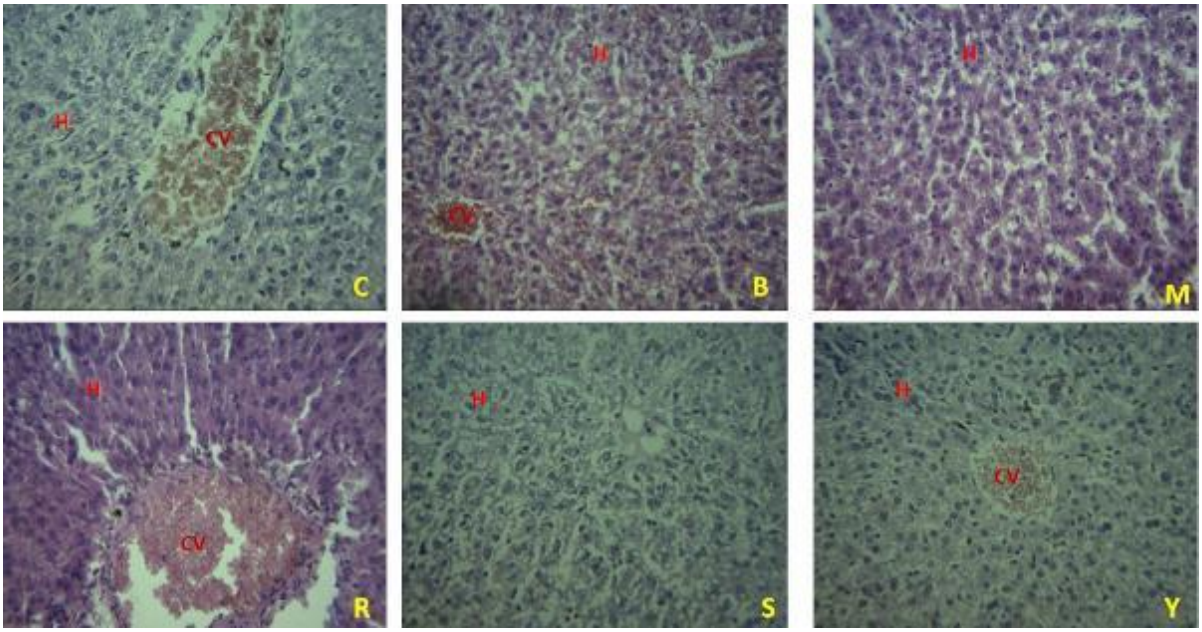

Plate 4.5.3: **Histomorphology of liver tissue of experimental animals using H&E stain**

C shows the normal morphology of the hepatocyte (H) arrangements with a well-defined central vein (CV). B shows slight different hepatocyte arrangement with active macrophage action indicating slight insult on the liver. R shows great insult on the liver as there is a loss of hepatocytes around the CV area which is radiating outward. S and Y show normal morphology like the control animals.

4.5.3 Histopathological assessment of the liver

Photomicrographs of liver from rats on different diets are illustrated in Plate 4.5.3. Liver from rats of group on control diet (plate C) and rats groups fed on maize diet, sorghum diet and yam diet (plate M, S and Y), displayed normal histopathological structure of hepatic lobules. They also show the normal morphology of the hepatocyte (H) arrangements with a well-defined central vein (CV). On the other hand, rats fed on basal diet shows slight different hepatocyte arrangement with active macrophage action indicating slight insult on the liver while rats fed rice diet show great insult on the liver as there is a loss of hepatocytes around the CV area which is radiating outward.

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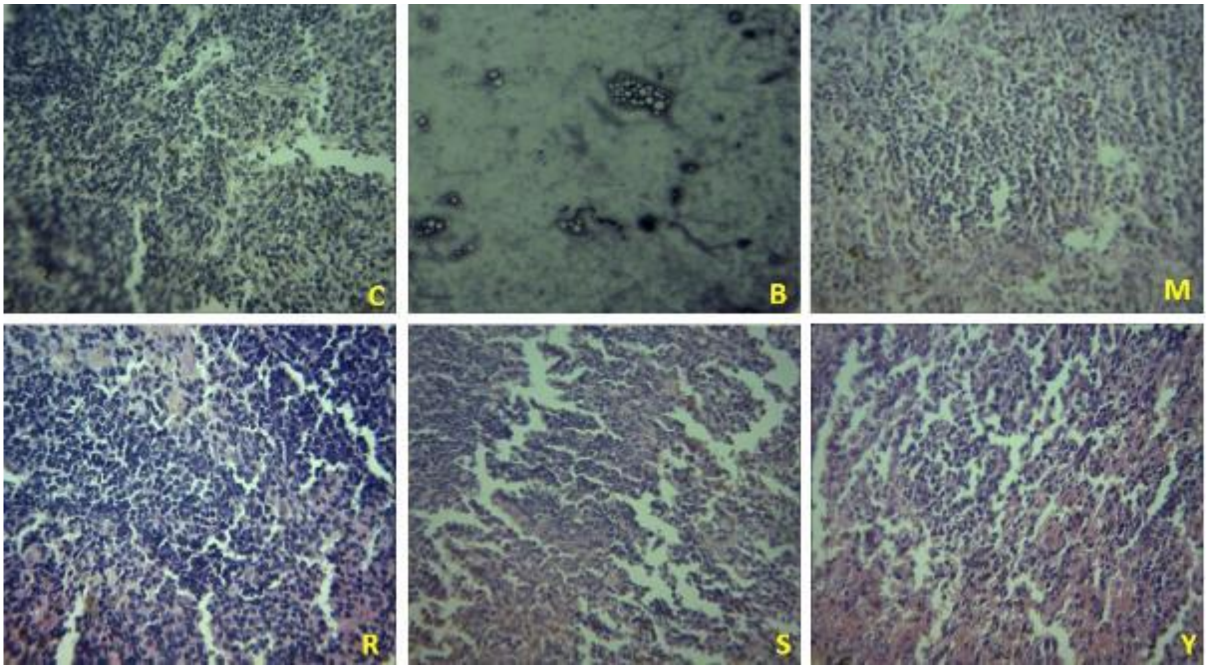

Plate 3: **Histomorphology of the spleen of animals**

Control (C) shows the normal arrangement of the lymphocytes with the branching pattern of the veins. Basal (B) is not well processed. Rice (R) group show normal morphology while Y shows irregular arrangement of the lymphocytes.

4.5.4: Histopathological assessment of the spleen

Plate 4.5.4 shows the effect of feeding control, experimental and basal diets on the lymphocytes and red to white pulp ratio in the spleen of rats. The photomicrography of rats' spleen fed with the control diet, maize and rice diets revealed no histopathological changes in their spleen, and they had normal arrangement of the lymphocytes with the branching pattern of the veins. Also, normal red to white pulp ratio was observed after the feeding trial. The rats on basal diet were poorly processed and could not be interpreted. On the other hand, the spleen of rats fed sorghum and yam diets showed irregular arrangement of lymphocytes.

Table 4.20: Sensory attributes of the formulated complementary food samples

Samples	Colour	Taste	Texture	Odour	Acceptability
MP+MB	5.83^{b}	6.57^{ab}	5.78^{e}	6.45^{d}	6.65^{c}
SP+MB	6.53^{a}	6.77^{ab}	6.76^{db}	6.90^{e}	6.84^{c}
BR+MB	6.42^{a}	6.18^{a}	5.65^{e}	6.50^{d}	6.39^{q}
BY+MB	6.61^{a}	6.63^{ab}	6.35^{db}	6.57^{d}	6.71^{c}

Values on the same column with different superscripts are significantly different $P<0.05$.*Macroterme Bellicosus* (MB), Maize pap (MP), Sorghum pap (SP), Boiled rice (BR) and Boiled yam (BY)

4.20: Sensory attributes and general acceptability of the four formulated complementary foods

The sensory attributes and general acceptability of the four formulated complementary foods is shown in Table 4.20 Boiled yam enriched with *M. Bellicosus* was rated highest in term of colour while maize pap enriched with *M. Bellicosus* has the least colour rating. There was no significant difference (*P*>0.05) between boiled yam enriched MB, boiled rice enriched MB and sorghum pap enriched MB in term of colour. Sorghum pap enriched with *M*. bellicosus has the best taste followed by boiled yam enriched with *MB.* Sorghum pap and boiled yam were the most acceptable in term of texture while boiled rice was least accepted. Sorghum pap enriched with MB was rated best in term of odour. Generally all the four formulated complementary foods were accepted, but the two most accepted were sorghum pap and boiled yam.

Table 4.21: Nutrient composition of *Macroterme bellicosus* enriched complementary foods (g100 g As consumed)

Sample	Moisture	C. P.	C.F.	Ash	T. C	G. E.
MB	3.86±0.00	31.8±0.10	16.4±0.03	3.8±0.03	43.0±0.10	450.7±0.00
MP+MB	64.30^{n}±0.04	5.92^{t}±0.11	5.54^{d}±0.03	2.35^{k}±0.04	21.78^{g}±0.02	163.31^{r}±0.01
SP+MB	63.80^{m}±0.04	5.03^{r}±0.10	6.13^{q}±0.02	2.52^{l}±0.02	22.52^{k}±0.09	168.48^{z}± 0.00
BR+MB	70.56^{c}±0.04	1.70^{h}±0.19	0.94^{a}±0.03	0.98^{s}±0.02	18.64^{p}±0.19	293.13^{s}±0.01
BY+MB	74.80^{a}±0.02	1.97^{j}±0.10	3.57^{c}±0.02	1.02^{w}±0.03	18.64^{y}±0.12	512.33^{g}±0.00

Values are means ± standard deviations of triplicate determinations. Means with different superscript on the same column are significantly different ($p<0.05$).Crude protein (C.P.), Crude fat (C.F.), Total carbohydrate (T.C.) Gross energy (G.E) *Macroterme bellicosus (MB)*, Maize pap (MP), Sorghum pap (SP), Boiled yam (BY) and Boiled rice (BR),

4.21: Nutrient Composition of the *Macrotermes bellicosus* enriched Complementary Foods

Maize pap enriched with *M.bellicosus* (MP+MB) and sorghum pap enriched with MB (SP+MB) were the best in term of crude protein, crude fat, ash and total carbohydrate. (Table 4.21). These parameters were significantly ($P<0.05$) higher in MP+MB and SP+MB than the values obtained in boiled yam enriched with *M. bellicosus* (BY+MB) and boiled rice enriched with M. bellicosus (BR+MB). The complementary foods had high moisture content. These complementary foods generally had low crude fat, protein and total carbohydrate.

Table 4.22: Mineral composition of the *Macroterme bellicosus* enriched complementary foods (mg/100 g)

Samples	Sodium	Potassium	Phosphorus	Magnesium	Manganese	Copper	Selenium (µg/)
MB	190±0.00	430±0.00	140 ±0.00	30±0.00	0.28±0.45	1.83±0.25	0.001±0.00
MP+MB	165.67^{j}±0.00	528.67^{h}±0.00	365.55^{n}±0.00	267.00^{a}±0.15	1.57^{z}±0.20	0.90^{q}±0.42	0.053^{b}±0.00
SP+MB	174.00^{k}±0.00	552.00^{p}±0.00	391.67^{t}±0.00	291.67^{a}±0.00	1.85^{g}±0.15	0.93^{e}±0.25	0.019^{z}±0.00
BR+MB	81.0^{d}±0.00	528.67^{h}±0.00	365.55^{n}±0.00	267.00^{a}±0.15	1.57^{z}±0.20	0.90^{q}±0.42	0.90^{q}±0.42
BY+MB	140±0.00	390^{z}.·00±0.00	310.00^{d}±0.00	249$^{a.00}$±0.00	1.39^{w}±0.30	0.70^{m}±0.31	0.02^{d}±0.00

Values are means ± standard deviations of triplicate determinations. Means with different superscript on the same column are significantly different *($p<0.05$).* *Macroterme bellicosus (MB)* Maize pap (MP), Sorghum pap (SP), Boiled yam (BY) and Boiled rice (BR), (g/100 g as consumed)

4.22: Mineral composition of the *Marcroterme bellicosus* enriched complementary foods

The mineral composition of the reconstituted formulated complementary foods is shown in Table 4.22. SP+MB and MP+MB gave the best values for all the minerals analysed.

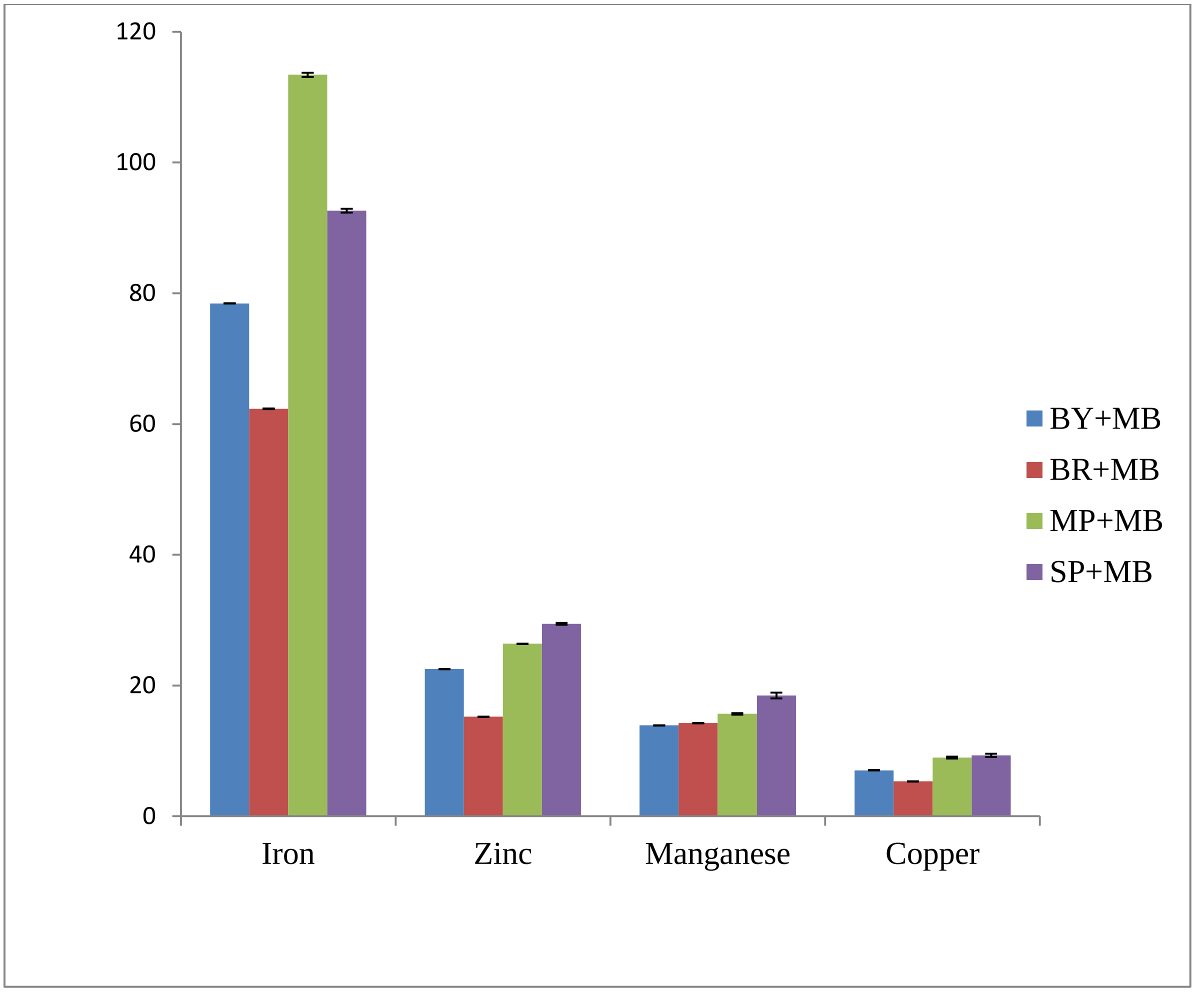

Figure 4.6.1: Mineral composition of the *Macroterme bellicosus* enriched complementary foods (mg/100 g). *Macroterme bellicosus (MB)* Maize pap (MP), Sorghum pap (SP), Boiled yam (BY) and Boiled rice (BR), (g/100 g as consumed)

4.6.4 Mineral composition of the *Macroterme bellicosus* enriched complementary foods

Maize pap enriched with MB (MP+MB) and sorghum pap enriched with MB gave the best values for the minerals, MP+MB being the richest source of iron while SP+MB gave the best values for Zinc, Manganese and copper. Boiled rice gave the least values for all these minerals.

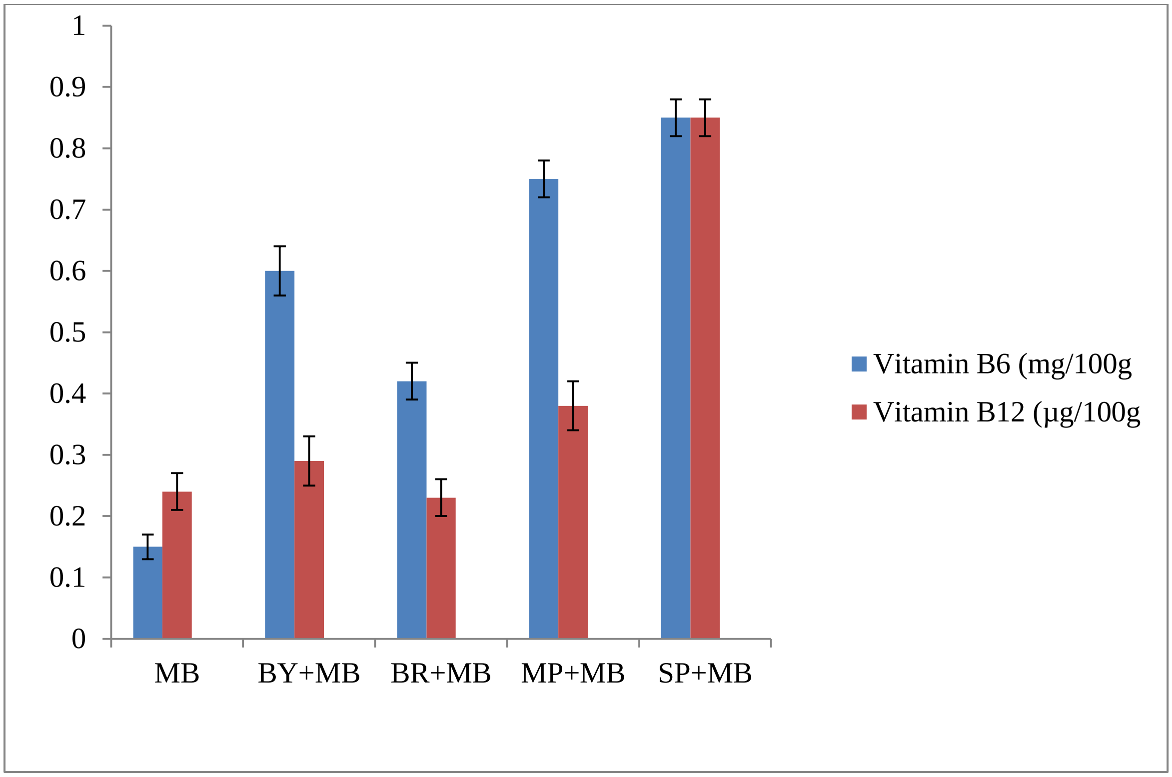

Figure 4.6.2: Vitamin B_6 and B_{12} of *Marcroterme bellicosus (MB,)* enriched complementary foods.

4.6.5: Vitamin B_6 and B_{12} of *Macroterme bellicosus (MB,)* enriched complementary foods

Figure 4.6.2 compares Vitamin B_6 and B_{12} content in *M. bellicosus* and the four reconstituted *M. bellicosus* enriched complementary foods. The individual MB enriched complementary foods was richer in vitamin B6 and B12 content than *Marcroterme bellicosus* itself. The difference between the vitamins content of MB and the four complementary foods was significant ($p<0.05$). SP+MB gave the best values for both vitamin B6 and B12 while BR+MB has the least values.

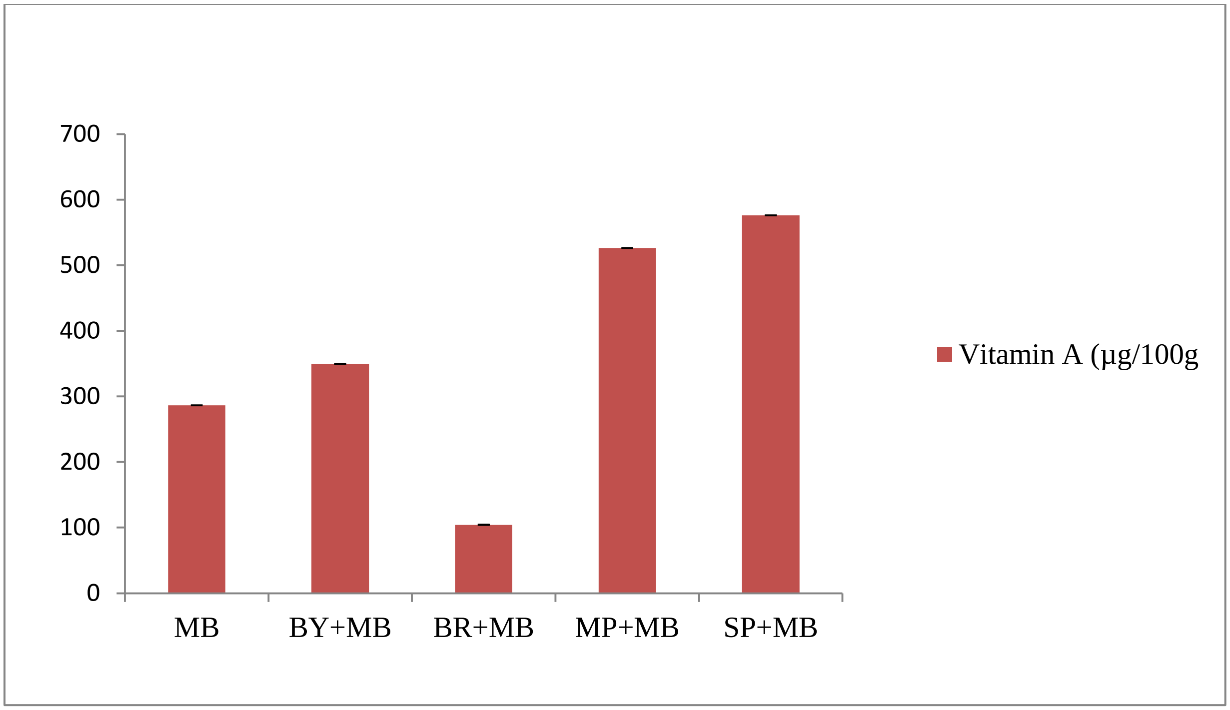

Figure 4.6.3: Vitamin A composition of *Macroterme Bellicosus (MB)* enriched complementary foods (µg/100g)

Macroterme bellicosus (MB) Maize pap (MP), Sorghum pap (SP), Boiled yam (BY) and Boiled rice (BR),

4.6.6: Vitamin A composition of *Marcroterme bellicosus (MB)* enriched complementary foods

Figure 4.6.4 compares Vitamin A content in *M. bellicosus* and the four *M. bellicosus* enriched complementary foods. The difference between the vitamin A content of MB and the four complementary foods was significant ($p<0.05$). SP+MB gave the best value for vitamins A while BR+MB has the least values.

Table 4.23: Antinutritional factors of *Macroterme bellicosus (MB,)* and enriched complementary Foods (mg/100)

Sample	Phytates	Oxalates	Tannins	Saponins	Trypsininhibitor (Tiu/100g)
MB	0.003±0.002	0.001±0.000	0.002±0.000	0.002±0.000	0.000±0.000
MP+MB	0.002^{d}±0.000	0.002^{a}±0.000	0.003±0.000	0.031^{a}±0.003	0.000±0.000
SP+MB	0.003^{d}±0.000	0.002^{a}±0.000	0.003±0.000	0.039±0.003	0.000±0.000
BR+MB	0.014^{f}±0.001	0.006^{c}±0.002	0.000±0.000	0.030^{a}±0.002	0.000±0.000
BY+MB	0.002^{d}±0.000	0.001^{a}±0.000	0.002±0.000	0.079±0.003	0.000±0.000
BR+MB	0.014^{f}±0.001	0.006^{c}±0.002	0.000±0.000	0.030^{a}±0.002	0.000±0.000

Values are means ± standard deviations of triplicate determinations. Means with different superscript on the same column are significantly different *($p<0.05$) Macroterme Bellicosus (MB)* Maize pap (MP), Sorghum pap (SP), Boiled yam (BY) and Boiled rice (BR) (g/100 g as consumed)

4.23: Antinutritional factors of *Macroterme bellicosus (MB)* and enriched complementary Foods

All the four MB enriched formulated complementary foods were very low in antinutritional factors. Trypsin inhibitor was not detected in any of the complementary foods.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

5.1.1 Socio demographic characteristics of respondents who consumed winged termites

Consumption survey of *M. bellicosus* has revealed that more female consumed the insect than male. Looking across the population distribution of those who consumed *M. bellicosus*, more teenagers followed by young adults, while the adults recorded the least number of participants who consumed the insect. Some adults held the belief that *M.bellicosus* is children's and young adult's snack. This believe was responsible for the least percentage recorded among the adult population who consumed *M.bellicosus*. Educational level or status one has attained in the society does not affect consumption of the insect. The insect was being consumed by all age groups, both illiterate and literate. The insect is well known in all the five Local Government Areas surveyed.

5.1.2 Knowledge of winged termite

The study revealed that 96.8% of the participants had perfect knowledge of Winged Termites. Participants who had consumed Winged Termites once or more times in their lifetime were 88.2%. The popularity of *M.bellicosus* in the area is as a result of the knowledge they had about the insect. *M. bellicosus* is widely accepted among the population surveyed. The result of our survey was similar to 89% participants reported by Niaba et al. (2012). This percentage is higher than those obtained by previous authors in the consumption of caterpillars in Central African Republic (85%) (N'Gasse, 2003) and in Democratic Republic of Congo (70%) (Monzambe, 2002).

5.1.3 Source of motivation for Winged Termite's consumption

The most compelling argument in favour of insects as food is their nutritional value and thus the potential to bolster food security and a balanced diet for better health (DeFoliart, 2005). About one-quarter (24.9%) of respondents reportedly consumed Termites by tradition (custom), 31.3% reported that termites are nutritious while 27.4% consume them for their flavours. Furthermore, 11.8% consumed Winged Termites by curiosity (Figure 4.1.3). The sources of motivation for M. bellicosus consumption are similar to Niaba et al. (2012) reported in their survey. According to Mbetid (2005), 50% of consumers consider the caterpillars very nutritious against 40% who preferred them for their taste.The percentages obtained for winged termites in the present study were below those obtained by Mbetid (2005)

5.1.4 Pattern of termites' consumption and methods of preparation

The termites are killed by boiling or roasting for a few minutes the morning after the swarming, and then they are sun-dried or smoke-dried, or both, depending on the weather (Silow, 1983). Sometimes they are then crushed to a mush with a pestle and motar and eaten with honey (Ogutu, 1986). Termites are fried in their own fat. This fat can also be used to fry meat (Bequaert, 1921), as by the Azande and pygmies in the D.R. Congo (Bergier, 1941). The pygmies also use the oil to treat their body and hair (Costermans, 1955). Termites can also be steamed or smoked in banana leaves (Uganda). The result of our servey revealed that 23.4% participants preferred fried termites seasoned with spices while 4.9% consume them fried without seasoning, 60.9% preferred frying the termites with little salt, 2.6% cooked it as sauce and 8.3% eat them freshly to fully benefit from their nutrients (Figure 4.1.5).

5.1.5 Mode of supply of winged termites

Various methods have been described by authors in the literature. In urban areas, they are attracted to electric light and are trapped in a receptacle with water which is placed under or near the light source. In rural areas, they are often caught at the termite mound itself. When emerging they are attracted by the light of a grass torch and then swept with a broom into a dug-out hole. In parts of the DRC a basket is put upside down over an emergence hole and the termites which cling to the bottom of the basket are then detached every few minutes by shaking the basket (Bergier, 1941). Another method is to build a dome-shaped framework of sticks or elephant grass covered with banana, Maranta leaves or a blanket to cover part of the emergence holes (Bergier, 1941; Osmaston, 1951; Roulon-Doko, 1998). In many East African towns and villages, sun-dried termites can be bought at the local market at the right season of the year (Osmaston, 1951; Owen, 1973). Our result showed that majority (85.8%) of our participants reported that they capture winged termites by attracting them with light, while few either trapped them at the entrance of termite's mounds or buy the dried or fried termites at the market.

5.1.6 Side effects upon short term consumption of winged termites

Although some insects such as *Zonocerus variegatus* in Cameroun (Barreteau, 1999) and Nigeria are considered toxic, they are still eaten. The larvae have to be prepared specially, such as by repeated cooking. The tessaratomid *Natalicola delagorguei* in Zimbabwe and South Africa excretes a pungent fluid (Bodenheimer, 1951; Faure, 1944) which can cause severe pain and even temporary blindness if it comes in contact with the eyes (Scholtz, 1984).The consumption of caterpillars with hairs containing toxic substances can be very dangerous and these have to be burned off by shaking them in a recipient with glowing coals (Tango Muyay, 1981). There have also been reports of seasonal ataxic syndrome after people consumed the silkworm *Anaphe venata* in southwest Nigeria (Adamolekun, 1993). This may occur in poorly nourished people who are marginally thiamine deficient as a result of a mainly carbohydrate diet. No literature has has reported toxicity of *M. bellicosus*. Result of this study revealed only 4.6% who reported side effect such as vomiting and stomach ache after consuming the insects. This report can be investigated further.

5.2.1 Proximate composition of roasted Macrotermes bellicosus

. The proximate nutrient composition of *Macrotermes bellicosus* is presented in Table 4.2 The result shows that roasted *M. bellicosus* was very low in moisture content. The moisture content reported in this study (4.02 g/100 was higher than moisture reported in National Food Composition Tables for Kenya (NFCT) (Sehi, 1993) for sun-dried termite consumed in western Kenya (1.70 g/100 g), but lower than 6.50-8.76 g/100 g values for four species of termites reported by Kinyuru et al (2013). The level of moisture content in any dried food is highly dependent on the drying environment among other factors. These are some of the reasons why there may have been a difference in the moisture content observed between the species.

The fat content of the termites in this study was lower than the values reported in NFCT for sun-dried termite (Sehmi, 1993) and that of Nausitermes spp. termite reported by Oyarzun et al. (1996), but higher than values reported for termite species studied by Banjo et al. (2006) in Nigeria. According to reports and analysis (Feng *et al.* 2001 a,b,c; 2000 a,b; 1999; Chen and Feng 1999; He *et al*. 1999; Lu 1992; DeFoliart 1991), during edible larvae and pupae stages, insects fat content is higher than the adult stage. Insects’ fat content values depend on insect stage of maturity, geographical location, diet and insect species. The fatty acid composition of insects appears to be influenced by the plants on which they feed (Bukkens, 2005).These factors may have contributed to the variation observed for fat contents of termites reported in this study.

Fat is an important component of the human body, storing and supplying energy as well as supporting and protecting different organs. Fat can also help in the absorption of vitamins. Phosphate, carbohydrate and cholesterol are components of many tissues and cells; combined with protein, they can form fat protein and cell membranes. Previous study show that phosphatide is good for the brain and liver, reduces blood fat, produces clean cholesterol, helps cells and skin to grow and postpones senility (Jin, 1987).

The value of crude protein reported in the present study was close to the values (33.51–39.74 g/ 100 g) observed by Kinyuru et al (2013) for four species of termites and to dried termite (35.70 g/ 100 g) reported in the NFCT (Sehmi, 1993). The protein content exhibited by the termites in this study was higher than that of raw red meats reported by Williams (2007) and therefore may offer an affordable source of protein. Other studies have reported that termites have high protein quality beneficial for human nutrition (Ramos-Elorduy et al., 1997; Verkerk et al., 2007; Kinyuru et al., 2010a), especially in an otherwise plant-dominated diet, typical in developing countries. In resource poor settings where maize is a staple food – such as Angola, Kenya, Nigeria and Zimbabwe – there are occasional widespread tryptophan and lysine deficiencies; supplementing diets with termite species like *Macrotermes bellicosus* should be a relatively easy step, as they already form accepted parts of traditional diets (Sogbesan and Ugwumba, 2008).

The ash content of the studied termite was similar to the values reported for dried termite (4.80 g/100 g) in NFCT (Sehmi, 1993). The total carbohydrate (carbohydrate and crude fibre content of the studied termites (43.0 g/100 g) was higher than both the range of values (0.72–8.73 g/100 g) reported by Kinyuru et al (2013) and for dried termite (3.50 g/100 g) reported by NFCT (Sehmi, 1993). However, carbohydrate in insects, including termites, has been reported in a highly variable range of 1.00–29.00 g/100 g of dry weight (Verkerk et al., 2007). *M. bellicosus* was very high in gross energy, and this is believed to be due to its high fat content, as fat contributes more calorie than twice the contribution of carbohydrates and proteins. The gross energy value of 529 ± 0.00 Kcal/100g is closer to 611 Kcal/100g obtained for the larva of *Anaphe venata* in Nigeria. The high protein, fat and gross energy content of the insect makes it a suitable source of dietary animal protein and energy in boosting the nutritional quality of complementary foods for infants and young children from local foodstuffs (Adepoju and Omotaya, 2014)

5.2.2 Mineral composition of fresh and roasted *M. bellicosus*

Roasted *M. bellicosus* can be a good source of dietary calcium and phosphorus which are required for strong bones and teeth by all ages (Table 4.3). It can also be a good source of zinc and copper which are required for proper enzymatic activities in the body. Zinc is required for growth, cell replication, fertility and reproduction, and hormonal activities among others (Rolfes et al., 2009, Insel et al., 2007); hence the insect can be a good source of this mineral in infant complementary foods. *M. bellicosus* can also be a source of haeme iron. However, the level of some important trace elements like iron, zinc and calcium in this study were not in agreement with previous studies on termites (Oyarzun et al., 1996; Onigbinde and Adamolekun, 1998; Christensen et al., 2006; Kinyuru et al 2013). Variability between the values obtained and the results of previous authors could be attributed to species difference as well as geographical variation. Inorganic salts and trace elements are important components of the human body. They are necessary materials to maintain normal physiological functions (Jin 1987). According to analysed results, edible insects have rich trace elements such as potassium (K), sodium (Na), calcium (Ca), copper (Cu), iron (Fe), zinc (Zn), manganese (Mn) and phosphorus (P). Many edible insects are also high in calcium, zinc and iron. Therefore, edible insects can supply necessary nutritive elements for human body functions.

The presence of selenium in *M. bellicosus* is an added advantage for its suitability for consumption by all, as selenium has been implicated in antioxidant protective characteristics, preventing lipid peroxidation (Insel et al., 2007). Its inclusion in infant complementary foods can serve as source of selenium in the foods.Of specific importance while focusing on the nutritional significance from edible insects is the potential contribution (in percentage) of its micronutrients to the diet of infants 7-12 months of age (Table 4.3). Consumption of 100g roasted *M. bellicosus* would provide Adequate Intake (AI*): zinc (500.0%), calcium (84 .3%), phosphorus (131.4%) and Recommended Dietary Allowances (RDA), iron (18.8%). Deficiencies of iron and zinc are core public health problems, especially for infant, young child and maternal health (Michaelsen et al., 2009). Reports on contents of zinc and iron in various insects generally indicate that insects are a valuable source of these minerals (Yhoungaree *et al.*, 1997; Christensen *et al.*, 2006). In addition, Christensen et al. (2006) suggests that bioavailability of these minerals from the insects is likely to be higher than from the plant foods. Therefore, cereal-based diets used for feeding infants and young children in developing countries could receive a boost with the addition of insects to the diets.

The sodium and potassium levels in the roasted *M. bellicosus* are 98.40 mg/100 g and 164.13 mg/100 g respectively, resulting in sodium to potassium ratio of approximately 0.6. This favourable sodium/potassium ratio renders the roasted *M. bellicosus a* potential component of diets for infant complimentary food

5.2.3 Selected Vitamin Composition of *M. bellicosus*

Studies on vitamins in edible insects are insufficient. Edible insects have been reported to contain vitamin A, carotene, vitamins B1, B2, B6, D, E, K, C, etc (Feng *et al.* 2001 a,b,c; 2000 a,b; 1999; Chen and Feng 1999; He *et al*. 1999; Lu 1992; DeFoliart 1991). Vitamin A content of *Macrotermes annandalei* Silvestri reaches 2 500 IU/100 gram, vitamin D reaches 8 540 IU/100 gram, vitamin E reaches 1 116.5 mg/100 gram and the vitamin C content of insect tea reaches 15.04 mg/100 gram. Edible insects are rich in vitamins for human health and nutrition. The result of this study revealed that *M.bellicosus* was a rich source of fat soluble vitamins, but low in water soluble vitamins (Table 4.5). Previous authors (Adepoju and Omotayo, 2014) confirmed that roasting of *M. bellicosus* brought about significant increase in the fat soluble vitamins and also resulted in significant reduction in water soluble vitamins (B-vitamins, as many of the water soluble vitamins are heat labile (Rolfes et al., 2009, Insel et al., 2007). The richness in fat soluble vitamins might be due to the levels of crude fat in the insect.

Antioxidants such as ascorbic acid, vitamin A (beta carotene), and tocopherol, coupled with dietary fibre have been associated with prevention of nutritionally associated diseases such as cancers, diabetes, coronary heart disease and obesity (Larrauri et al., 1996 and McDougall et al., 1996) and evidence of vitamins E and C playing a key role in decreasing the incidence of degenerative diseases is considered to be strong (Halliwell, 1997**)**. The presence of vitamins A and E in substantial amount in *M. bellicosus* coupled with selenium is suggestive of the fact that the insect possesses good antioxidant properties.

5.2.4 Fatty acid composition of *Macrotermes bellicosus*

The result presented in Table 4.4 shows that termite oil contained more polyunsaturated fatty acids (PUFA) than monounsaturated fatty acids. Saturated fatty acids account for 12.47% of the fat. However, the levels of these saturated fatty acids were low in the *M. bellicosus*. Polyunsaturated fatty acid (n-3, 52.30%) and (n-2, 24.91%) were the predominant lipid found in the insect while 7.5% were monounsaturates. Ekpo and Onigbinde (2007) found that oleic and palmitic acids are the major fatty acids in Macrotermes bellicosus termite oil. The findings of this study therefore did not correlate with their studies. Other researchers have reported significant amounts of linolenic and linoleic acids (DeFoliart, 1991).

Polyunsaturated to Saturated fatty (PU/SA) ratio under 0.20 has been associated with high cholesterol level and high risk of coronary heart disorders (Mann, 1993). The insects had PU/SA ratios above 6.20. Oyarzun et al. (1996) reported a PU/SA ratio of 0.20 for Nasutitermes spp. of termite from Venezuela, while Ekpo and Onigbinde (2007) reported a PU/SA ratio of 0.80 for a Macrotermes spp. of termite from Nigeria. The PU/SA ratios of >0.20 for any food items suggest that such food can be associated with a lower risk for certain coronary heart diseases.

5.2.5 Antinutrient composition of roasted *Marcrotermes bellicosus*

The insect was very low in antinutritional factors studied (Table 4.6). The levels of phytate, oxalate, saponins and tannins were highly insignificantly small since they are majorly found in foods of plant origin. The level of trypsin inhibitor is very low and cannot constitute any malabsorption or bioavailability of proteins from other sources. This result also corroborated the result of Adepoju & Omotayo (2014).

5.2.6 Mean feed intake, weight change, and protein efficiency ratio of rats

The conspicuous weight loss of rats fed the basal diet confirmed the absence of protein in their diet, while the significant weight gain in the experimental and control groups indicated that these rats' diets contained protein of high biological value, which is usually of animal origin,which is required for proper growth and development of infants and young children (Roth &Townsend, 2003).

The rats in the basal diet group had the least feed consumption index (CI), growth rate (GR) and efficiency of conversion of ingested feed (ECI). This could be attributed to a lack of protein in their diets. Protein rich diets are more appealing and palatable (Roth &Townsend,

2003). However, the experimental and control diets were relished by the rats in these groups, and their consumption indices were significantly higher than that of basal diet group ($p<0.05$), confirming that protein-containing foods are attractive and palatable. The higher CI and GR values of the control diet did not translate to significantly different ECI between the experimental and control groups, but rather, the higher ECI for experimental group led to significantly higher weight gain in experimental group compared with control group.

The comparatively higher mean weight gain in the experimental rat group above that of control group is an indication that M. bellicosus protein is of high biological value and is bioavailable for the rats' growth. The level of protein in M. bellicosus coupled with its high gross energy qualify it as source of essential integral part of complementary foods, hence, its inclusion in locally formulated complementary foods will reduce the prevalence of protein-energy malnutrition among the vulnerable population.

5.2.7 Serum micronutrient bioavailability of rats

The mean serum calcium, iron, zinc, and vitamin A content of rats fed the experimental diet were significantly higher than both basal and control groups ($p<0.05$), showing the contribution of M. bellicosus to these micronutrients. The contribution of M. bellicosus to rat serum level of these micronutrients was an indication that these micronutrients were bioavailable in rats, and by extrapolation, will be bioavailable in humans at the 15% inclusion level (Sanchez-Muniz et al.,

5.3.1 Proximate Composition of Fermented Maize and Sorghum Flours and *MB-enriched* Complementary Foods

The result of proximate composition of fermented maize and sorghum flour is as shown in Table 4.8 and 4.9. The moisture and crude fat content of the two cereal flour were low; the crude protein value was moderately high while the ash and total carbohydrates content were very high. The moisture and crude fat values of the maize flour were not significantly different from that of sorghum, while sorghum was significantly higher in crude protein ($p<0.05$) but insignificantly higher in ash, total carbohydrates and gross energy compare with maize flour.

The values of moisture, crude protein, fat total carbohydrate and gross energy of fermented maize and sorghum flours were similar to the ones reported in the literature (Adepoju & Daboh, 2013). The fermented flours were low in moisture and fat content, which is an indication that they can be kept for a period of time before they go bad. They were moderate in protein content compared with that of other plant-based staples used for complementary foods such as yam and rice (Adepoju, 2012; Otegbayo et al., 2001). However, the two fermented flour were high in ash, total carbohydrates and gross energy content. The high values of total carbohydrates and gross energy content underscore the reason why they are used as basic staples for locally made complementary foods.

The reduction in moisture content of the *M. bellicosus* enriched complementary foods was similar to the trend reported by Adepoju and Daboh (2013) for *Cirina forda* powder enriched maize and sorghum complementary foods. The lower moisture content in the enriched complementary foods was an indication that the dried form of the enriched flour can keep long before use. The increase in protein, fat, ash, total carbohydrate and gross energy content of enriched complementary foods is an indication that addition of *M. bellicosus* resulted in significant improvement in the nutrient content of the formulated foods. The same trend of increase in nutrient content of the enriched flour was observed by Adepoju and Daboh (2013) in *Cirina forda* powder enriched fermented maize and sorghum flour. Inclusion of *M. bellicosus* powder in enriching locally formulated cereal-based complementary foods seems to be a promising way of increasing the protein content of animal origin and gross energy content of the complementary foods. Feeding infants and young children with the *M. bellicosus* enriched cereal-based complementary foods twice daily will likely provide the greater part of their recommended value of macronutrient needs on daily basis, especially at 20% level of inclusion.

5.3.2 Proximate Composition of *M. bellicosus*, boiled yam, boiled rice and Complementary Foods

The low moisture content of roasted *M. bellicosus* underscores it high value of dry matter content, and hence high value of macronutrients. The value obtained for the macronutrients of the insect was very similar to those reported for roasted *M. bellicosus* in the literature (Adepoju (2016), and within the range stated for termite (*Trinervitermes germinates,* Afiukwa *et al*., 2013). The protein content exhibited by the termites in this study was higher than that of raw red meats reported by Williams (2007) and therefore they may offer an affordable source of protein, while the value for moisture was similar to that of Adepoju & Omotayo (2014). Generally, the insect is rich in crude protein, crude lipid, ash, total carbohydrates and gross energy. The high values of the macronutrients of the insect can contribute significantly to macronutrients of infant complementary foods.

The values of moisture of the cooked rice and yam (dry matter basis) was low, however, these values are closer to the recommended norms (≤5 g) (Table 4.3.4). Food commodities which are intended to be used in the preparation of dry weaning foods should be properly dried to reduce eventual development of populations of bacterial and mould, and then only small quantities should be prepared at a time to avoid prolonged storage (WHO, 1998; Solomon, 2005). The low moisture content of the raw material may aid the storage quality of the formulated complementary foods, because low moisture content in foods will reduce microbial growth.

The crude protein, fat, and ash of the cooked yam and rice were below the recommended value (CODEX CAC/GL 08. 1991; Adepoju, 2012; Otegbayo et al., 2001)) (Table 4.3.3). The foods may not meet the RDA of infant (0-6months) without being supplemented with other animal sources. However, both cooked rice and yam were high in total carbohydrates and gross energy content. The high values of total carbohydrates and gross energy content underscore the reason why they are used as basic staples for locally made complementary foods.

The reduction in moisture content of the *M. bellicosus* enriched complementary foods was similar to the trend reported by Adepoju and Daboh (2013) for *Cirina forda* powder and Adepoju and Ajayi (2016) for *M. bellicosus* of enriched maize and sorghum complementary foods. The low moisture content of the raw material may aid the storage quality of the formulated complementary foods, because low moisture content in foods will reduce microbial growth. Addition of *M. bellicosus* resulted in significant increase in protein, fat, ash, total carbohydrate and gross energy content of enriched complementary foods. Boiled yam and rice enriched with M. bellicosus met the recommended values for protein, ash, total carbohydrate and supply about 50% crude fat requirement (CODEX CAC/GL 08. 1991). Feeding infants and young children with the *M. bellicosus* enriched complementary foods twice daily will likely provide the greater part of their recommended value of macronutrient needs on daily basis, especially at 20% level of inclusion.

The high energetic value of the formulated foods can be explained by the high content of carbohydrates and lipids. The FAO and WHO have recommended that foods fed to infants and children should be energy-dense ones (FAO/WHO, 1998). According to the recommendation, this is necessary because low-energy foods tend to limit total energy intake and the utilization of other nutrients. Energetic diets are necessary for children to cover their need considering the size of their stomachs (Solomon, 2005). High nutrient density is also a desirable characteristic in flours that are used as a base for infant food formulation

5.3.3 Minerals Composition of Fermented Maize and Sorghum Flour and MB-enriched Complementary Foods

The fermented maize and sorghum flour were high in potassium and phosphorus, moderate in iron but low in sodium, zinc, manganese and copper, and very low in calcium and magnesium in comparison with their daily requirements. The very low values of essential macro and micro minerals explains the inadequacy of these basal staples being used for complementary foods with little or no source(s) of calcium and complete protein, hence they are usually high in energy but low in other essential nutrients. Use of this type of complementary foods will result in undernutrion and growth failure in old infants and young children being fed with this kind of foods. Addition of *M. bellicosus* powder at various levels of inclusion improved the values of all the minerals in fermented maize and sorghum flour significantly, showing that its inclusion will be beneficial to locally formulated cereal-based complementary foods, especially at 20% w/w level of inclusion. Formulations with 20% inclusion level of the insect gave the highest nutrient content for the formulated complementary foods, and hence are the best. A high intake of potassium has been reported to protect against increasing blood pressure and other cardiovascular risk (Insel et al., 2007). Feeding infants and young children the enriched maize and sorghum complementary foods will supply greater percentage of daily mineral needs, except calcium and magnesium. However, milk, which is a rich source of calcium is expected to be fed to the infant and young child on complementary feeding, hence the low value of calcium in this enriched foods should not be a barrier to its adoption.

The recommended daily allowance (RDA) of iron for children aged 1-3years old is 7 mg/day (Faber et al., 2008). According to (Codex Alimentarius, 1991), complementary food which satisfied two third of minerals and/or vitamins RDA is acceptable. The iron content of the formulated diets ranged between 2.81 and 4.13 mg per 100gm which fulfilled the minimum RDA for children aged 1-3yeear, but this may not meet the requirement for infants between ages 6 and 11 months old based on the recommended value. The amount of zinc in the enriched foods meets the requirement for infants at age 6-11 months and 1-3 years.

5.3.4 Mineral Composition of Roasted M. bellicosus, boiled rice, boiled yam and formulated Complementary Foods

Boiled rice and yam are good sources of magnesium, phosphorus and calcium, moderate in potassium, sodium iron and zinc respectively (Table 4.10). Boiled yam was significantly higher in all the minerals than boiled rice ($p<0.05$).

Addition of *M. bellicosus* to boiled rice and boiled yam (Tables 4.15a and 4.15b) resulted in significant increase in values of the minerals ($p<0.05$) in all the formulated complementary diets, the values increased with increase in inclusion level of *M. bellicosus*. However, all the formulated diets at levels of inclusion of *Marcrotermes bellicosus* met the recommended values for zinc, manganese and copper. (FAO/WHO 1991).Intake of the formulated complementary foods twice daily will satisfied requirement for calcium and phosphorus, while daily intake of the formulated diets 2-3times at 20% inclusion of the insect will meet the requirement for iron. Zinc is an essential mineral, vital to human metabolism, growth and immune function (Aggett and Comerford, 1995). Zinc deficiency may significantly contribute to growth stunting in young children.; such that what has been termed Protein Energy Malnutrition especially low height-for-age, may be due to poor diet quality including low levels of bioavailable zinc rather than an inadequate quantity of either protein or energy. Mild to moderate zinc deficiency may present clinically as impaired growth, which have previously been attributed to other factors (Brown et al., 1998).

Iron deficiency is the most common cause of anemia although other nutrition and non-nutrition related cause can be involved in the origin of anemia. Hallberg and Rossander (1984) reported that anemia is most prevalent in children between 6 and 24 months of age and the major causes are inadequate dietary intake of bioavailable iron, malaria and parasitic infections. Infants with iron-deficiency anemia are easily fatigued, are more irritable, and have shorter attention spans. They also do less well in tests of psychomotor development during later childhood than those who are not iron-deficient in infancy (Lozoff et al., 1991). Fernandez et al., (2002) reported that the prevalence of anaemia is higher during infancy and early childhood than at any other time in the life cycle. The high prevalence is consistent with data showing dietary iron to be inadequate and of low bioavailability in most complementary foods (Lutter, 2003).

5.3.5 Vitamin Composition of Fermented Flour and Enriched Complementary Foods

The fermented maize flour was high in β-carotene content while that of sorghum flour was very low (Tables 4.16a and 4.16b). The fermented flour were however low in all the water soluble vitamins except vitamin B_{12}. The low level of these vitamins is believed to be due to the extent of soaking of the fermented sample, as it has been reported that soaking food samples for a period of time leads to leaching of the water soluble micronutrients into the soaking water (Adepoju et al., 2010, Adepoju, 2012). Addition of *M. bellicosus* powder led to remarkable increase in the β-carotene content of the enriched foods, the increment being due to the vitamin A content of the insect, which has been previously reported to be a good source of vitamin A (330.42±0.12 μg/100g, Adepoju & Omotayo, 2014).

Vitamin A is an antioxidant which prevent cells from damage by free radicals, essential for maintaining healthy eyes and skin, needed for normal growth and reproduction, promote healthy immune system and prevention of infections (Rolfe et al., 2009; Roth & Townsend, 2003). Enriched maize complementary foods will supply substantial amount of vitamin A to daily requirements of infants and young children, whereas, the enriched sorghum ones will require a rich source of vitamin A to be able to meet the nutritional needs of the children. Significant reduction observed in most of the water soluble vitamins of the enriched complementary foods was due to lowering of the fermented maize vitamins by the very low levels of water soluble vitamins of the insect (Adepoju & Omotayo, 2014). Only vitamin B6 daily requirement can be met by the complementary foods, hence, other sources of meeting the other water soluble vitamins are needed to augment the one in the complementary foods.

5.3.6 Vitamin Composition of boiled rice, boiled yam and formulated Complementary Foods

The boiled yam and rice were moderate in β-carotene content. Roots and tubers are not rich sources of fat soluble vitamin. Most roots and tubers contain only negligible amounts of beta carotenes. Some yam varieties have beta-carotene in quantities of 0.14-1.4mg per 100g (Murtin and Ruberte, 1972). Vitamin C is found in appreciable amount in the boiled yam, but addition of *Marcrotermes bellicosus* tend to reduce its level in the *Marcrotermes bellicosus* enriched complementary foods. Vitamin C occur in appreciable amounts in several root crops and when correctly prepared can make a significant contribution to the vitamin C content of the diet. Yam contains 6-10mg of vitamin C per 100gm (Murtin and Ruberte, 1972). The high vitamin C concentration in some root crop may help to render soluble the Iron and make it more available than in cereals and other vegetable.

The two samples were low in water soluble vitamins. Cooking of the samples may have reduced the level of these vitamins probably as a result of leaching of the water soluble micronutrients into the cooking water (Adepoju et al., 2010, Adepoju, 2012). Addition of *M. bellicosus* powder led to remarkable increase in the β-carotene content of the enriched foods, the increment being due to the vitamin A content of the insect, which has been previously reported to be a good source of vitamin A (330.42±0.12 μg/100g, Adepoju & Omotayo, 2014).

Vitamin A is an antioxidant which prevent cells from damage by free radicals, essential for maintaining healthy eyes and skin, needed for normal growth and reproduction, promote healthy immune system and prevention of infections (Rolfe et al., 2009; Roth & Townsend, 2003). The enriched boiled yam and rice will meet recommended value for vitamin B6 (CODEX CAC/GL 08. 1991). Alternative sources of water soluble vitamins must be included as the levels in the formulated complementary foods will not meet the daily recommended value. Only vitamin B6 daily requirement can be met by the complementary foods, hence, other sources of meeting the other water soluble vitamins are needed to augment the one in the complementary foods.

5.3.7 Antinutrient Composition of Fermented Flour and Enriched Complementary Foods

The fermented flours were low in all the antinutrients studied. This is believed to be due in part to the fermentation of the grains, as processing has been found to reduce the level of antinutrients in foods (Adepoju & Adeniji, 2008; Adepoju et al., 2010). The flour did not contain trypsin inhibitors at the mg/100g level of detection. The reduction observed in the antinutrient content of formulated complementary foods as the level of inclusion of *M. bellicosus* powder increased was believed to be as a result of very low level of these antinutrients in the insect (Adepoju & Omotayo, 2014). Inclusion of *M. bellicosus* powder is therefore beneficial in reducing the level of antinutrients in complementary foods. The level of the antinutrients were very low and negligible, and hence, cannot constitute any hindrance to nutrient bioavailability in the enriched complementary foods.

5.3.8 Antinutrient Composition in boiled food samples and Enriched Complementary Foods

The results show that both boiled rice and yam and *Marcrotermes bellicosus* enriched samples were low in all the antinutrients studied. Studies have shown that most of the phytic acid (90%) is localized in the germ in maize and in the outer aleurone layer in rice and wheat (O'Dell et al, 1972). Hence, degerming maize flour, refining wheat flour, and polishing rice, removes most of the phytic acid. However, it also removes some essential nutrients. Foods based on starchy roots, tubers and sago with no added legumes have a much lower phytic acid content (Gibson et al., 1998). Addition of *M.bellicosus* to the samples did not increase the level of antinutritional factors in the formulated diets. The level of antinutrients were very low and slight and hence, cannot constitute any hindrance to nutrient bioavailability in the enriched complementary foods.

5.4.1 Growth performance of the experimental animals fed with formulated complementary foods

The weight gain observed in rat groups fed with the four formulated diets enriched with *M. bellicosus* confirmed that *M. bellicosus* protein complement the protein content of the four formulated complementary foods adequately. Enriching locally made complementary foods with *M. bellicosus* will yield complementary foods of higher nutrient density because *M. bellicosus* protein is of high biological value (Adepoju, 2016). The weight gained in maize and control groups were significantly higher than rats in sorghum, yam, rice and basal group ($p<0.05$). These differences in weight gain between the four experimental diets (i.e maize, sorghum, yam and rice) may be due to variation in the level of feed intake, which indirectly may be due to the texture and taste of the foods. Food intake is primarily influenced by factors such as taste, smell and texture Naim et al (1977).

As expected, the obvious weight loss of rats fed the basal diet established the absence of protein in their diet, while the significant weight gain in the experimental and control groups indicated that that these rats' diets contained protein of high biological value, which is indispensable for proper growth and development of infants and young children (Roth &Townsend, 2003).

5.4.2 Bioavailability of selected minerals and vitamin in formulated complementary foods, basal diet and control diets

The serum micronutrients level such as iron, zinc, calcium and vitamin A in rats groups fed the four experimental diets were significantly higher than the level in rat group fed basal diets ($p<0.05$), confirming the contribution of *M. bellicosus* to serum micronutrients in the experimental groups. These results has demonstrated that addition of M. bellicosus to complementary foods at 20% inclusion level will improve the bioavailability of the important micronutrients of public health importance such as iron, vitamin A, and zinc. Micronutrient needs are high during the first 2 years of life to support the rapid growth and development during this period. Iron and zinc are among micronutrients whose daily requirements' are difficult to meet with plant-based complementary foods alone (Dewey 2005). Complementary food diets need to contain foods rich in these nutrients (generally animal-source foods), or be fortified in some way (Dewey 2005). Micronutrients are essential for growth, development, and prevention of illness in young children (WHO, 2009). Adequate intakes of micronutrients, such as iron, zinc, and calcium, are important for ensuring optimal health, growth, and development of infants and young children (Caballero *et al*., 2005 and Rolfes *et al*., 2008)

5.5 HISTOPATHOLOGICAL ASSESSMENT OF RATS' ORGANS

Organs such as small intestine liver, kidney and spleen were examined by histological approach and the photomicrographs of hematoxalin and eosin stained, specimens were illustrated in plate (4.5.1- 4.5.4).

5.5.1 Histopathological assessment of small intestine

Rats on different diet groups were fed control diet (lactalbumin standard protein 20%), four experimental diets (M. bellicosus protein 20%) and basal diet (no protein source). The normal histopathological layer (mucosa, submucosa and musculosa) observed in control, maize and sorghum diets confirmed that inclusion of *M. bellicosus* in complementary food will not induce any undesirable effect on the small intestine. However, the degerating intestine observed in rat group fed yam diet enriched with M. bellicosus needs further investigation.

5.5.2 Histopathological assessment of Kidney

The present results show the effect control diet and experimental diets on kidney morphology, it demonstrated that both the control and the experimental diets will not alter the kidney morphology. The results also show that intake of diet without protein for a period of time can have negative effect on kidney morphology**.** The kidney is an excretory organ that removes metabolised and nonmetabolised toxic materials from the body (Robbins et al., 1985); hence this organ would be exposed to high concentrations of the noxious materials that could have caused the lesions. This result has confirmed that *M.bellicosus* was not nephrotoxic to the kidney.

5.5.3 Histopathological assessment of the liver

Liver represents a suitable model for monitoring the effects of a diet, due to its key role in controlling the whole metabolism. The changes in the liver, as a site responsible for biotransformation and detoxification, suggest alterations in the metabolic processes. The liver, being the first organ that encounters all absorbed materials from the gastrointestinal tract, has been shown to respond to toxicological insults in a number of ways including cellular degeneration and necrosis, bile duct hyperplasia and fibrosis (Jubb et al., 1995). The effect of feeding rats with different diets enriched with M. bellicosus at 20% inclusion has further demonstrated that M. bellicosus is safe for consumption as there is no pathological lesions observe in control, maize, sorghum and yam diets. Basal diet shows slight different hepatocyte arrangement with active macrophage action indicating slight insult on the liver; this may be due to absence of any protein source in their diet. However, insult observed on the liver of rats on rice diet may be due to contamination of either their feed or the water, further investigation may be required to confirm this result.

5.5.4 Histopathological assessment of the spleen of rats

The anatomy of the spleen is complex, with its open-structured red pulp and interwoven branches of white pulp, separated by the marginal zone. White pulp is involved in the crucial function of developing specific immune responses and is densely populated with lymphocytes. Red pulp on the other hand has open connections with the bloodstream and is involved in the clearance of bacteria and the removal of abnormal and senescent blood cells (Kraal, 1992). The result of this study has revealed that rats' spleen fed with the control diet, maize and rice diet reveal no histopathological changes in their spleen, and they also show the normal arrangement of the lymphocytes with the branching pattern of the veins. Also, normal red to white pulp ratio was observed after the feeding trial. The further confirmed that both the control diet and all the experimental diets will not encourage the growth unpleasant bacteria when consumed if properly prepared.

Histopathological evaluation of the small intestine, liver, kidney and spleen showed that *M. bellicosus* had no toxic effects on these organs, but instead, appeared to be beneficial in complementing locally formulated diets thereby supporting the growth of experimental rats. This discovery is novel as the literature seems to lack such information, and it suggests that *M. bellicosus* may be a promising animal source of protein and important micronutrients with no negative histopathological effect on the organs.

5.6.1 Maternal food acceptability

Results of maternal evaluation of the foods are reported in Table 4.6.1. The scores for MP+MB (5.83) was the least of all the four food. The reason being that addition of MB changed the appearance of the pap from the usual white appearance to dark brown colour. Addition of MB also affects the texture of SP+MB and MP+MB, their scores being 5.65 and 5.78 respectively sho that they are less preferred in term of texture compare to BY+MB and SP+MB. So, in term of appearance and texture, there is a significant difference between SP+MB and MP+MB ($P>0.05$). The study revealed that all the foods prepared were moderately liked by the nursing mothers agreed with previous observations that mothers often find complementary foods processed from local foodstuffs attractive (Mensa and Tomkins, 2003; Owino et al., 2007).

5.6.2 Nutrient Composition of the *Macrotermes bellicosus* enriched Complementary Foods

The nutrient composition of the complementary foods as shown on Table 4.6.2 revealed that the complementary foods have high moisture content. This as a matter of course means that the infants will need to consume a large quantity of the food to meet up their nutritional requirements. However, this may not be possible considering their stomach size, which is small. The high moisture content of the local diets may also affect the storage quality of the foods. High moisture content in foods has been shown to encourage microbial growth (Temple et al., 1996). This is an important consideration in local feeding methods in Nigeria because some mothers may want to prepare large quantities of infant foods and keep in containers to avoid frequent processing, in order to have spare time and energy for other domestic activities (Ogbonnaya et al., 2012).

The protein contents of the complementary foods varied considerably with low levels of protein found in BR+MB (1.70±0.19 g/100g), BY+MB (1.97±0.10 g/100g), to a moderate level found in MP+MB (5.92±0.11 g/100g) and SP+MB (5.03±0.10 g/100g). Maize pap enriched with *M. bellicosus* (MP+MB), however had the highest protein content. If these meals are consumed 3-4 times daily, the infants will be able to meet their protein requirement of 14mg/day (WHO/UNICEF, 1998) except for the BR+MB and BY+MB. The result of our study as expected, show an improve in protein content in maize pap and sorghum pap due to the addition of M. bellicosus compare to the values reported for unsupplemented sorghum pap (1.06g/100g) and maize pap (1.08g/100g) by Ogbonnaya et al. (2012).

The WHO recommendations for complementary feeding of the breast fed child suggests that infants between 6 and 8 months receive at least 200kcal/day from complementary foods and 300kcal/day for infants 9 to 12 months (Dewey and Brown, 2003). These recommendations also assume good maternal nutritional status and adequate breast milk intake and composition of breast milk. Based on these WHO recommendations, infants feeding on the four M. bellicosus enriched reconstituted complementary foods will be able to meet their energy requirement, for infants 6 – 12 months of age consuming such meals 2 – 3 times daily. Moreover, the guiding principles for complementary feeding of the breast fed child (PAHO / WHO, 2003) recommends energy density of 0.8kcal/g and that they be fed 2 or 3 times a day to infants 6 to 8 months of age, and 3 to 4 times a day to infants 9 to 11 months and children 12 to 24 months. The energy density of the four M. bellicosus enriched reconstituted complementary foods in our study exceeded this basic requirement.

5.7 CONCLUSION

Winged termites (*Marcrotermes bellicosus*) has proven to be a rich source of protein, fat and essential micronutrients including the important trace minerals which are needed in infants diets. The insect is a good source of fat soluble vitamins like vitamin A, D, E and K, but low in water soluble vitamins. The energy density of M.bellicosus per 100 gram of sample can easily supply daily energy requirement of a growing infant. The fatty acids profile of MB revealed that the insect contained favourable polyunsaturated fatty acids (PUFA) to saturated fatty acid ratio, the attributes that further made the insect an excellent alternative for complimenting local staples used in the preparation of CFs in Nigeria. The study has further demonstrated that MB is very low in all antinutritional factors studied, thus consumption of the insect cannot pose any health challenge or bioavailable issue to nutrients in the CFs.

Evidence of animal experiments revealed that M. bellicosus-enriched diet support rats growth at 15% levels of inclusion and compete favourably well with those rats fed standard protein diet, meaning that MB protein is of high biological value.

Complementary foods prepared from Local staples such as maize, sorghum, rice and yam cannot supply all the macro and micronutrients requirement of a growing infant, therefore, they must be supplemented with animal source food. The results of this study has demonstrated increment in nutrient composition of locally formulated complementary foods after adding MB at all levels of inclusion. These four formulated diets also supported rats' growth at 20% levels of addition of the insect. The reduction in moisture content of the *M. bellicosus* enriched complementary foods was similar to the trend reported by previous authors. The lower moisture content in the enriched complementary foods was an indication that the dried form of the enriched flour can keep long before use. The increase in protein, fat, ash, total carbohydrate and gross energy content of enriched complementary foods is an indication that addition of *M. bellicosus* resulted in significant improvement in the nutrient content of the formulated foods.

Histopathological evaluation of the small intestine, liver, kidney and spleen showed that *M. bellicosus* had no toxic effects on these organs, but instead, appeared to be beneficial in complementing locally formulated diets thereby supporting the growth of experimental rats. This discovery is novel as the literature seems to lack such information, and it suggests that *M. bellicosus* may be a promising animal source of protein and important micronutrients with no negative histopathological effect on the organs.

Marcrotermes bellicosus is popular in Ekiti State where the study was conducted. Consumption survey of *M. bellicosus* has revealed that more female consumed the insect than male. Almost all our studied participants known the insect very well and have consumed the insects before. Larger percentage of our studied participants consumed the insects because it is nutritious and only a few number of the respondents had experienced side effect after the consumption of the insect. The side effect might not be due to the consumption of the MB. Therefore, further investigation may be needed to establish the claim. The four formulated complementary foods were well rated among the nursing mothers; therefore all the mothers were willing to present them to their children.

5.8 RECOMMENDATIONS

1. Most of the traditional complementary foods assessed were deficient in both macro and micro nutrients and not meet the requirements of a growing infant. Therefore it is recommended that foods from animal sources such as inseect fish, liver etc which are known to be good sources of iron and zinc should be used to enrich these traditional complementary foods so as to make it nutritionally adequate for the infants consuming them.
2. Appropriate pre-processing steps such as fermentation, soaking germination etc. should be incorporated as means to reduce anti-nutrients in traditional grains before processing to complementary foods.
3. Large scale production of M. bellicosus through mini-culture should be introduced to make the insect available all the year round
4. The use of insect as an alternative to other sources of animal protein should be promoted among the nursing mother or caregiver.

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APPENDIX 1

Images of traditional foodstuffs utilized in development of the Complementary foods

Winged termite

Maize Grains

Sorghum grains

Yam tuber

APPENDIX 2
Animal Experiment

APPENDIX 3

CONSENT FORM

Dear Sir/Ma,

I am a post-graduate student of the Department of Human Nutrition, University of Ibadan. I am conducting a project titled **POTENTIAL CONTRIBUTION OF WINGED TERMITES *(MACROTERMES BELLICOSUS*) TO NUTRIENT COMPOSITION OF LOCALLY FORMULATED NUTRIENT-DENSE COMPLEMENTARY FOODS USING ANIMAL MODEL** .This questionnaire is designed to collect information relating to the title from you. No name will be collected nor recorded. The data and information collected during this study will be kept confidential; identifier will not be used in any publication or reports from this study.

Thanks for your cooperation.

………………………………..	………………………………..
Respondent’s sign & date	Investigator’s signature

Subject Number:
……………………………………………………………………………………

Date of interview (dd/mm/yy):
……………………………………………………………………….

STATEMENT OF THE PERSON GIVEN CONSENT

I have read the description of the research and I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it.

………………………………..	………………………………..
Respondent’s sign & date:	Investigator’s signature:

CONSUMPTION SURVEY OF WINGED TERMITES *(MACROTERMES BELLICOSUS*) IN EKITI STATE

INSTRUCTION: Kindly tick the response that corresponds to your experience in the appropriate box below

SECTION A: SOCIO-DEMOGRAPHIC

1. Gender: Male ( ), Female ( )

2. Age: 20 – 29 ( ), 30 – 39 ( ), 40 – 49 ( ), 50 – 59 ( ), 60 and above.

3. Marital Status: Single ( ), Married: ( ), Widow/Widower : ( ), Divorced/seperated: ( ).

4. Household Size: 2 ( ), 3 ( ), 4 ( ), 5 ( ), 6 ( ), 7 and above ( ).

5. Educational level: (1) No formal education (2) Primary **(**3**)** Secondary (4) Tertiary education

6. **Occupation:** (1)Civil servant(2)Farming (3) Trading (4) Artisan **(**5**)** Student

7. Monthly Income: (1) < N10,000 (2**) N10,000 – N20,000 (3)N21,000 – N30,000 (4) >N31,000**

8. **Religion**: (1) Christianity (2) Islam (3) Others specified……………………….

9. Housing: (1) modern **(**2**)** poor housing or slum (3) rural housing

10 Ethnic group: (1) Yoruba (2) Hausa (3)Igbo (4) Others specify………………..

SECTION B.CONSUMPTION PATTERN AND FREQUENCY OF CONSUMPTION OF WINGED TERMITES *MARCROTERME BELLICOSUS* (ESUNSUN)

11. Do you know winged termites (Esunsun)? Yes ( ) No ( )

12. Have you eaten winged termites (Esunsun) before? Yes ( ) No ( )

13. During swarming of winged termites, do you consume as you can find? (i) Yes (ii) No (iii)

14. Since when have you been eaten winged termites (Esunsun)? (i) Childhood (ii) Adolescence (iii) Adulthood

Do other members of your household eat winged termites (Esunsun)? Yes ( ) No ( )

15. During swarming, how often do they eat winged termites (Esunsun) ? Regularly ( ) As much as it is available ( ) Seldom ( )

16. Who are the class of people do you think eat winged termites (Esunsun) ? (i) Rich (ii) Average (ii) Poor (iv) All of the above

17. Which of these groups do you think eat winged termites (Esunsun)? Children ( ) Young people ( ) Adult ( ) everybody ( )

18. In what form do you /they consume winged termites (Esunsun)? (i) Fresh (ii) Fried with seasoning (ii) fried without seasoning (V) Fried with little salt (v) cook as sauce

19. What motivate you to consume winged termites (Esunsun) ? (i) Custom (ii) Nutrition facts (iii) Flavour (iv)curiosity

SECTION C.AVAILABILITY AND ACCESSIBILITY

20. How popular and/or acceptable is winged termites (Esunsun) in your locality?

(i)Very popular (ii) popular (iii) rarely known (iv) not known at all

21. What season of the year are they mostly available? Rainy ( ) Dry ( )

22. During swarming, how do you have access to the winged termites

(i) Catching (ii) Buying (iii) Trapping

23. Can winged termites (Esunsun) be stored? Yes ( ) No ( ) not sure ( )

24. If yes, in what form is it stored? Dried ( ) Wet ( ) others ( )

25. Do winged termites (Esunsun) available in the market all year round? Yes ( ) No ( )

26. Do winged termites affordable in the market for all? Yes ( ) No ( )

SECTION D: LIMITATION AND RESTRICTION TO CONSUMPTION

27. Does religion belief affect its consumption? Yes ( ) No ( )

28. If yes, in what ways? ....,……………………………

29. Does any traditional belief/myths attached to its consumption? Yes ( ) No ( )

30. If yes, what do people say about its consumption? ………………

31. Have you ever experienced any side effects upon short time consumption of winged termites? Yes ( ) No ( )

32. If yes, in what ways? (i) Stomach ache (ii) Vomiting (iii) Skin rashes (iv) head ache

33. Has any member of your household experienced side effects upon short time consumption ofthe winged termites? Yes ( ) No ( )

34. If yes, in what ways? (i) Stomach ache (ii) Vomiting (iii) Skin rashes (iv) head ache

35. Does availability affect its consumption, Yes ( ) No ( )

36. If yes, in what ways ……………………………………………………..,

37. Does cost affect it consumption? Yes (   ) No (   )

38. If yes, in what ways? (i)   It is too expensive (ii) The price is cheap (iii) Other specified………

39. What is the general opinion of people in your locality concerning winged termites …………………………

A picture of one the interviews conducted during a consumption survey in Ekiti State

APPENDIX 4

Sensory evaluation questionnaire for the developed complementary Foods

Hedonic Test

You are provided with four (4) coded samples of complementary porridge. **A.** Please rate the samples (1-7) according to the scale provided below by filling in the table against each sample and attribute ranging from Like very much to Dislike very much. Rinse your mouth with clean water after tasting each food.

SENSORY EVALUATION OF FORMULATED COMPLEMENTARY FOODS

SAMPLE A

Parameter	Colour	Taste	Texture	Odour	Acceptability
Like Very Much					
Like Moderately					
Like Slightly					
Neither Like Nor Dislike					
Dislike Slightly					
Dislike Moderately					
Dislike Very Much					

SAMPLE B

Parameter	Colour	Taste	Texture	Odour	Acceptability
Like Very Much					
Like Moderately					
Like Slightly					
Neither Like Nor Dislike					
Dislike Slightly					
Dislike Moderately					
Dislike Very Much					

SAMPLE C

Parameter	Colour	Taste	Texture	Odour	Acceptability
Like Very Much					
Like Moderately					
Like Slightly					
Neither Like Nor Dislike					
Dislike Slightly					
Dislike Moderately					
Dislike Very Much					

SAMPLE D

Parameter	Colour	Taste	Texture	Odour	Acceptability
Like Very Much					
Like Moderately					
Like Slightly					
Neither Like Nor Dislike					
Dislike Slightly					
Dislike Moderately					
Dislike Very Much					

A cross section of nursing mothers who participated in the sensory evaluation of the formulated complementary foods

Pictures of nursing mothers feeding their infants with the formulated foods

Samples of formulated CFs prepared for chemical analysis