2018-08

Optimal model formula for ensuring nutritional adequacy for infants and young children (6 – 23 months)

Makori, Nyabasi

NM-AIST

https://doi.org/10.58694/20.500.12479/298

OPTIMAL MODEL FORMULA FOR ENSURING NUTRITIONAL ADEQUACY FOR INFANTS AND YOUNG CHILDREN (6 – 23 MONTHS)

Nyabasi Makori

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences and Engineering of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

August, 2018

ABSTRACT

Infant and young child feeding practices rank among the most effective interventions to promote child growth and development. Improving the quality of complementary food has been observed as an important strategy for promoting child growth in resource-poor settings. Therefore, the objective of this study was to design a safe and nutritious model formula that meets nutritional requirement for infants and young children aged 6 – 23 months. The objective was achieved by conducting a cross-sectional study aimed to assess the nutritional status of 394 children and identify factors associated to under-nutrition. Then, complementary flours were collected randomly from selected households and analyzed for macro- and micro-nutrients, phytate content and presence of aflatoxins. Exposure assessment was done to evaluate the contribution of aflatoxins exposure on nutritional status of children. Finally, the model formula was designed using commonly consumed foods to meet the nutritional requirements for children. Data analysis was performed by using SPSS (IBM version 21), ENA for smart, Microsoft Excel and LINDO (version 6.1). The results showed that prevalence of stunting was 40.4% (95% CI; 29.8% - 50.9%). Children aged between 12 - 23 months were more stunted compared to those aged between 6 – 11 months ($p \leq 0.001$). The age of introducing complementary foods (AOR = 13.3, 95%CI, 2.6 – 67.6), maternal education (AOR = 5.5, 95% CI, 1.0 – 9.8) and residence in Chamwino district (AOR = 3.2, 95%, 1.3 – 5.9) were identified as factors associated with stunting. Analysis of cereal-based complementary foods indicated that protein and fat content ranged from 1.17 - 11.17 g/100 g and 0.61 - 11.19 g/100 g respectively. A significant difference in protein content between composite cereals and other types of complementary foods was observed ($p \leq 0.001$). Sorghum had the highest iron and phytate content of 8.37 mg/100 g and 1176.8 mg respectively. Aflatoxins contamination was detected in 42.5% of home-made complementary flours at the levels ranging from 0.3 μg/kg to 2,128 μg/kg. There was a significant association between stunting and infants dietary exposure to aflatoxin B_1 ($p = 0.05$, 95% CI; 0.019 - 0.028). Cereal-based complementary foods consumed by the studied children were observed to have dual burdens in a sense that they contained high level of phytate and being contaminated with aflatoxins to the levels of health concern that may contribute to impairment of growth. Developed optimized model formula serves as a basis for ensuring adequate nutrient intake, minimized intake of phytate and reduced dietary exposure of infants

Keywords: Stunting; dietary diversity; complementary foods; nutritional adequacy; phytate; child growth; aflatoxins; dietary exposure; mineral bioavailability, linear programming; optimal diet.

DECLARATION

I, **Nyabasi Makori** do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

………………………………………………………… …………………………..

Nyabasi Makori Date

The above declaration is confirmed

…………………………………………………… …………………………….

Dr. Athanasia Matemu Date

…………………………………………………… …………………………….

Dr. Neema Kassim Date

10th August, 2018

…………………………………………………… …………………………….

Prof. Joyce Kinabo Date

COPYRIGHT

CERTIFICATION

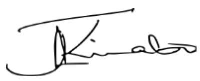
The undersigned certify that they have read and found it to be acceptable for examination; hereby recommends for examination of the dissertation entitled **Optimal model formula for ensuring adequate nutritional adequacy for infants and young children (6 – 23 months)**. In fulfilment of the Award of Doctoral of Philosophy in Food and Nutrition Sciences at The Nelson Mandela African Institution of Science and Technology

.. **…………..……………………..**

Dr. Athanasia Matemu **Date**

………………………………………. **…………………………………**

Dr. Neema Kassim **Date**



10th August, 2018

………... **………………………………**

Prof. Joyce Kinabo **Date**

ACKNOWLEDGMENT

First and foremost, my deepest acknowledgment goes to my almighty God for his blessings, guidance, protection and encouragement. Thank you Lord for granting me good health throughout the entire period of my study. My LORD, I will always glorify your name.

I would like to express my profound gratitude and appreciation to my supervisors; Dr. Athanasia Matemu, Dr. Neema Kassim and Prof. Joyce Kinabo, for their valuable guidance, inspiration, encouragement, commitment and constructive inputs which enabled successfully completion of this work. I'm so grateful to have had such wonderful supervisors.

I would like to extend grateful acknowledgment to the government of Tanzania through Nelson Mandela African Institution of Science and Technology for granted me a scholarship throughout my PhD study.

My appreciation goes to laboratory technologist Mr. Amri Juma from TFNC, for his assistance in the analysis of aflatoxin and Dr. Beatus Lyimo for assisting analysis of phytate at NM-AIST laboratory. God bless you.

I highly acknowledge the support of Regional Medical Officer of Dodoma region, District Medical officer and District Executive Officer of Dodoma municipal and Chamwino district for their support in facilitating data collection exercise for this study. I would also like to express my sincere gratitude to parents/guardian and children who participated in this study. Thank you for your patience, tireless, great cooperation and good understanding during the entire period of data collection. I acknowledge the support of village leaders and ward executive officers from all study areas for their guidance and support during data collection.

I am also very grateful to my parents especially my wonderful mothers, Felicitas Fabian and Mrs. Wassaga for their moral support during the period when I gave birth to my son in 2016. Thank you mothers you played a great role to ensure my kid got proper attention and at the same time you ensured that I achieve my career goal. I acknowledge the support of my father, who is also my mentor and career advisor, thank you for your advice, counselling, close follow up and laying up a good foundation of my education career. I acknowledge a profound support, encouragement and love from my relative.

I thank my Husband Mosses John for your love, prayers, encouragement, support and motivation throughout the period of my study. Thank you for being there for me, really the journey would not have been possible without you. I am so thankful to my wonderful kids July, Fely, Glory and Elisha, thank you for your patience and tolerating my busy schedule.

DEDICATION

This work is dedicated to my lovely family, thank you God to have you in my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

AFB_1	Aflatoxin B_1
AOAC	Association of Official Analytical Chemist
AOR	Adjusted Odd Ratio
Ca	Calcium
CF	Complementary Food
CI	Confidence Interval
COR	Crude Odd Ratio
Cu	Copper
DDS	Dietary Diversity Score
DMT1	Divalent Metal Transporter 1
DNA	Deoxyribonucleic Acid
EFSA	European Foods Safety Authority
ENA	Emergency Nutrition Assessment
EU	European Union
FAO	Food and Agriculture Organization
Fe	Iron
GAP	Good Agricultural Practice
GNP	Gross National Product
HPLC	High Performance Liquid Chromatography
IgA	Immunoglobulin A
IARC	International Agency for Research on Cancer
IYCF	Infant and Young Child Feeding
K	Potassium
LINDO	Linear Interactive Discrete Optimizer
LOD	Limit of Detection
LOQ	Limit of Quantification
MAD	Minimum Acceptable Diet
Mg	Magnesium
ML	Maximum Limit
Mn	Manganese
MoE	Margin of Exposure

MS	Microsoft
Na	Sodium
NBS	National Bureau of Standards
NIMR	National Institute for Medical Research
PBS	Phosphate Buffer Saline
RDA	Recommended Dietary Allowance
RDI	Recommended Dietary Intake
RNI	Recommended Nutrient Intake
RSD	Relative Standard Deviation
SD	Standard Deviation
SPSS	Statistical Package for Social Studies
TDHS	Tanzania Demographic Health Survey
TFCT	Tanzania Food Composition Table
UNICEF	United National
USDA	United States Department of Agriculture
WFP	World Food Program
WHO	World Health Organization
Zn	Zinc

CHAPTER ONE

1.1. Introduction

1.1.1. Background information

Under-nutrition is an adverse health outcome occurs to an individual due to exposure of associated factors such as infectious diseases, inadequate dietary intake, lack of health services, food insecurity and improper child care practices (Fekadu *et al*., 2015). Worls Healh Organization (WHO) categorized under-nutrition into different forms, including wasting (low weight for height), stunting (low height for age), underweight (low weight for age) and deficiency of essential vitamins and minerals (Schwarz *et al*., 2008). Under-nutrition remains unacceptable throughout the world, it continues to undermine the survival, growth and development of children. Linear growth failure during childhood is the most prevalent form of under-nutrition globally, particularly in low and middle income countries it has remained as the main public health challenge (Prendergast and Humphrey, 2014). Stunted children may never grow to their full cognitive development potential (UNICEF/WHO/World Bank Group, 2017). Globally it is estimated that 155 million children of under five years of age are suffering from stunting. In Asia it has been reported that 87 million infants and young children are stunted and in Africa are 59 million infants were reported to be stunted (UNICEF/WHO/World Bank Group, 2017). In Tanzania particularly, according to Tanzania demographic health survey (TDHS) more than one third (34.4%) of infants and young children are stunted (NBS, 2016). Additionally, under-nutrition underlies 45% of all deaths among children below five years of age. The consequence of stunting are life long, profound and irreversible, mostly it occurs during the first 1000 days considered as critical period for growth and development (Black *et al*., 2013). Stunting hinders developmental potential and human capital of the entire societies due to its increasing risk of morbidity, mortality, impair physical growth and cognitive development (Prendergast and Humphrey, 2014). Nutrition in early childhood has a lasting impact on health and well-being of an individual in adulthood.

1.1.2. Complementary feeding

Adequate nutrition during the first years of life is very crucial in laying foundation of good health and ensuring optimal child growth (Dewey, 2013). Complementary foods should contain all essential nutrients that meet the recommended nutrient intake (RNI) for children (Okwunodulu and Iwe, 2015). The period of complementary feeding is a major global health concern on attainment of adequate nutrient intake and meeting the child nutritional needs (Zone *et al.*, 2015). Nutritional deficiency normally arises when body physiological requirements are not met in a long term. Additionally, inadequate dietary intake is considered to be a risk factor for nutritional deficiency, burden of disease and adverse health consequences (Kumssa *et al*., 2015; Muthayya *et al*., 2013; Bhutta *et al*., 2013). However, poor nutrition increases the risk of diseases, growth faltering, impaired cognitive development and is either directly or indirectly associated with 9.5 million deaths that occurred in 2006 among children less than five years of age (Black *et al*., 2008).

The period of transition from exclusive breastfeeding to complementary foods referred to as the period of complementary feeding, it covers the age of 6 – 23 months. Infant's nutritional requirements start to increase at the age of six months when nutritional needs exceeded what is supplied by breast milk (EFSA, 2009). Complementation of exceeded nutritional needs is necessary for achieving optimal growth and development, this can be attained by the introduction of adequate and appropriate complementary foods at the age of six months (Grimshaw *et al*., 2013; Przyrembel, 2012; Jonsdottir, Thorsdottir *et al*., 2012). World Health Organization (WHO) recommend that the introduction of complementary food should be timely, meaning that it should start when an infant is at the age of six months, in order to meet the increased nutritional needs and to complement from what breast milk is providing (Allen, 2012).

1.1.3. Nutritional requirements for infants

Infancy is a period of rapid growth and development, it is characterized by high nutritional requirements. It imposes unique nutritional needs to meet the body requirements. Children below five years of age are the most vulnerable population due to increased nutritional demands to sustain high growth rate (Ahmed *et al.,* 2013; Black *et al*., 2008). Nutrition play crucial role in determining body defense mechanism against infection. However, ensuring adequate nutrient intake during the period of complementary feeding is a global health priority. Optimal feeding is an evidence-based measure for improving child nutrition and survival. Appropriate complementary foods should be introduced to infants in order to facilitate optimal growth. Complementary foods should include a variety of foods in order to ensure the nutritional requirements of infants and young children are met, by considering adequate intake of energy, protein, fat, vitamins and micronutrients that correspond to RNI.

Lack of suitable complementary foods in term of adequate nutrients to meet the nutritional requirement of infants is a major challenge. Most of the complementary foods are formulated locally and are based on staple foods, usually cereals constitute a major portion of the formulation (Okomo *et al*., 2016). Cereal-based gruels are low in protein and other essential nutrients which are crucial for growth and development of a child (Muhimbula *et al*., 2011). The cereal-based complementary foods are frequently associated with micronutrient deficiency, especially zinc and iron which are essential for growth promotion (Hlaing *et al.*, 2016). Anigo *et al.* (2009) reported the nutrient content of complementary food used in Nigeria contained very low substantial amount of protein, fat, amino acids and minerals, which did not meet recommended daily allowance (RDA) for infants. In addition, Kulwa *et al.* (2015) found that porridge made from cereals were mainly used as complementary food, reported to contain low amount of nutrients in terms of energy, protein, iron and calcium. Inadequate dietary intake has been reported to be a major contributing factor to under-nutrition, this might be contributed by underlying factors including lack of dietary diversity, dependency on cereal-based complementary foods, presence of anti-nutritional factors and low intake of animal food sources (Gibson *et al*., 2010; Maseta *et al*., 2016).

1.1.4. Under-nutrition and its effect on child growth and development

World Health Organization (WHO) classifies children whose height for age, weight for height, weight for age Z-score are below -2 SD from the median of WHO reference population are considered to be stunted, wasted and underweight respectively (WHO, 2008). Inadequate dietary intake and diseases are considered as immediate causes of under-nutrition. The underlying causes include; food insecurity, inadequate care, poor hygiene and lack of health services (UNICEF, 2013). Globally, over 3.1 million death of children under the age of five years in 2011 were associated with under-nutrition (Black *et al.*, 2013). The effects of under-nutrition are not only linked to child mortality but have pervasive effects on child survival (Moran and Dewey, 2011). Under-nutrition affect physical development, limit the rate of motor development which also alters brain development, it's effect has been linked to poor cognitive ability and educational attainment (Asfaw *et al*., 2015). Stunting in childhood is also most likely to influence adulthood stature, diminishes their chances of success in later life due to effect that has been imparted in cognitive development (de Onis and Branca, 2016). The pathway by which under-nutrition limits socio-economic growth is through impairment of cognitive development during childhood (Casale *et al*., 2014). Therefore, meeting child's daily nutritional requirement is a key factor to achieve normal child growth.

1.2. Anti-nutritional factors

Anti-nutritional factors are substances that hinder the absorption of nutrients in the body. Cereals are used as staple foods and have been observed to contain anti-nutritional factors such as phytate, oxalate, tannins, polyphenols and trypsin inhibitors. Additionally, cereals are used as a major component in children's diet and are mainly used in the formulation of complementary foods. Presence of anti-nutritional factors in complementary food can have a negative impact on the nutritional status of children by interfering with the absorption and utilization of minerals such as iron, zinc, calcium (Roos *et al*., 2013). A small amount of phytate in the diet, can reduce the absorption of iron (Hurrell and Egli, 2010). Consumption of high levels of anti-nutritional factor with breast milk, can affect the bioavailability of minerals from the breast milk (Gibson *et al*., 2010). Low bioavailability, interference of absorption and utilization of nutrients and minerals due to the presence of anti-nutritional factors is another factor that limits the quality of plant-based diets (Platel and Srinivasan, 2015; Getenesh *et al*., 2014). Gibson *et al. (*2010), Lazarte *et al.* (2015) and Roos *et al.* (2013) found high level of phytate above the threshold in cereal-based complementary foods which might inhibit the bioavailability of minerals.

1.3. Aflatoxins

Aflatoxins are secondary metabolite of fungi produced by *Aspergillus flavus* and *Aspergillus parasiticus* under exposure of pre and post-harvest conditions such as high temperature, humidity and moisture content (Kumar *et al*., 2017). There are four major types of aflatoxins including aflatoxins B_1, B_2, G_1 and G_2. "B" and "G" refer to the blue or green fluorescence observed with exposure of the toxin to ultraviolet irradiation (Reddy *et al*., 2010). Fungal invasion may occur in the field, during harvesting and when food commodities are stored under conditions that promote fungal growth. Aflatoxins contaminate a wide range of agriculture produce including cereals, oil seeds, nuts, dried fruits, it has been observed that aflatoxins B_1 is the predominant form in cereals and oil seeds (Geary *et al*., 2016). Human being get infected with aflatoxins through ingestion of aflatoxins contaminated foods such as cereals, cereal-based products, milk and milk produce, eggs (Bhat *et al.*, 2010). The exposure of infants to mycotoxins can occur at different stages in their life including during utero life (pregnancy), breast feeding and the period of complementary feeding (Smith *et al*., 2017; Khlangwiset *et al*., 2011). Breastfed infants may be exposed to aflatoxin M_1 through breast milk if lactating mother consumed food which is contaminated with aflatoxins (Magoha *et al*., 2014). The major route that expose children to aflatoxins during the period of complementary feeding is through consumption of contaminated complementary foods. In addition, the exposure to aflatoxins can also occur through inhalation of aflatoxins contaminated dust during sorting, handling and processing of contaminated crops (Bbosa *et al*., 2013). Aflatoxin B_1 is the most potent toxic and carcinogen, it is listed as group one carcinogen by the international agency for research on cancer (IARC) (Zain, 2011).

Mycotoxins are of global importance due to their impact on human health, animal health and their associated economic development losses (Darwish *et al*., 2014). Food and Agriculture Organization (FAO) has estimated that 25% of the world's food crops are significantly contaminated with aflatoxin (WHO, 1999). In Tanzania maize is the main staple food mainly for people living in rural areas and are depending on subsistence farming (Kimanya *et al*., 2009). Furthermore, maize and groundnuts have been observed to be the basic ingredients in formulation of complementary flour, and have also been reported to be susceptible to aflatoxin contamination (Obade *et al*., 2015; Shirima *et al*., 2015).

1.3.1. Impact of Aflatoxins on child health

Studies have found that under normal conditions, about 50% of ingested aflatoxin B_1 is estimated to be rapidly absorbed in the duodenum and goes to the liver through portal vein (Coulombe *et al.*, 1991). Aflatoxicosis is a condition resulted after ingestion of aflatoxins contaminated food (Çelik *et al*., 2005). It is categorized in two forms; acute and chronic intoxication. The acute form is a rapid onset from a single exposure which tends to cause abruptly liver damage and death while a chronic form is a long term exposure it causes subsequent illness for a long time. However, chronic exposure to aflatoxin B_1 is causing cancer of the liver, immune suppression, growth faltering and increases the risk of diseases i.e., non-communicable diseases in adulthood (Kimanya, 2015; Magoha *et al*., 2014). Duration of exposure and dosage have an effect on the magnitude of exposure as well as adverse health consequences associated with toxicological effect. In Tanzania, Kimanya *et al*. (2014) and Shirima *et al.* (2014) reported the extent at which infants are exposed to different levels of mycotoxin contamination through complementary foods and its associated risk of growth impairment.

1.3.2. Aflatoxin regulations and maximum limits

Worldwide maximum levels of aflatoxins in food commodities have been established, the number of countries reported having mycotoxins regulations has increased from 15 countries in 2003 to 99 countries in 2011 (Nortaa and Sowley, 2016). The potential effect of aflatoxins in human health which is a widespread concern has driven many countries to establish control measures and set limits by international authorities. Countries have diverse regulations for aflatoxins contamination, often specific and detailed to various foodstuffs intending to protect the health of consumer from the harmful effect associated with mycotoxins. The legislation includes specific maximum levels in individual food stuff for certain type of mycotoxins. European Union (EU) legislation regarding mycotoxins has set maximum limits for aflatoxin B_1 and total aflatoxins in various food stuff including cereals, cereals products, processed cereal-based foods for infants and young children (EC, 2010). The maximum limits set by EU for aflatoxin B_1 and total aflatoxins in cereal-based food are 2 µg/kg and 4 µg/kg respectively. In Tanzania, the maximum limits set for aflatoxin B_1 and total aflatoxins are 5 µg/kg and 10 µg/kg respectively (TFDA, 2012). Developed countries usually set lower levels than developing countries where the most of the vulnerable commodities are produced, this might be due to higher exposure rate aggravated by lack of appropriate technologies to prevent pre- and post-harvest contamination.

1.4. Problem statement

Most of CFs in developing countries are formulated without considering critical factors such as dietary diversity, anti-nutritional content, aflatoxin levels and nutrient absorption enhancers. Thus consumers, do not always meet a minimum acceptable diets. Recent studies have shown that, only 16% of infants met the MAD in Africa, only 10% in Tanzania, 4% in

Ethiopia, 14.7% in Kenya and 29% in Nigeria (NBS, 2016; NNBS, 2014; Matanda *et al*., 2016; Mokori *at el*., 2016). This might have contributed to the observed high levels of malnutrition in Africa and Asia, which contribute about one third of the global prevalence of malnutrition. Child under-nutrition is unacceptable all over the world (Rajan *et al*., 2015). Globally, 155 million children under five years of age are stunted and 52 million are wasted. Additionally, in Asia 87 million children under-five years of age are stunted and in Africa are 59 million (UNICEF/WHO/World Bank Group, 2017). Tanzania Demographic and Health Survey reported that 34.4% of under-five children are stunted, 13.7% are underweight, and 58% anaemic (NBS, 2016). According to TDHS (2010), Dodoma region had the highest prevalence of stunting (56%) compared to other regions in Tanzania (NBS, 2011). Over a period of time, Dodoma region has been observed to have high stunting prevalence, affecting lives of many infants and young children. Additionally, stunting is still being a major public health concern not only in Tanzania but also in low and middle-income countries (Black *et al*., 2013). Under-nutrition has been associate with the increased risk of morbidity, mortality, weakened immune system, impairment of physical growth and cognitive development (Negash *et al*., 2015). The consequences associated with growth faltering during infancy is likely to be extended in adulthood and it is an irreversible process (Adair *et al*., 2013). The impact of stunted growth has lifelong consequences not only to the health of an individual but also it affects human capital. It has been observed that adults who were stunted during childhood have less ability to work and produce following the effect that has been imparted on cognitive development during childhood. Stunting has been observed to have effect on economic growth and pose other developmental constraints. In Asia and Africa, lose from all forms of malnutrition have been estimated up to 11% of GDP (Horton and Steckel, 2011). Globally, the estimated enormous costs resulted from economic growth foregone and losses of human capital investment is as high as USD 3.5 trillion per year or USD 500 per individual (Global Panel, 2016). Additionally, stunting may affect household's wealth, through caring for malnourished children which consequently affect time allocated for production. Therefore, intervention that promotes optimal child growth is likely to be of benefit for multiple outcomes, including on child health, improved household economic wealth, and therefore contributing to the economic growth of the nation.

1.5. Justification of the study

The optimized model diet ensures adequate nutrient intake that correspond to RNI at the same time the levels of phytate and aflatoxins were reduced. Therefore, meeting daily infants nutritional requirement is a measure of improving child nutrition, growth and survival. Deviations from normal growth pattern especially during childhood are associated with many factors, however inadequate dietary intake being the major contributing factor (Asfaw *et al*., 2015; Sudfeld *et al*., 2015). Cereals-based complementary foods limit the attainment of adequate nutrient intake since they are less in nutrient content to meet the RNI for infants and young children. In addition, poor bioavailability of minerals in cereal-based complementary foods could be another factor which limits the intake of adequate nutrients. On the other hand, cereals have been observed to be highly contaminated with aflatoxins despite the development of regulatory limits for aflatoxins contamination. Mycotoxins contamination still remains prevalent in complementary foods, occurring at intolerable levels above the regulatory limits. Meeting daily nutritional requirement for optimal growth during infancy and childhood is a key challenge. Therefore an optimized model diet to ensure adequate nutrients intake that meet RNI at the same time reduced the levels of phytate and aflatoxins in complementary foods is of the most importance.

1.6. Objective of the research

1.6.1. General Objective

To design a safe and nutritious model formula that meets nutritional requirement for infants and young children aged 6 – 23 months.

1.6.2. Specific Objectives

i. To assess nutritional status among infants and young children aged 6 to 23 months and explore factors associated with under-nutrition.
ii. To analyse nutrient composition and anti-nutritional factors of homemade complementary foods and to assess parent’s knowledge and practices on preparation of complementary foods.

iii. To explore the contribution of dietary aflatoxins exposure and its influence on nutritional status among infants and young children.
iv. To design an optimal model formula using commonly consumed foods to meets the nutritional requirements for infants and young children.

1.6.3. Research questions

i. Which factors may influence nutritional status of children of age range between 6 – 23 months?
ii. What is the nutrient content and phytate level of homemade complementary food, its associated practices on preparation and the influence of parent's knowledge?
iii. At what level infants and young children are exposed to aflatoxins through diet and how does this influence their nutritional status?
iv. To what extent can optimal model formula designed from commonly consumed foods meet nutritional requirements for infants and young children of 6 – 23 months?

1.6.4. Significance of the study

The study developed useful modeled diet that intends to meet the RNI for infants of age group 6 – 11 months and young children 12 -23 months. Adequate nutrition for the first 1000 days of infant's life (from conception to second year) is fundamental for a child to achieve optimal growth and development. Meeting daily RNI during childhood is a key focus for prevention of nutritional deficiencies, especially micronutrients. Therefore, the results from the present study provide information on how to utilize locally available food to improve the nutritional status and wellbeing of infants and young children. This explore the opportunity for setting priorities on meeting child nutritional requirements through utilization of optimized diet.

CHAPTER TWO

Dietary Related Factors in typical African Complementary Foods and their Implications on Child Health and Development – A Review[1]

Abstract

Micronutrients deficiency is a predominant problem in low and middle income countries. Poor minerals bioavailability of plant-based foods resulted from dietary related factors is the major reason of their wide prevalence. Typical African complementary foods contain compounds that inhibit mineral bioavailability. The presence of phytate in the diet forms unabsorbed phytate-minerals complexes in the gastrointestinal tract due to lack of endogenous phytase enzyme. This review aimed at elucidating the effects of phytate on iron and zinc bioavailability and its associated adverse health effect on child health and development. Deficiency of micronutrients is associated with impairment of child growth, cognitive development, immune functioning and physiological effects that can be life threatening to infant and young children. However, dietary diversification, use of nutrients that enhance absorption and application of preliminary food preparation and processing methods such as fermentation, germination, soaking and use of phytase enzymes could reduce phytate content in plant-based foods thus minimizing their adverse effect in human health.

Key words: Complementary food, infants, phytate, mineral bioavailability, iron and zinc.

[1] *Submitted to International Journal of food Science and Nutrition; CIJF-2017-0873*

2.1. Introduction

Adequate nutrition during childhood is fundamental for survival, growth and development (Skau *et al*., 2014; Michaelsen, 2015). Good nutrition of an individual is a result of eating nutritious food that meet daily recommended dietary intake (RDI) for macro and micro-nutrients. Infants are introduced to complementary foods at the age of six months when there is increased nutritional requirements which cannot be met by breast milk alone (Adimasu *et al*., 2016). Complementary foods are foods apart from breast milk that are introduced to infants for the purpose of providing adequate nutrients to support growth (Dewey, 2013). World health organization (WHO) recommends that complementary foods must be sufficient to provide protein, energy, folate, vitamins and micronutrients such as iron and zinc, to cover child's nutritional requirements (WHO, 2009). The growth of children in developing countries often declines around the age of 6 months when they are introduced to complementary foods and continues up to the second year of life (Krebs, 2014). Furthermore, the period of complementary feeding is characterized by challenges, some of them are context specific but in most cases they tend to cut across setting. In developing countries cereals are mostly used as ingredients in formulation of complementary foods, and constitute a greater portion while the intake of vegetables, fruits and animal food sources is inadequate *(*Melese, 2013; Adimasu *et al*., 2016).

In Africa, more than 80% of children (6 – 23 months) did not attain a minimum acceptable diet during the period of complementary feeding (de Onis & Branca, 2016). Studies have indicated that very few children (6 – 23 months) in Tanzania 10%, Ethiopia 4%, Kenya 14.7% and Nigeria 29% meet the minimum acceptable diet during the period of complementary feeding (NBS, 2016; NNBS, 2014; Matanda *et al*., 2016; Mokori *at el*., 2016). Infant and young child feeding (IYCF) practices being the most important determinants for improving child nutritional status, growth and development. Attainment of adequate nutrient intake is affected by various factors including the presence of anti-nutritional factors in the diet; this includes polyphenols, phytate, and oxalate, which are also considered as dietary related factors that limit the bioavailability of nutrients in plant-based diets (Okomo *et al.*, 2016; Gibson *et al.*, 2006). Most of plant-based food contains high level of phytate, a potent of micronutrients inhibitor (iron and zinc) absorption by affecting their bioavailability. Phytic acid affect the absorption of micronutrients by forming insoluble phytate-minerals compounds in the gastrointestinal tract, that impede the bioavailability and utilization of nutrients in the body (Maseta *et al*., 2016; Pelig-Ba, 2009). Child under-nutrition is a real burden in developing countries it is associated with 35% of death (Black *et al*., 2008). The fact that, 34% of children under five years of age are stunted, 5% wasted, 14% underweight and 57% anemic, this further justifies the magnitude of under-nutrition in Tanzania (NBS, 2016).

Globally, more than 2 billion people have been reported to suffer from deficiency of one or multiple micronutrients (WHO, UNICEF and WFP, 2007). Zinc, iron, folate, iodine and vitamin A deficiencies are the most prevalent micronutrient deficiencies contributing to poor growth, impairment of cognitive development and increased the risk of morbidity and mortality (Morales-Ruán *et al*., 2012; Rohner *et al*., 2013; Wong *et al*., 2014; Wieser *et al*., 2013; Miller and Welch, 2013). Iron and zinc are the two micronutrients that have been recognized as problematic micronutrients in low resource settings (Fahmida *et al*., 2014; Santika, 2009). Iron deficiency is the widespread micronutrient deficiency worldwide contributing to iron deficiency anemia, reduce capacity for work, as well as impair cognitive and immune function. Whereas zinc which is essential for optimal immune function, its deficiency is associated with an acute respiratory infections and increased incidence of diarrhea, it has been reported as the major causes of death in children of under five years of age (Bailey *et al.*, 2015). The identified gaps on attainment of adequate nutrients intake for infants have been a characteristic of human diets since the revolution of agriculture. Therefore, in this review we discussed the commonality of cereal-based complementary foods in Africa context and potential adverse health effects associated with the presence of phytate in relation to child health and development.

2.2. Phytic acid and mineral bioavailability

Phytic acid (myo-inositol hexakis dihydrogen phosphate, $C_6H_{18}0_{24}P_6$) (Fig. 1a) is organic substance naturally occurring in plants, have chelation properties with some minerals cations (Bohn *et al.*, 2008). It consist of six reactive phosphate ester groups with inositol ring. It usually present as a salt of the mono- (Na^+, K^+) and divalent (Mg^{2+}, Zn^{2+}, Fe^{2+} and Ca^{2+}) cations and it accumulates in the seeds during the ripening period (Thavarajah, 2014). In cereals, seeds and legumes the phytic acid is normally stored in the form of phosphorus, its content varies, and the anti-nutritive effect is based on its molecular structure (Pallauf and Rimbach, 1997). In cereals, phytic acid is stored in the aleurone layer (bran) and it accounts for 0.5% to 5% of total weight of grains and seeds (Gemede, 2014). Phytic acid works in a broad pH-region as a highly negatively charged ion. In a complete dissociation state, its phosphate groups carry high density of negatively charged ion (Fig. 1a) which binds di-and trivalent cations such as $Fe^{2+/3+}$, Mn^{2+}, ·Zn^{2+}, Ca^{2+}, Cu^{2+}, and Mg^{2+} into a very stable complexes in the human's gastrointestinal tract (Fig. 1b) rendering them unavailable for intestinal uptake due to lack of endogenous phytase enzymes (Fig. 1b) (Kumar *et al*., 2010).

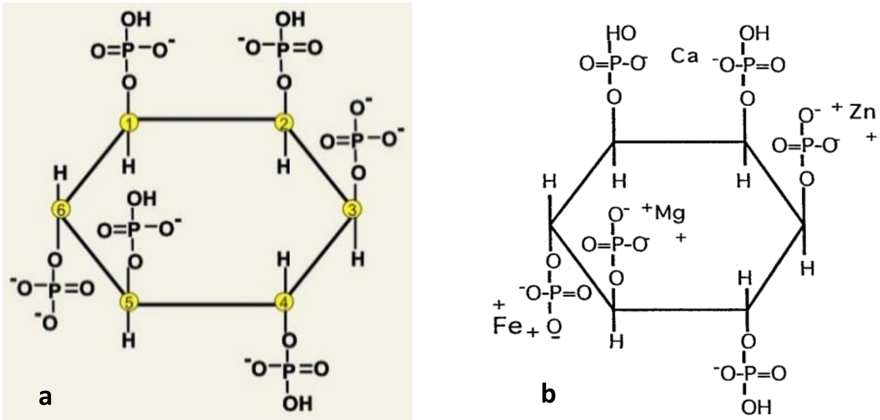


Figure 1. Structure of phytic acid (a) and phytic acid after binding divalent cations (b) such as Fe, Zn and Ca (Abdul and John, 1992).

Cereals and legumes offer a reasonable amount of carbohydrate, dietary fibers, protein, water soluble vitamins and minerals, moreover they are also rich in phytic acid (Abdel-Gawad *et al*., 2013). Most of typical African infant's diets are cereal-based complementary foods, the existence of phytic acid in cereals make it challenging for attainment of adequate bioavailable nutrients to facilitate growth and development (Champ, 2002). The presence of phytate either from the same food matrix or other foods in the same meal may affect the absorption of minerals by forming phytate-minerals insoluble complexes (Fig. 2) which is not absorbed in the gastrointestinal tract (Kana Sop *et al*., 2012). Consumption of high levels of phytate with breast milk has been shown to compromise minerals bioavailability from the breast milk (Gibson *et al*., 2010).

Daily consumption of phytate can exceed up to 4500 mg per day depending on the quantity of the food consumed and method of processing (Greiner and Jany, 2006). Phytic acid should be lowered for the best health effect to less than 25 mg per 100 g whereas at this level, micronutrient losses are minimized (Onomi *et al*., 2004). This implies that, as the intake of phytate increases, the bioavailability of micronutrients in the diet is more affected. The challenge of achieving micronutrient requirement from predominantly plant-based diets are of major concern particularly due to lack of endogenous phytase enzyme in human being (Gibson *et al*., 2010). The role of phytase enzyme is on dephosphorylation of phytic acid and therefore decreases its binding affinity to divalent and trivalent metal cations.

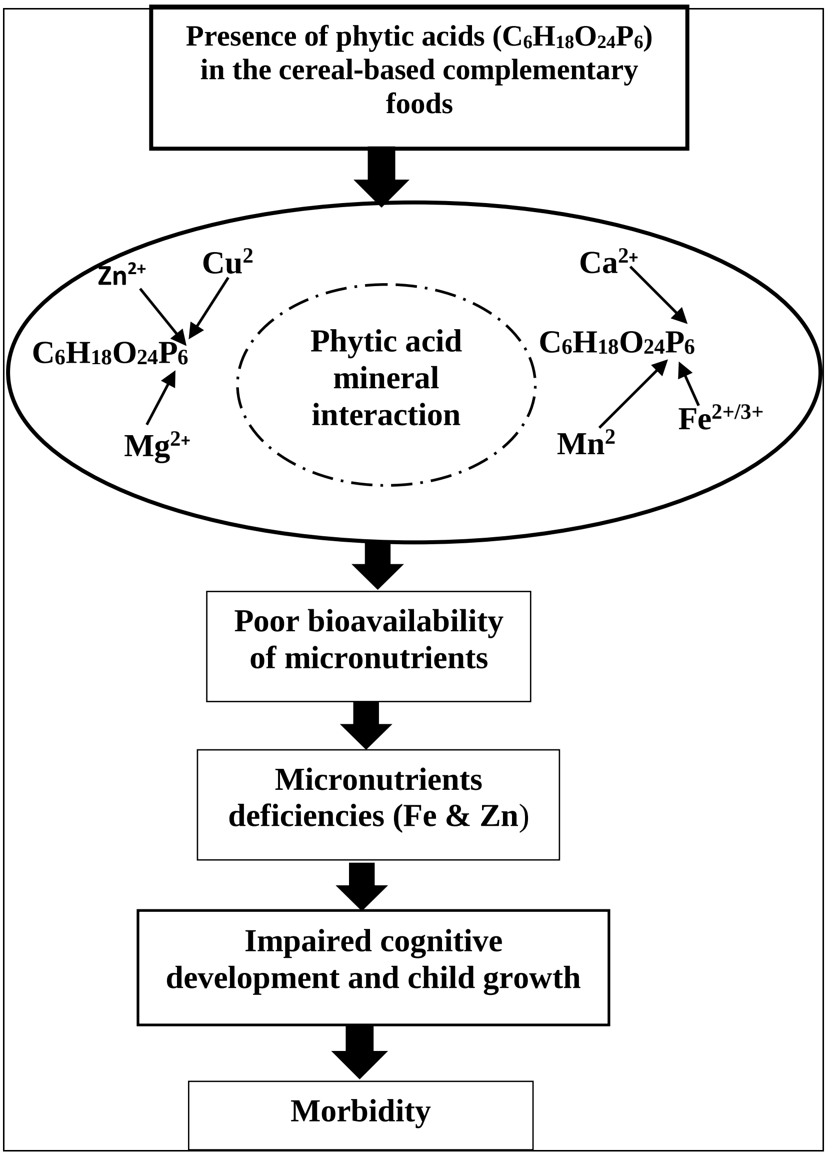


Figure 2. The conceptual framework describing effect of phytate as a dietary related factors contributing to micronutrient deficiency and child under-nutrition

Studies on local and processed complementary foods in developing countries reported high phytate content per each 100 g of dry weight of cereals and legumes food products, whereby the phytate content for unrefined cereals was 600 mg, refined cereals was100 mg and < 20 mg for root and tubers starchy (Gibson *et al*., 2010). Unrefined complementary foods had the highest level of phytate than refined cereals though both exceeded 25 mg per 100g per day, a recommended phytate threshold (Onomi *et al*., 2004). Therefore, consuming food containing high levels of phytate may increase the likelihood of poor bioavailability of minerals and affect micronutrients absorption due to chelation effect (Roos *et al*., 2013).

2.3. Child Health and Micronutrients Needs

Micronutrients are required by the body in a very minute amount, and their role is to maintain all physiological processes in the body (Khan *et al*., 2015). Infants have significantly higher micronutrients requirements to meet their physiological needs in the first two years of life, considered as the period of rapid growth (Moran and Dewey, 2011). Deficiency of micronutrients is normally occur when physiological requirement of the body is not met in a long term. Infants are prone to micronutrient deficiencies due to poor minerals bioavailability, inadequate dietary intake and low intake of animal food sources (Soetan and Oyewole, 2009; Labadarios *et al*., 2011).

Ensuring proper child growth requires provision of all essential nutrients in the right amount at all times to facilitate growth (Golden, 2009). Micronutrients plays a vast role in achieving optimal growth as its deficiency has been shown to increase the rate of illness, contributing to the burden of diseases and death (Bailey *et al*., 2015). Micronutrients deficiency accounts for 7.3% of the burden of diseases worldwide (Ahmed *et al*., 2013). According to World Health Organization (WHO) it is estimated that, a total of 1.62 billion people are anemic which is equivalent to 25% of total population (McLean *et al*., 2009), out of which pre-school children (47.4%) have the highest overall prevalence. Insufficient zinc intake has also been reported to affect 17.3% of the global population, with the highest estimate in African 23.9% followed by Asia 19.4% (Bailey *et al*. (2015) where young children are the most vulnerable group.

Supplementation of micronutrients is the common widely practiced intervention used to manage micronutrients deficiencies. The effectiveness of micronutrients powder supplementation to children (6 – 59 months) and its potential impact on stunting and linear increment was reported by Rah *et al*. (2012). The intervention reduced the prevalence of anemia from 64 - 48% ($P < 0.001$) in Bangladesh (Rohinga refugee camp), whereas the rate of stunting was reduced from 39 - 23% ($P < 0.05$) in Nepal and significance reduction on incidence of diarrhea from 30 - 18% (Rah *et al*., 2012). This confirms that, adequate supply of micronutrients facilitate optimal growth, since children have high demands of micronutrients following increased metabolic requirements.

2.3.1. Importance of iron and zinc on child health

Iron is essential mineral required for ensuring oxygen transportation (hemoglobin), energy transfer within cells (cytochromes) and oxygen storage (myoglobin) (Huang *et al*., 2013; Thejpal, 2015). Iron is vital for normal development and functioning of motor and cognitive during childhood (Beard, 2008; Black, 2012). Proper differentiation of brain cells and neurogenesis requires adequate supply of iron (Linert and Kozlowski, 2012). Normal iron homeostasis in the body is essential to sustain various cellular functions, such as a continuous stability between iron intake, transportation, storage, and utilization (Nadadur *et al*., 2008). The quantity of iron which is absorbed is used as a surrogate measure for bioavailability.

During the first six months of life, iron reserve covers iron demands while infants continuous with breastfeeding. The requirement of iron increases at the age of six months as a result of rapid growth rate, at this age iron supply is mostly depend on complementary foods. Based on the recommended dietary intake of iron for infants between the age of 7 – 11 months and 12 – 23 months are 11 mg/day and 7 mg/day respectively (Domellöf *et al*., 2014). Inadequate dietary intake to foods rich in bioavailable iron has been the major risk factor for iron deficiency. Deficiency of iron is a condition whereby there is insufficient supply of iron in the body to maintain normal physiological function of the tissues, generally characterized by hemoglobin level less than 11.0 g/l (Lundström, 1994).

In developing countries, vulnerability to iron deficiency particularly to children had shown to increase in the first 5 years of life when there is increased iron needs and nutritional requirements (Abbaspour *et al*., 2015). Diet has been considered as the major factor influencing individual's iron status (Petry *et al*., 2014; Beck and Heath, 2013). Inadequate iron intake might be associated with type of the food consumed, routinely consumption of plant-based diets and low intake of animal-rich foods as enhancers of iron absorption. Therefore, in a situation where accessibility and bioavailability of iron are subject to the presence of phytate in the diet, may results into poor absorption and iron deficiency anemia, which is prevalent and persistent in most of the poor resources families in the developing countries.

Zinc is important in maintaining different biological processes of the body including cellular homeostasis, immune functioning, protein synthesis, cell replication and deoxyribonucleic acid (DNA) (Wyman *et al*., 2008; Kayaalti *et al.*, 2015). Zinc deficiency is among factors contributing to morbidity in developing countries (Gopalsamy *et al*., 2015). Children aged between 7 – 23 months require 3 mg/day of zinc to meet recommended dietary intake (USDA, 2009). Exclusively breastfed infants for the first six months obtain sufficient amount of zinc from the breast milk, after wards complementary foods need to contain adequate absorbable zinc required to meet nutritional requirements (Warthon-Medina *et al*., 2015). In healthy individuals, zinc absorption is determined by individual zinc status and bioavailability of zinc from the diet (Solomons, 2013). Deficiency in zinc impedes various organ systems particularly during infancy stage when nutritional needs are very high to meet the nutritional requirement (Huskisson *et al*., 2007).

2.3.2. Phytic acid and Iron absorption

Phytic acid has a very marked inhibitory effect on the absorption of non-heme iron in humans and it occurs when phytate binds iron and affecting its bioavailability (Gulec *et al*., 2014). The negative inhibitory effects of phytate on mineral bioavailability not only depend on the amount of phytate present in the meal but also on the molar ratios of phytate: minerals which predict the level of interaction. The molar ratio of phytate: iron is not supposed to exceed 1, above this recommended level the absorption of minerals will be highly interfered (Mahfuz *et al.*, 2016). Absorption of iron is subjected to numerous factors such as iron content in the diet, bioavailability of dietary iron, iron stores in the body and the degree of erythrocyte production (Thankachan *et al*., 2008). Cereal-based complementary foods contain high levels of phytate and molar ratios sufficient to inhibit iron absorption. The recommended dietary intake of phytate should not exceed 25 mg per 100 g (Onomi *et al*., 2004). (Lazart *et al.* (2015) reported highest levels of both phytate at 2,060 mg/100 g and phytate to iron molar ratio of 68.8 ± 2.4 in peanuts, 44.4 ± 4.2 in maize and 36 ± 8.1 in wheat grain (Lazarte *et al*., 2015). Furthermore, Amare *et al.* (2015) found highest phytate content of 235 – 893 mg/100 g and the molar ratio in a range of 0.89 – 7.07 in seven locally produced cereals-based complementary foods in Ethiopia (Amare *et al*., 2015). These high levels of phytate and its molar ratio in complementary foods were above tolerable limit hence might have increased the inhibitory effects of iron on the absorption process. Poor bioavailability of iron from plant-based foods contributes significantly to iron deficiency.

Once released from food, non-heme iron is present as ferric (Fe^{3+}) which cannot be absorbed because of its insolubility character, until it undergo reduction process to ferrous (Fe^{2+}) (Fuqua *et al*., 2012) by the reaction of ferric-reductase. Reduced iron form which is ferrous (Fe^{2+}) is transported across the membrane into the enterocytes through apical membrane of the duodenum by the divalent metal transporter 1 (DMT1) (Wyman *et al*., 2008). The main transporter for ferrous iron in the intestine is divalent cation (Kayaalti *et al*., 2015). In the presence of phytate the reduced iron which is divalent cation interacts with negative charges of phosphate group to form phytate-iron insoluble complexes hence minimizing bioavailability of iron in the body (Lazarte *et al*., 2015).

The effect of phytate on iron absorption is dose dependent, it occurs at very low level (Bohn, *et al*., 2008) even when ratios of phytate to iron are 0.2:1.0, phytate exhibits inhibitory effect. Effect of iron absorption is predicted by using phytate molar ratio to iron (Abbaspour *et al.*, 2015). Lowering the molar ratio of cereal and legume based meals with no enhancers to the ratio of 1:1 or less than 0.4:1 improves iron absorption (Maren *et al*., 2015). Zimmermann *et al*. (2005) studied the alteration of iron status to children who were fed with a diet contained high phytate. Children were tracked for 15 months, and the results revealed that, the source of iron in the diet comprised cereals by 57% and legumes by 13%. After 15 months of the intervention results indicated that, the prevalence of anemia increased from 0% at the baseline to 43% at the final survey (Zimmermann *et al*., 2005). Findings demonstrate that, high levels of phytate in plant foods could be a contributing risk factor for iron deficiency due to its effect on poor bioavailability, absorption and utilization in the body.

2.3.3. Phytic acid and zinc absorption

On the other hand, phytic acid is among the dietary factors which have shown to affect zinc absorption through chelation effect. A metabolic study investigated zinc homeostasis by administration of high phytate maize-based diet to Malawian children aged between 2 -5 years, results showed low plasma zinc concentration than the mean of reference populations, indicating that the presence of phytate in the diet increases the likelihood of zinc deficiency (Manary *et al.,* 2002). Similarly, it was observed that fractional absorption of zinc is negatively affected by the presence of phytate in the diet (Erdman and Poneros-Schneier, 2013; Maret, 2013). In another study by Kirksey *et al.* (1994) it was shown that, mothers' diets during pregnancy has influence on the motor performance of early infant's behavior and development. Poor motor performance was observed in infants whose mothers consumed plant-based foods (Kirksey *et al*., 1994). Therefore, consumption of diets containing relatively low bioavailable zinc affects plasma zinc concentration, hence the quantity of zinc consumed and amount absorbed is inversely related.

2.4. Health consequences of Iron Deficiency

Deficiency of iron occurs when iron stores are depleted, and red blood cells are inadequate to regulate the normal physiological process and functioning of body tissues (Rao and Parikh, 2015). The most susceptible cause of anemia is iron deficiency although there are other causes such as chronic and acute infections that cause inflammation, micronutrients deficiencies and genetically inherited traits which tend to affect hemoglobin synthesis and production of red blood cells (Kevin, 2014). Iron deficiency anaemia imparts health consequences to human as described below;

Decrease fitness: Low hemoglobin concentration below recommended cut-offs levels leads to reduced oxygen transportation from lungs to the tissue where it is required. Decrease in the circulating hemoglobin concentration in the blood is associated with a reduced oxygen carrying capacity and declined the level of physical activity which affect performance due to decreased oxygen consumption and aerobic power (Dellavalle, 2013). The mechanism by which iron deficiency anemia reduce fitness/physical activity is by reducing tissue oxidative capacity by impairing aerobic capacity, low oxygen carrying capacity affect energetic efficiency and endurance due to decreased oxygen delivery (Haas and Iv, 2001). Therefore, children may have a problem related to decrease fitness/physical activity due to decreased supply of oxygen caused by iron deficiency.

Impaired cognitive and motor development: Childhood anemia poses an increased risk of adverse consequences on motor and cognitive development (Thejpal, 2015; Nyaradi *et al*., 2013). Vulnerability in brain development increases during last trimester (in fetal life) and continues up to 24 months, this is very critical stages of growth in life (McCann *et al*., 2006). Poor cognitive development and lower motor test score to infants who were anemic have been confirmed (Sachdev *et al*., 2005). According to (Chang *et al*. (2013) a significantly lower mental developmental index ($p = 0.036$) in pre-natal group with iron deficiency anemia was reported (Chang *et al*., 2013). Additionally, lower mental developmental score at 12, 18 and 24 months was observed in anemic pre-natal. The attributable cognitive effect to 9 months children with iron deficiency anemia were having low attention and recognition memory, which were mediated with effect of iron deficiency (Carter and Jacobson, 2010). Furthermore, effect of iron deficiency anemia during infancy is associated with alteration of neuro-functional development and coordination, its effect is not recognized during that particular time of deficiency instead it is observed later in life (Algarin *et al*., 2013). Myelination of parts of the brain mostly occurs in the first 24 months of life when there is iron deficiency (Walczyk *et al*., 2014). Experimental studies confirmed the cognitive impairment in childhood is caused by iron deficiency, which damage brain mitochondrial (Warthon-Medina *et al*., 2015). The impairment of cognitive development occurs at an early stage of life (childhood), the process is irreversible throughout the life time regardless the application of adequate medical therapy (Lozoff *et al*., 2000). Horton and Ross (2003) projected the productivity loss outcome due to anemia cases to be 2.32 US$ per capita or 4.05% of Gross Domestic Product (GDP) (Horton and Ross, 2003).

Poor life outcome: Iron deficiency during childhood can lead to poor life outcome at individual level and society as well. A study in Costa Rica (Lozoff *et al*., 2013) assessed lives of people who had iron deficiency during infancy, however they were treated and followed up for 25 years to monitor the consequences of iron deficiency in the entire life. Poor life outcome have been observed to those who developed iron deficiency in childhood and the condition were corrected. At an average of 25 years, 42% did not accomplish secondary school education and 68% were not attending any informal or formal educational related matters. For individuals who developed chronic iron deficiency, 67% did not complete secondary school education, 84% were not engaged in any informal or formal education and 25% were jobless. Furthermore, they ranked their emotional health as feeling (nervous, dispassion, sadness and detachment). Findings revealed that iron deficiency and chronic iron deficiency during childhood expose infants to high risk of becoming adults with poor life outcome due to poor cognitive development. Therefore adequate supply of iron during infants is very crucial in achieving optimal growth.

2.5. Health consequences of Zinc deficiency

The effect of zinc deficiency is of most significance during infancy when zinc requirement is very high as a result of limitations in body stores and if a child is susceptible to infectious diseases (Bailey *et al*., 2015). Interference of metabolism of thyroid hormones occurs when zinc is deficient, which has potential effect on impairment of growth, suppression of immunity and increase episodes of diarrhea and rate of infectious diseases *(*Brown *et al.*, 2009*;* Deshpande *et al*., 2013; Fischer *et al.*, 2009). Pituitary gland contains higher concentration of zinc, that enhance it to function, secretion of growth hormone is limited when zinc deficiency occur (Miletta *et al*., 2013). Effectiveness of zinc supplementation on growth increment has been reported (Hamza *et al*., 2012; Brown *et al*., 2002). Similarly, zinc supplementation has shown a positive effect on accelerating growth, by enhancing normal functioning of thyroid and growth hormone to facilitate optimal growth (Roohani *et al*., 2013). In addition, zinc deficiency affects multiple system involved on immunity of the body including natural killer cells and neutrophils. Production of macrophages, cytokine, phagocytosis and intracellular is limited when zinc is deficiency (Mayer *et al*., 2014). Similarly, T cells generation and cell mediated immunity is reduced when there is zinc deficiency, frequency and number of infectious diseases including diarrhea tend to increase due to inability of macrophages to capture and destroy parasites (Kulkarni *et al*., 2012). Diarrhea is known to contribute to growth failure approximately by 25 – 30% in developing countries (Patwari, 1999). A positive effect of zinc supplementation on reducing the rate of acute diarrhea cases by shorten time (15%), persistent (15.5%) and frequency of passage of stool (18.8%) have been observed (Lukacik *et al*., 2008). Therefore, if the body is supplied with adequate zinc that meet physiological needs would be able to fight and prevent the re-occurrence of infectious diseases to growing children.

2.6. Options for reducing phytic acid in cereal-based complementary foods

Phytic acid is widely present in plant-based food, it can be reduced by mechanical, physical and enzymatic processing such as milling, soaking, fermentation and germination/malting respectively as presented in Table 1. Methods that can reduce phytic acid in cereals while increase the bioavailability of minerals would be the most beneficial. These strategies that intended to increase the physicochemical accessibility of micronutrients by decreasing the content of anti-nutritional factors, such as phytic acid hence improve bioavailability, digestibility and absorption. Therefore, wide applications of appropriate technologies are essential to enhance bioavailability of minerals for a potential nutrient intake through the diet.

Table 1. Pre-preparation methods to reduce phytate level and enhance mineral bioavailability

Method	**Mode of action**	**Type of food**	**% of Phytate reduction**	**Reference**
Germination	Degradation of phytate through phytase activity, decrease binding affinity of phytate to minerals	Horicot bean	79	Getenesh *et al*. (2014)
Fermentation	Reduce number of phosphate group, increase solubility of phytate and being unable to bind minerals	Cereals	Up to 90	Hotz and Gibson (2007)
Soaking	Water soften the outer cell wall, allowing leaching of water soluble components including phytate. -Phytate is soluble in water	Brown rice	42 – 59	Liang *et al.* (2008)
Dehulling	Removes the outer layer (bran) from grain, accumulated with anti-nutritional factors including phytate	Cereals	50	Lestienne *et al*. (2007)
Malting	A combination of soaking and germination, phytase degrade phytate	Oat	98	Larson and Sandberg (1992)

2.7. Conclusion

Nutrition in early life has extended and ever lasting impact at individual and society level. Normal growth pattern is achieved by provision of all essential nutrients that are necessary to meet the infant’s nutritional requirements. Cereals and legumes play important role in the diet, contribute substantial amount of protein, carbohydrate, minerals and phytate as well. Presence of phytate in complementary food has negative nutritional impact to the child health and development. Eliminating cereals from the diet may not be feasible, thus the focus should be on maintaining phytate at acceptable levels or tolerable daily intake which will not impose deleterious health effect to infants and young children. This can be achieved by application of food processing methods and use of phytase enzyme which enhance bioavailability of minerals, by reversing the strong inhibitory effect that phytate and other anti-nutritional factors exerts on cereals-based meals.

CHAPTER THREE

Assessment of Factors Associated with Stunting in Dodoma Municipality and Chamwino District, Dodoma Region[2]

Abstract

Child under nutrition is major public health concerns, claiming lives of numerous children below five years of age in the developing world. The objective of this study was to assess the factors associated with stunting in children of age 6 – 23 months in Dodoma Municipality and Chamwino District of Dodoma region. Dodoma region located in the central zone of Tanzania was chosen for this study because of its high prevalence (56%) of stunting among children of under five years of age. Simple random sampling was used to choose villages and children 6 -23 months of age in those villages were included, resulting in 394 households participating in this cross sectional study. A standardized questionnaire was used to collect socio-demographic information and infant feeding practices. Anthropometric measurements of children were taken as per WHO standard procedures. Repeated 24 hour dietary recall was used to assess food intake among study population. The overall prevalence of stunting was 40.4% (95% CI; 29.8; 50.9), Chamwino District had higher prevalence of stunting (44.3%) compared to Dodoma municipality (26.3%). Multivariate logistic regression analysis showed that, age of introduction of complementary food [AOR = 13.3; (95% CI: 2.6 – 67.6)], maternal education [AOR = 5.5; (95% CI: 1.0 – 9.8)], residence in Chamwino District [AOR = 3.2; (95% CI: 1.3 – 5.9)] were factors associated with stunting. About half of the study population (49%) was introduced to complementary foods early, the median age was four months instead of 6 months as recommended by WHO. Infant's diet were mainly cereal based, other foods such as animal food source, fruits and dairy products were consumed by 18% 11% and 7.1% of children respectively. The dietary pattern of infants was not diversified, 47.7% of infants scored 1 to 3 points out of 12 points categorized as low dietary diversity. Failure to attain normal growth pattern is the most prevalent form of under-nutrition in childhood. Associated factors are many, diverse and interrelated, the present study identified maternal education, early introduction of complementary foods and being a

---

[2] *Accepted with minor corrections; AJFAND 17000*

resident of Chamwino were factors identified to be associated with stunting. Prioritization of identified factors serves as highlight for better planning of nutrition intervention programs that intend to promote child growth.

Key words: Stunting, nutrition status, 6 – 23 months, dietary diversity and complementary foods

3.1. Introduction

Under nutrition continues to threaten growth, survival and development of infants and young children in developing countries (Bhutta and Salam, 2012). Globally, 165 million children of age below 5 years have been reported to be stunted (Black *et al*., 2013). In addition, 45% of death of children in this age group is associated with under nutrition (Black *et al*., 2013). The prevalence of under nutrition is high between the ages of 6 – 23 months. This is a period of rapid growth and high nutrient requirements; therefore it is a critical period for growth faltering and nutritional deficiencies if the nutritional needs of children are not attained (Dewey, 2013). Stunting in childhood is caused by many factors including diseases, food insecurity, lack of access to health services, inadequate maternal/child caring practices and also inadequate dietary intake which is an outcome of prolonged deprivation of essential nutrients to meet demands for growth. Stunting has adverse health consequences that may extend to the adulthood (Prendergast and Humphrey, 2014). Poor cognitive ability and educational attainment have been confirmed in longitudinal studies as being the consequence of stunting in children, which tend to impart extended detrimental effect in adulthood (Casale *et al*., 2014; Grantham-McGregor *et al*., 2007; Sokolovic *et al*., 2014; Sudfeld *et al*., 2015; Walker *et al*., 2015).

In Tanzania, stunting is still a public health concern affecting 34% of children of age below five years (NBS, 2016). Reports of the Tanzania Demographic Health Survey (TDHS) have shown trends of child stunting, in 1996 and 1999 the prevalence of stunting was 43.4% and 43.8% respectively (NBS, 1997; NBS, 2000). In 2010 and 2015/2016, 42% and 34% of children were reported to be stunted respectively, the prevalence has been declined by 9.3% for the past 20 years (NBS, 2011; NBS, 2016). Dodoma region is one of the regions in Tanzania was having high prevalence of stunting. In 2010, about 56% of children were reported to be stunted and in 2015 the prevalence has been reduced to 36%. The decline of prevalence of stunting might be contributed by establishment of clear coordination of nutritional activities and increased allocation of human and financial resources on nutritional related intervention programs in the country.

The prevailing causes of stunting are multifactorial ranging from Infections (parasitic, HIV, helminths), insufficient intake and poor absorption of nutrients, sub-optimal breastfeeding practices, poor child care practices and poverty (Fekadu *et al*., 2015). The need to investigate factors contributing to under nutrition among children is very crucial especially in breaking the intergenerational cycle of malnutrition. However, factors associated with stunting have been studied and observed to vary from one area to another based on geographical location, ethnicity and cultural practices. Therefore, this study assessed the independent factors associated with stunting among children of age 6 to 23 months in Dodoma municipality and Chamwino District of Dodoma region for development and strengthening appropriate nutritional strategies and programs intended to promote child growth.

3.2. Methodology

3.2.1. Study population

A total of 394 children within the age range of 6 to 23 months were participated in the study. In each household, only one child was assessed. Criteria for selection of respondents were based on age and area or residence (Chamwino and Dodoma Municipality). Twins, disabled and seriously sick children were not included in the study.

3.2.2. Study Design and Sampling Procedure

A cross sectional study was conducted in Dodoma region to assess factors associated with stunting among infants and young children. Dodoma region was purposively selected for being a study area because it was ranking the first region in the country having the highest prevalence (56%) of stunting in children of under five years of age (NBS, 2011). The sample size for this study was determined by using formula for cross sectional studies by Varkevisser *et al.* (1991) whereby the minimum sample size was 378 subjects, however a total of 394 children (204 male and 190 female) were recruited in the study (Varkevisser *et al*., 1991). Simple random sampling technique by using random numbers was used to select two

Districts out of seven Districts in the region, Dodoma Municipality and Chamwino district were picked to be a study area. Sample size among the two districts was distributed by using the prevalence of stunting of the respective district, therefore a total of 175 (82 female and 93 male) children were recruited in Dodoma Municipality and 219 (108 female and 111 male) children in Chamwino District. A systematic random sampling technique was used to select ward and villages participated in this study, a list obtained from ward executive officers was used to select respective wards and villages involved in this study whereby after every n^{th} interval ward and village was selected from the list. Therefore three wards of Dodoma Municipality (Makole, Chigongwe and Mahoma Makulu) and four wards of Chamwino District (Mvumi Mission, Mlowa, Ikowa and Manchali) were chosen to be a study area. A total of 14 villages were covered in this study and in each selected ward two villages were randomly sampled. A list of all households that were having children of age between 6 and 23 months was obtained from the respective village offices, systematic randomly sampling was used to select households recruited in the study area.

3.2.3. Household Interviews and Anthropometric Measurement

A structured questionnaire was pre-tested and refined before it was administered to mothers/caregivers at the household to collect socio demographic data. Information on food consumption was collected twice in two non-consecutive visits using a 24 hour dietary recall method. During interview, each parents/guardian was asked to recall all types of foods and beverage consumed by their infants in the past 24 hours. Mothers were asked to provide detailed information about applied food preparation methods and type of ingredients used. Amount of food reported to be consumed in 24 h dietary recall was estimated by using household utensils such as cups, bowls and spoons. The estimated portion was weighed using a kitchen scale (CAMRY, model EK3131) and the weight was recorded accordingly. Dietary Diversity Scores (DDS) was estimated by using dietary information collected from the 24 hour dietary recall. Dietary diversity of study population was measured using a scale of twelve food groups as per the Food and Agriculture Organization (FAO) to measure DDS (FAO, 2008). Assessment of dietary diversity was done based on the number of food groups consumed over the past 24 hours. These food groups include; cereals based foods, roots and tubers, legumes, fruits, vegetables, fish, eggs, meat and milk product, milk and milk products, sugar, oil and fats, spices and condiments. To each food group consumed over a reference period, a point was given, and then, the DDS was calculated as a summation of all points scored. Dietary diversity categories were derived from 12 points as follows; low dietary diversity 1 – 3 points, medium dietary diversity 4 – 6 points and high dietary diversity 7 – 12 points. Diversity of the diet was judged based on the scores.

Anthropometry measurement of children was performed according to the World Health Organization guideline (WHO, 1995). Recumbent length of a child was measured using a length board (210; Seca, Hamburg, Germany) to the nearest 0.1 cm. A combined weight of both mother and a child was measured using a standard weighing scale (HD-386-BK; Tanita, Tokyo, Japan) to the nearest 0.1 kg. Weight of a child was then recorded as a result of subtracting mother's weight from a combined weight of the mother and a child.

3.2.4. Ethical Clearance

Ethical approval for conducting this study was obtained from the National Health Research Ethics Sub-Committee (NatHREC) of the National Institute of Medical Research (NIMR) of Tanzania, reference number NIMR/HQ/R.8a/Vol.IX/1973. Parent or care taker of each individual child signed an informed consent. Confidentiality was observed throughout the study.

3.2.5. Statistical analysis

Data and responses from questionnaire were coded and double entered into an Epidata version 3.1 by two independent person. Emergency Nutrition Assessment (ENA) for SMART version 2011 was used to analyze data obtained from anthropometric measurement. Z-scores and prevalence of stunting was calculated according to the child growth standards of the WHO (Schwarz *et al*., 2008). Data from the household survey were then exported to SPSS for Windows software (IBM version 21) for analysis. Difference between groups was compared using Pearson Chi-square statistical test whereas Crude Odds Ratio (COR) and Adjusted Odds Ratio (AOR) with 95% Confidence Interval (CI) were used to assess the strength of association. Differences were considered statistically significant if $p \leq 0.05$. Variables that were significant in the univariate analysis were analyzed using multivariate analysis. Multivariate logistic regression modeling was used to assess factors of stunting. The procedure used was backward stepwise selection with removal testing that was based on the probability of the likelihood ratio statistic. The significance level of a likelihood ratio statistic was compared to a cut-off value of $p \leq 0.1$. Seven variables (Table 2) were considered in initial model, only 3 variables were considered significant based on the *p* value for likelihood ratio and nutritional epidemiological importance. District was not considered in univariate analysis, rather in the multivariate model because it was an important exposure.

3.3. Results

3.3.1. Demographic characteristics

A total of 394 children were involved in the study, 204 (51.8%) were males and 190 females (48.2%). The mean age of children was 13.7 ± 5.3 months and that of mothers was 28.2 ± 7.3 years respectively. Table 1 summarizes the social demographic information of the studied children and their mothers/care givers. Majority (82.3%) of the households were living below the poverty line as they spend less than 1 USD per day. Number of household members ranged between 2 and 20 people, with the mean of 5 people per household.

Table 2. Social Demographic information

Variable	Dodoma Municipality	Chamwino	Total
Children, N = 394	**n = 175**	**n = 219**	**n = 394**
	(%)	**(%)**	**(%)**
Sex of the child			
Male	53.1	50.7	51.8
Female	46.9	49.3	48.2
Age of the child			
6 - 11 years	44.6	35.2	39.9
12 - 23 years	55.4	64.8	60.1
Mothers/care takers, N=394	**n = 175**	**n = 219**	**n = 394**
Age of the mother			
14 – 25 years	44.0	41.6	42.6
>25 years	56.0	58.4	57.4
Education level			
None	34.9	28.8	31.5
Primary education	52.6	65.3	59.6
Secondary and above	12.5	5.9	8.9
Occupation			
Employed	6.3	3.7	4.8
Agriculture	66.3	89	78.9
Livestock keeping	0.6	0.9	0.8
Business	26.8	6.4	15.5
Marital status			
Married	85.1	93.2	89.6
Single	8	5.9	6.9
Divorced	4	0	1.7
Widow	2.9	0.9	1.8

3.3.2. Feeding Practices and Dietary Pattern

Results indicated that 91% (n=357) of children were still breastfeeding, 49% of infants were introduced to complementary foods before the age of 6 months. The median age of initiation of complementary food was four months. Factors associated with early introduction of complementary food include; mother's own perception that her infant was not satisfied with breast milk, mothers felt that they did not have enough milk, cases of crying baby, demand for mothers to resume their work, and some mothers wanted their infants to get used to complementary feeding before recommended age. The number of meals given to infants ranged between one to five meals per day, with the mode of three meals per day. Feeding frequency differed among age groups whereby for infants who were less than one year, 1.4% were fed four times, 54% three times, 43% twice and 1.6% once per day. Those infants who were above one year, 2.8% were fed fifth times, 42% fourth times, 46.2% three times and 9% twice per day. Furthermore, the study found that 65% of mothers do not know exactly how many times a child is supposed to be fed in a day.

The typical dietary pattern of children was characterized by high consumption of cereal based products such as maize or millet along with vegetables. The overall mean for dietary diversity was 3.6 ± 1.0 with a range of 2 – 8 points. Based on dietary diversity categorization, 51.5% of children were categorized as having medium dietary diversity score (4 – 6 points), 47.7% on low dietary diversity score (1 – 3 points) and 0.8% on high dietary diversity score (7 – 12 points). The distribution of DDS for Chamwino and Dodoma Municipality is shown in Fig. 3, Chamwino District had a higher proportion of children who had low DDS (50.5%) compared to Dodoma Municipality (40%). A high proportion (58.3%) of children in Dodoma Municipality had medium DDS. Food groups consumed by infants and young children is presented in Table 3. Cereals were the most dominant food and were consumed by 100% of children. Animal source food were least consumed (18%) except for breast milk. Other foods such as fruits and dairy products were consumed by a small proportion of children by 11% and 7.1% respectively.

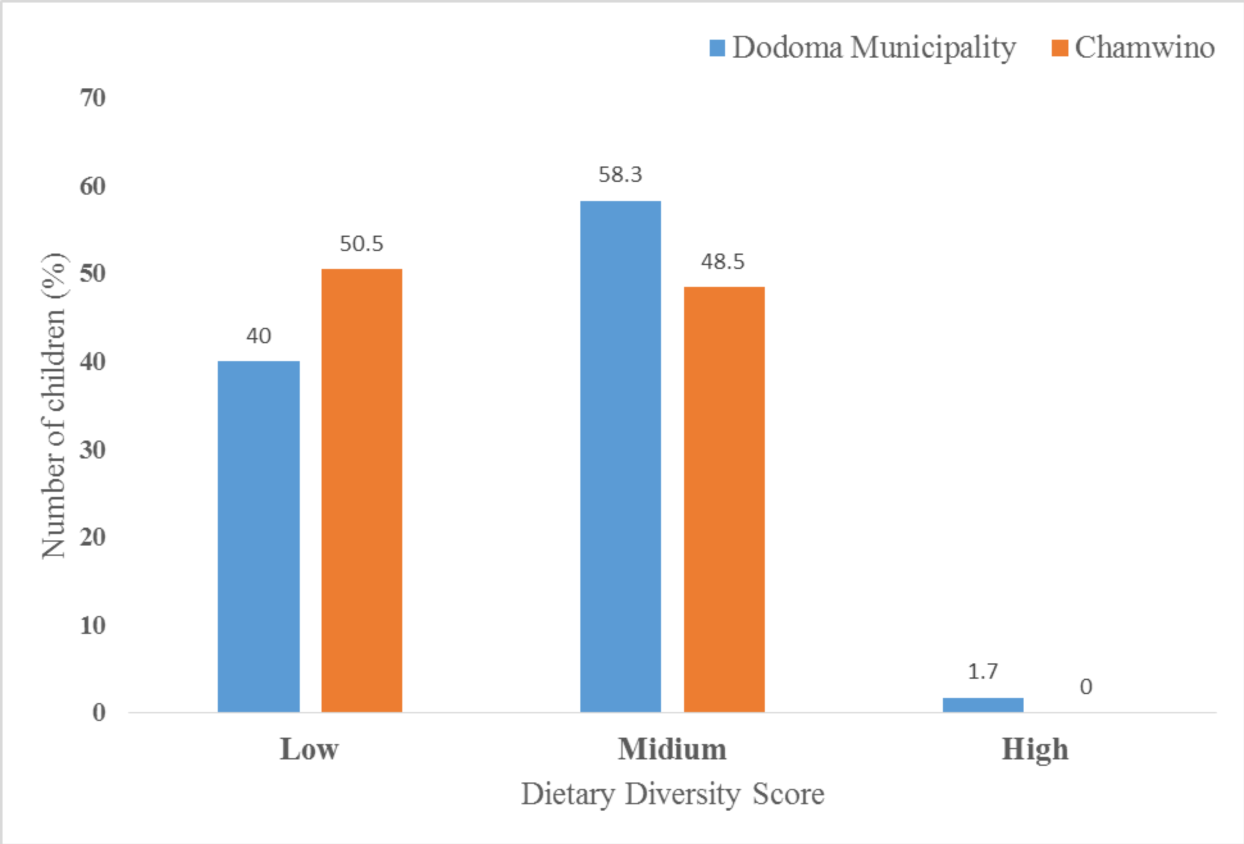


Figure 3. Dietary Diversity Scores of Dodoma Municipality and Chamwino District

Table 3. Consumption of different types of food groups by children

Food group	**DDS: % of children consuming each food group**
Cereals based foods	100
Roots and tubers (banana, potatoes, cassava)	18.1
Legumes	29.4
Fruits	7.1
Vegetables	57.9
Meat and meat product	18
Fish	8.9
Eggs	2.3
Milk	11
Oil and fats	72
Sugars	49
Others (Spices and Condiments)	29.4

3.3.3. Nutritional Status

The distribution of height-for-age of the studied population is shown in Fig. 4 which shows the curve is deviated from the median, skewed to the left and less flatter in comparison to the WHO, Z score reference population of the same age group. The mean Z-scores was -1.61 ± 1.30, and the overall prevalence of stunting was 40.4% (95% CI; 29.8% - 50.9%). Variation on the prevalence of stunting between the two Districts was observed, whereas Dodoma Municipality had prevalence of 26.3% (95% CI; 23.2% - 29.6%) and that of Chamwino was 44.3% (95% CI; 37.9% - 50.9%). Children of age between 12 – 23 months were more stunted (82%) compared to those of age 6 – 11 months ($p \leq 0.001$). The stunting rate of boys was higher 46.9% (95% CI; 34.4% - 59.5%) compared to that of girls 32.8% (95% CI; 19.7% - 45.8%). Factors associated with stunting varied between the two Districts as presented in Table 4.

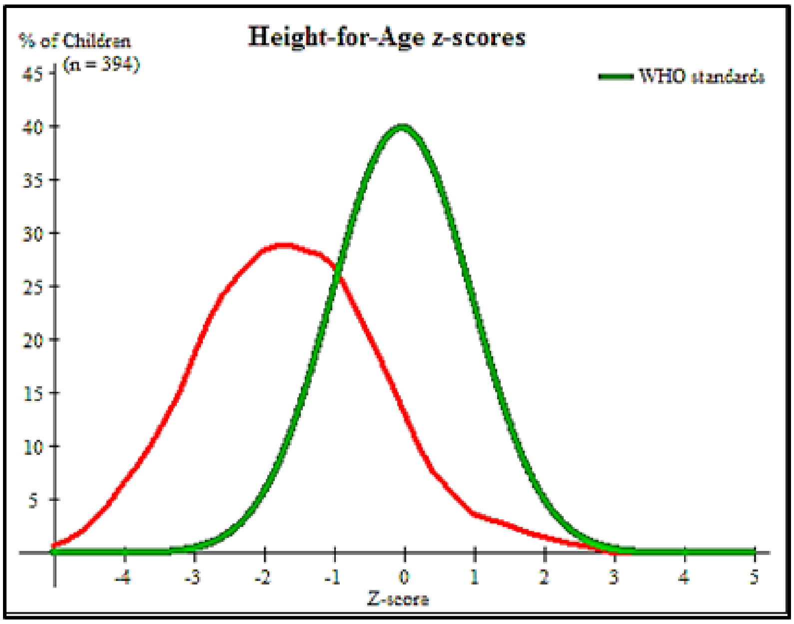


Figure 4. Distribution of height for age Z-scores compared to WHO growth standards in Chamwino and Dodoma Municipality.

Table 4. Factors associated with stunting in Dodoma Municipality and Chamwino District

	Dodoma Municipality			**Chamwino District**		
Variable	**% stun**	**COR(95%CI)**	**AOR(95%CI)**	**% stun**	**COR(95%CI)**	**AOR(95%CI)**
Children N=394	**n=175**			**n=219**		
DDS**						
Low	41.9	1.4(0.7-2.8)	0.4(0.01-0.2)	34.5	3.3(1.9-5.7)*	0.1(0.02-0.2)
Medium	17.4	1		17.4	1	
Birth weight						
< 2.5	23.4	1.4(0.6-3.2)	0.1(0.01-0.5)	4.6	3.5(1.6-7.8)*	0.1(0.01-0.9)
> 2.6	20.0	1		34.2	1	
Age of a child						
6 - 11 months	14	1		32.6	1	
12- 23 months	86	0.1(0.0-0.2)*	0.4(0.2(0.9)	67.4	0.5(0.3-1)*	1.3(0.6-2.8)
Feeding practices						
Below	68.6	0.9(0.2-3.6)	1.3(0.7-2.4)	49.8	0.9(0.3-2.4)	0.3(0.1-0.5)
Normal	24.6	1		41.1	1	
Age of 1ntroduction of CF						
1 - 5 months	41.7	5.3(2.1-14.5)*	0.2(0.1-0.5)	40.6	0.02(0.01-0.05)*	0.2(0.1-0.5)
> 6 months	32.0	1		14.2	1	
Sex of a child						
Male	63	1.7(0.8-3.4)	18.1(6.0-54.2)	58.6	0.5(0.3-0.9)*	20.3(6.8-60.5)
Female	37	1		41.4	1	
Mother N=394	**n=175**			**n=219**		
Age of the mother						
< 25 years	33.1	0.8(0.4-1.7)	9.4(3.5-24.8)	22.4	0.9(0.5-1.6)	1(0.6-1.8)
> 26 years	40.6	1		32.4	1	
Maternal level of education						
No formal	62.8	0.01(0.0-0.4)*	0.1(0.03-0.4)	50.9	0.004(0.00-0.01)*	0.2(0.1-0.5)
Primary education	7.7	1		2.8	1	
Source of income						
Agriculture	50.3	0.7(0.4-1.5)	18.1(7.15-35.9)	47.5	1.7(0.7-4.3)	0.01(0.00-0.03)
Non agriculture	10.3	1		3.7	1	

Significance at $p < 0.05$, Stun stunting, *CF* complementary food, ** *Some information (with extremity) were excluded*

3.3.4. Multivariate analysis for factors of stunting

Results from multivariate analysis of factors for stunting are presented in Table 5. The multiple logistic regression analysis showed that factors that were statistically significant and independently associated with stunting were age at which a child introduced to complementary foods (AOR = 13.3, 95%CI, 2.6 – 67.6), maternal education (AOR = 5.5, 95%CI, 1.0 – 9.8) and residence in Chamwino District (AOR = 3.2, 95%, 1.3 – 5.9).

Table 5. Multivariate logistic regression of factors of stunting, N=394

Variable	N	% stun	COR(95%CI)	AOR(95%CI)
DDS**				
Low	180	43.8	1.9(1.3-2.8)	0.6(0.2-1.3)
Medium	210	56.2	1	
Maternal level of education**				
No formal	153	93.1	0.006(0.003-0.01)	5.5(1.0-9.8)*
Primary education	217	6.9	1	
Age of a child				
6 - 11 months	155	20	1	
12- 23 months	239	80	0.24(0.2-0.4)	0.2(0.06-1.2)
Age of 1ntroduction of CF				
1 - 5 months	226	95.9	0.02(0.01-0.06)	13.3(2.6-67.7)*
> 6 months	168	4.1	1	
District				
Chamwino	219	68.3	2.3(1.5-3.5)	3.2(1.3 – 5.9)*
Dodoma Municipality	175	31.7	1	
Occupation				
Agriculture	311	48.7	1.3(0.8 -2.3)	0.4(0.1-1.9)
Non agriculture	83	6.6	1	
Feeding				
Below	362	58.1	0.9(0.4-2.0)	0.6(0.3-1.5)
Normal	32	3	1	

*1 Reference, *Significant at p < 0.05, ** Some information (with extremity) were excluded, stun* Stunting

3.4. Discussion

The distribution of height-for-age Z-scores of studied population deviated from that of the WHO reference population indicating under nutrition is prevalent among children whereby studied population failed to acquire normal height for age. Stunting prevalence was high (40.4%) according to WHO national growth estimates (Schwarz *et al.*, 2008). The level of stunting reported in this study is similar to that reported in Kilimanjaro region (40.1%)

(Abubakar *et al*., 2012; Asfaw *et al*., 2015). Stunting remains a significant public health concern in Dodoma region with prevalence of 56% in 2010, 45.2% in 2014 and 40.4% in 2015 as reported by the current study (NBS, 2011; Welfare, 2015). This show a substantial decline of stunting rate but also this demonstrate a continuous failure to attain optimal growth due to increased risk of exposure to associated adverse health and nutritional conditions for a long time. Factors associated with stunting originate from diverse yet interrelated variables, some of which have been identified from this study as maternal education, age of introduction of complementary food and area of residence.

Multivariate regression analysis indicated that the odds of stunting were 13.3 times higher among children who were introduced to complementary food before the age of six months than for those whose introduction was timely (p = 0.002). Time for introduction of complementary foods is determined by several factors including ethnicity, geographical location, social and economic situation (Muhimbula and Issa-zacharia, 2010). The rate of early introduction of complementary foods was higher (67%) in Chamwino District compared to Dodoma Municipality (48%). This might be due to most of the mothers in rural areas spending much of their time on farms with less time to breastfeed. Introduction of complementary food before the recommended age of six months has been shown to be a contributing factor of poor growth rate in developing countries (Patel *et al*., 2012). Most of the complementary foods given to infants are nutritionally inadequate in term of micronutrients and protein content to meet infant’s physiological requirement, thus may predispose child to the risk of growth faltering (Anigo *et al*., 2009). Early introduction of complementary food reduce the volume of breast milk ingested by infants due to limited gastric capacity and predispose infants to reduced protective benefits of breast milk (Tang *et al*., 2015). However, poor hygienic practices during preparation and feeding of complementary food, increases the risk of infant exposure to infection and diarrhea, also contributing to poor growth rate (Romulus-Nieuwelink *et al*., 2011). Islam *et al*. (2012) observed that, 40% of complementary foods given to children were contaminated with coliform bacteria as a result of poor hygienic practices.

The present study has shown that the rate of child stunting was high in children under the care of uneducated mothers compared to those that are being cared by educated mothers. Children whose mothers did not attend any formal education had a 5.5 folds higher odds of being stunted than those who attended primary education (p = 0.004). A mother is a primary care provider to child needs, but the type of care depends on literacy and awareness of the mother (Bbaale, 2014). Maternal education has greater influence on child nutritional status but also it depends on level of education attained, however high education level has impact on socio economic status compared to low level (Ali *et al*., 2011; Chen and Li, 2009). Various factors are associated with maternal education and child nutritional status, these include utilization of health knowledge, small family size and increased income (Anwar *et al*., 2013). Literate mothers have improved knowledge related to health care utilization, child care and feeding practices which significantly affect child's health and nutrition status. Abuya *et al.* (2012) observed that, low maternal education was a significant factor for chronic under nutrition of infant and young children. Negash *et al.* (2015) observed a strong relationship between child nutritional status, mother's education and social economic status. Frost *et al*. (2005) reported that the primary pathway connecting maternal education and child nutritional status is socio-economic status, though the utilization of health care information accounted for portions of the maternal education effect.

Variation of stunted growth to children between Dodoma Municipality and Chamwino Districts has been observed in this study. The prevalence of stunting was very high (44.3%) in Chamwino District as compared to Dodoma Municipality (26.3%). District of residence was significantly related to stunted growth, whereby children who resided in Chamwino District were more stunted (3.2 times) compared to those in Dodoma Municipality (p = 0.002). The differences in prevalence of stunting between Chamwino District and Dodoma Municipality could be attributed to several factors including young age of the mother, early pregnancies, low maternal education, income differences and cultural practices. Also households in Chamwino District reported to have inadequate food consumption as most could access only two meals per day. Other studies also reported similar observations that children residing in rural areas were more stunted than those in urban (Amare Zelellw, 2011; Nouri *et al*., 2014).

Although there was no significant association between feeding frequency and stunting in multivariate analysis, the dietary pattern of studied population was characterized by low DDS. About 48% (p = 0.08) of household, consumed less than three food groups hence the probability of attaining adequate nutrient intake was low due to lack of dietary diversity. Cereals and vegetables were the food groups mostly consumed by the studied population. Food items such as meat, eggs, fruits, milk and milk products (except breast milk) were less consumed. These food items like eggs are usually sold at the market to generate household income. The current study found that very few children (18%) reported to consume animal food source, this might be due to low purchasing power that forced them to access other foods. The observation made in Mpwapwa District in Dodoma region found that only 6.7% of children consumed animal food source, the proportional is lower compared to the current study (Kulwa *et al*., 2015). Under normal conditions, there is no single food offering all nutrients at a time except breast milk in the first six months. Thus, the more diverse the diet provides the greater opportunity of offering diverse nutrients in the same meal, hence increases the likelihood of attaining high dietary diversity scores. Taruvinga *et al.* (2013) investigation reported that, high dietary diversity score is likely to be attained by educated household and less likely to uneducated households. Dietary diversity is directly related to the quality of diet and inversely related to malnutrition in terms of inadequate nutrient supply and nutrient deficiencies (Labadarios *et al*., 2011).

Children aged 12 – 24 months had a higher rate of stunting (82%) than other age groups; 6 – 8 months (8.3%) and 9 – 11 months (9.7%). The risk of stunted growth increases as children approach to their second year of life, growth often starts to decline at the age of 6 months and this continues up to 24 months. Deterioration of nutritional status after six months of age could be due to inappropriate timing of introduction to complementary food or consumption of nutritionally inadequate complementary foods to infants which does not meet their nutritional requirements for growth. Growth shortfall begins at early age under long term exposure to associated factors of under nutrition and its adverse consequences being realized later. Nagahori *et al*. (2015) also found a higher rate of stunting in older children (p = 0.0078). Additionally, TDHS (2015) found highest rate of stunting 33% and 43% to children aged 12 – 17 and 18 – 23 months respectively, as compared to children aged 9 – 11 months who had the lowest proportional of 14.4% (NBS, 2016).

3.5. Conclusion

The rate of stunted growth was high (40.4%) according to WHO categorization criteria, Chamwino district had highest prevalence of stunting (44.3%) as compared to Dodoma Municipality (26.3%). Identified factors associated with stunting in multivariate analysis in the current study were early introduction of complementary food, low maternal education level and residing in Chamwino District. Generally, factors associated with under nutrition are diverse and interrelated, this also include food insecurity, diseases, health seeking behavior and water, sanitation and hygiene practices. This implies that by increasing maternal education, adherence to the recommended time of introduction of complementary food would reduce the rate of stunting. Therefore, the current findings serve to highlight the critical predisposing factors which may be prioritized for intervention by different actors.

CHAPTER FOUR

Nutrient Composition of Cereals-based Complementary Flour and its Nutritional Adequacy in Infants Nutrition[3]

Abstract:

Evaluation of the quality of complementary flour and its nutritional adequacy in relation to infant's nutritional requirements was done to four different types of complementary flour (composite cereals with groundnuts, maize, millet and sorghum). The complementary flour were analyzed for determination of macronutrient, micronutrients and phytate content. The mean protein content in all types of complementary flour was 7.30 ± 2.52 /100 g. A significant difference in protein content between composite cereals ($p \leq 0.001$) and other types of cereals was observed. Fat and iron content were found to be inadequate in all types of complementary flour. Phytate content ranged from 59.47 – 1176.8 mg/100 g, the highest content was observed in sorghum. On the other hand, the mean iron and zinc content in all types of complementary flour was 5.25 ± 1.35 mg/100 g and 2.99 ± 1.36 mg/100 g respectively. The molar ratios of phytate:iron and phytate:zinc were at a level that would tend to inhibit bioavailability of iron and zinc in 97% and 45% of analyzed samples respectively. An association between anaemia and phytate:iron molar ratio (AOR = 4.2, 95%, 1.2 – 6.9) was observed. The quality and adequacy of nutrients in cereal based complementary flour has shown to be compromised by the presence of phytate.

Keywords: Complementary flour, proximate composition, nutrient requirement, protein, phytates, anaemia, child growth

[3] *Published Journal of Food Research; doi:10.5539/jfr.v6n6p45*

4.1. Introduction

In developing countries, malnutrition is still a serious health problem affecting infants and young children (Ahmed *et al*., 2013; Black *et al*., 2013; Steiber *et al*., 2015). Globally and in sub Saharan Africa, 26% and 40%, of children below five years of age are stunted (UNICEF, 2013). In Tanzania, 34.4% of children below five years are stunted and 13.7% are underweight (NBS, 2015). Though causes of malnutrition are diverse and interrelated, inadequate dietary intake during the complementary feeding period is considered to be major contributing factor (UNICEF, 2013). In most cases, cereals and legumes constitute a major portion in formulation of complementary foods, therefore it might be difficult to attain nutritional adequacy for children due to dietary related factors (Okomo *et al*., 2016). Nutritional deficiency, diseases and disability are considered to be a risk factor of inadequate dietary intake (Kumssa *et al*., 2015; Muthayya *et al*., 2013).

Nutrition in early life has the greatest influence on child growth, development and survival (Michaelsen, 2015; Skau *et al*., 2014). Following six months of exclusive breast feeding, appropriate and adequate nutritious complementary food should be introduced. The nutrient content of complementary foods should be adequate and diverse enough to meet the child's nutritional needs. Infants and young children are vulnerable to inadequate nutrient intake during the period of complementary feeding (6 -23 months), the needs of nutrients during this period is very high to support the high rate of growth while the supply is insufficient to meet the demand (Michaelsen, 2015). The first two years in life provides an opportunity for the child to achieve maximum growth potential but at the same time it is a critical period for growth faltering if the nutritional needs are not attained (Dewey, 2013). Proper physical, cognitive and intellectual development is attained by adequate nutrition in childhood (Onoja *et al*., 2014; Sudfeld *et al*., 2015). Therefore, in order to meet nutrient requirements, the complementary foods need to contain all essential nutrients such as carbohydrates, protein, fat, vitamins and minerals appropriate to facilitate optimal growth and development.

Lack of dietary diversity and dependence on plant-based complementary foods are some of the key factors that limit the quality of complementary diet in terms of nutrient content and bioavailability (Gatahun *et al*., 2015; Shiriki *et al*., 2015). Furthermore, the presence of anti-nutritional factors such as phytate, limits nutrients bioavailability as it chelates divalent cations (Fe, Zn, Ca). Phytate in cereals tend to affects the bioavailability of minerals and reduce the chances of being absorbed in the body (Platel and Srinivasan, 2015). More than 80% of children (6 – 23 months) in Africa do not attain a minimum acceptable diet during complementary feeding period, especially in areas where there is a problem of food scarcity (de Onis and Branca, 2016). Relying on cereal-based complimentary foods might limit micronutrient bioavailability and attainment of nutrients adequacy which may collectively have a negative effect on nutritional status of a child during the critical period of growth. In this context, complementary flour are basically cereal-based used to prepare porridge and other traditional complementary foods. In low resource settings, they are stirred up solely with water and boiled as plain porridge or added with minimum ingredients such as milk, lemon juice, cooking oil, margarine or butter. Therefore, the aim of this study was to evaluate the nutritional quality of cereal-based complementary flour used to prepare complimentary food for infants and young children (6 - 23 months) and to assess its nutrient adequacy in relation to infant's/child's nutritional requirements, in order to advocate for improvement.

4.2. Materials and Methods

4.2.1. Study area, design and sampling procedure

Dodoma region was purposively selected for being a study area due to the fact that it was having high prevalence of stunting (56%) in children of under five years of age (NBS, 2011). Out of seven districts in this region, Dodoma Municipality and Chamwino District were randomly selected to be the study area. Three wards (Chigongwe, Makole and Mahoma Makulu) from Dodoma Municipality and two wards (Ikowa and Mlowa) from Chamwino district were also randomly selected. Five villages were recruited in this study, whereby in each ward one village was randomly selected. A list of all households that were having children of age between 6 and 23 months was obtained from the respective village offices, a systematic randomly sampling technique was used to select all households recruited in this study followed the criteria of having a child who is receiving a complementary food. A cross-sectional study involved a total of 100 children (48% male and 52% female) who were between the age ranges of 6 - 23 months. A total of 100 complementary flour samples were used for preparation of complementary food were collected from households. Four different types of cereals-based complementary flour were collected from the households [composite cereals with groundnuts (n = 51), maize (n = 22), millet (n = 18) and sorghum (n = 9)]. From each selected household, approximately 100 – 150 g of locally made complementary flour was collected and kept at room temperature. The collected samples were then taken to the laboratory, stored at 4 °C for further analysis. Infants were assessed for haemoglobin status by using Hemo Control Hemoglobin and Hematocrit Analyzer (*EFK* Diagnostic, GmbH Germany), association between phytate intake and haemoglobin level was determined.

4.2.2. Dietary pattern

Repeated 24 h dietary recall method was used to capture detailed information provided by a mother/care-giver for all food items and drinks consumed by a child for the past 24 h in two non-consecutive visits. Amount of food reported to be consumed in 24 h dietary recall was estimated by using household utensils such as cups, bowls and spoons. The consumption rate of a child was calculated by considering weight of the food per serving and meal frequency per day. Nutrient adequacy of complementary foods was calculated based on the amount of food consumed per day. Calculated nutrient intake was compared with Recommended Dietary Intake (RDI) of respective age category.

4.2.3. Proximate composition analysis

Complementary flour samples were analyzed for protein, moisture content, crude fat, ash and carbohydrates. Determination of nutrient composition of the samples was performed using standard procedures as described by the Association of Official Analytical Chemists – AOAC (2005) (AOAC, 2005). Protein content was determined by Kjeldhal method (method no. 960.52) using 0.3 g of samples. Percentage of protein was calculated by multiplying with the factor of 6.25 for conversion of nitrogen content of complementary food sample. Moisture content of the samples was determined by hot air oven (method no. 925.10) whereby 5 g of each sample was placed in oven (Memmert, Germany) at 105 °C for 6 h. Ash content was determined using Muffle furnace (ThermoScientific, England). A charred 1 g of sample was placed in a Muffle furnace at 550 °C for 4 h (method no. 923.03). The appearance of gray white ash indicated complete oxidation of all organic matter in the sample. Crude fat was determined by extracting 5 g of each sample in 75 mL of petroleum ether (C_6H_{14}) (Uni-Chem, India), boiled at a temperature between 40 °C to 60 °C using Soxhlet (Foss, Sweden) (method no. 2003.05). Fiber was determined by digesting 1 g of sample with 200 mL of refluxed 1.25% H_2SO_4 (Uni-Chem, India) for 30 min then followed by addition of 200 mL of

1.25% sodium hydroxide (NaOH) (Sigma-Aldrich, South Africa) for 30 min (method no. 962.09). Carbohydrate was determined by subtraction of percentage of protein, fat, moisture, ash and fiber from 100. Results of analysis of proximate composition were expressed per 100 g of the dry weight matter.

4.2.4. Determination of phytate

Phytate content in complementary flour was determined by using a method described by Holt's (1955) with some modifications (Davies and Reid, 1979). Briefly, 0.25 g of sample was extracted with 10 mL of 0.5M nitric acid (HNO_3) (Ajax Finechem, India) by continuous shaking placed over night in a Shaker (ThermoScientific, USA). The extract was filtered through whatman filter paper no. 3 (Sigma-Aldrich, South Africa). An aliquot (1.0 mL) of the filtrate was then diluted with distilled water to a final volume of 1.4 mL, then 1 mL of a ferric ammonium sulphate solution ($NH_4Fe(SO_4)_2$) was added and well mixed. The test tubes contained the mixture was immersed into water bath (Memmert, Germany) at 100˚C for 20 min, then cooled at a room temperature. Five milliliters of amyl alcohol ($C_5H_{11}OH$) (Sigma-Aldrich, South Africa) and 0.1 mL of ammonium thiocyanate (NH_4SCN) (Abron Chemicals, India) solution were added and immediately mixed by inversion and shaking and then centrifuged at 3000 rpm for 5 min. The intensity of colour in the amyl layer was determined at 465 nm using Spectrophotometer (SQ-2800 Single Beam, UNICO) against an amyl alcohol 'blank' exactly 15 min after addition of NH_4CNS. Phytate content in a sample was expressed per mg/100 g of dry weight matter.

4.2.5. Determination of minerals (Zinc and Iron)

Zinc and iron contents were determined by a method described by Association of Official Analytical Chemists –(AOAC, 2000b). A gram of homogenized sample on the crucibles was placed in Muffle furnace at 550 °C for 4 h. Appearance of grey white ash indicated complete oxidation of all organic matter in the sample. The crucibles containing ashes were left to cool at room temperature (26°C), then 10 mL of 0.1 N HCl (LaborChemie, Germany) was added in order to dissolve ashes. The solution was filtered using Whatman filter paper no. 1 (Sigma-Aldrich, South Africa). The filtrate was used for iron and zinc quantification using Atomic Absorption Spectrophotometer (ThermoScientific, USA) at wavelength of 248.3 nm and 213.9 nm, respectively.

4.2.6. Determination of molar ratio of phytate: mineral

Mole of phytate, zinc and iron was calculated by dividing the weight of phytate and minerals (zinc and iron) with its molecular weight (660 g/mol for phytate, 65 g/mol for zinc and 56 g/mol for iron) (Mahfuz *et al*., 2016; Norhaizan and Norfaizadatul, 2009). The molar ratio was obtained by dividing the mole of phytate with the mole of zinc as well as mole of iron. Phytate: minerals ratio was used to estimate the bioavailability of minerals. The recommended limits of phytate: iron and phytate:zinc were 1 and 15 respectively (Mahfuz *et al*., 2016).

4.2.7. Determination of hemoglobin level

The hemoglobin concentration of infants (6 – 23 months) was determined using Hemo Control Hemoglobin and Hematocrit Analyzer (*EFK* Diagnostics, GmbH Germany). The middle finger at the top side was cleaned (using a cotton wool) and then pricked using sterile disposable lancet. A drop of blood was filled at the center of cuvette, immediately was placed into a holder and pushed to its position for reading. Hemoglobin concentration was expressed as g/dL. Categorization of hemoglobin level was done as per WHO reference standards (WHO, 2011), as presented in the Table 6.

Table 6. Haemoglobin cut off points to define anaemia

Category	**Haemoglobin (g/dl) cut off point**
Non anaemia	11.0 or above
Mild anaemia	10.0 – 10.9
Moderate anaemia	7.0 – 9.9
Severe anaemia	Below 7.0

4.2.8. Ethical Clearance

Ethical approval for conducting the present study was obtained from the National Health Research Ethics Sub-Committee (NatHREC) of the National Institute of Medical Research

(NIMR) of Tanzania, reference number NIMR/HQ/R.8a/Vol.IX/1973. Parent /care taker of each individual child signed an informed consent. Confidentiality was observed throughout the study.

4.2.9. Statistical analysis

Data were coded and entered into an Epidata version 3.1, then exported to Statistical Package for Social Studies (SPSS) for windows software (IBM version 21) for analysis. Difference between groups was compared using Chi-square statistical test, the strength of association were assessed using Crude Odds Ratio (COR) and Adjusted Odds Ratio (AOR). Differences were considered statistically significant if $p \leq 0.05$. One way ANOVA was used to compare means between types of complementary flour, significant difference were established when $p \leq 0.05$.

4.3. Results

4.3.1. Dietary Pattern

Dietary pattern of studied population was cereal-based products where by all infants and young children (6 - 23 months) were introduced to cereal-based complementary foods. The mean consumption rate of complementary foods per child was 145 g/day, the minimum value was 91 g/day and maximum was 293 g/day. The composite complementary flours were formulated by using different types of cereals ranging from 2-7 types (i.e., maize, millet, sorghum, rice, finger millet, wheat, beans) groundnuts was the main ingredient. The consumption of dairy products, fruits, vegetables and other animal foods source were consumed by 11%, 7.1%, 57.9% and 18% of infants respectively. The mean carbohydrate content of composite cereals-based complementary flour provided 2.8 kcal/g (2.3 - 3.19 kcal/g). Based on the consumption data, the mean protein intake among infants was 8.43 ± 0.31 g per day. The RDI of fat for infants and young children was not attained by all types of complementary foods fed to infants. Fat content was able to supply 19% of energy required by infants. Iron intake was inadequate whereby only 6% (n = 100) of infants (12 - 24 months) met the RDI. The mean zinc intake among infants was 2.99 ± 1.36 g/day, where by 75% (n = 100) of infants met the RDI of zinc.

4.3.2. Proximate composition

Results of proximate composition of four different types of complementary flour are presented in Table 7. The protein content of composite cereals complementary flour was higher 7.30 ± 2.52 g/100 g compared to maize, millet and sorghum. A significance difference ($p \leq 0.001$) in protein content between composite cereals and other types of complementary flour was observed. The mean difference of protein content in different types of complementary food is presented in Table 8. The protein content of cereals-based complementary foods ranged from 1.17 - 11.17 g/100 g of dry weight matter. Fat content ranged from 0.61 - 11.19 g/100 g and composite cereals had the highest fat content. Insignificant difference ($p = 0.51$) in fat content between composite cereals and other types of complementary samples was observed. On the other hand, the fiber content ranged from 1.03 - 13.27 g/100 g, whereas the highest fiber content was found in maize. The minimum and maximum carbohydrate content was 59.68 and 79.97 g/100 g respectively, with maize showed slightly higher value as opposed to composite cereals. Furthermore, the moisture content of complementary flour was found to be within acceptable range 7.01 – 14.01 g/100 g (McLean *et al*., 2009). The ash content was found in a range of 0.23 - 3.57 g/100 g.

Table 7. Proximate composition [g/100 g] of different types of complementary flour

Type of CF	n	Protein	Fat	Fiber	Ash	Moisture	CHO
Composite cereals	51	8.70 ± 2.24	4.7 ± 2.12	3.6 ± 2.09	1.78 ± 0.65	8.54 ± 0.90	70.54 ± 3.27
Maize	22	7.09 ± 2.05	3.34 ± 2.06	5.09 ± 1.50	1.87 ± 0.68	9.6 ± 1.98	74.01 ± 3.74
Millet	18	5.87 ± 1.66	3.7 ± 2.36	3.69 ± 0.88	1.5 ± 0.58	9.11 ± 1.02	72.39 ± 4.49
Sorghum	9	6.97 ± 2.05	3.67 ± 1.88	3.78 ± 0.51	1.9 ± 0.55	8.7 ± 1.30	73.40 ± 2.96
Over all	100	7.30 ± 2.52	4.15 ± 2.19	4.0 ± 2.30	1.78 ± 0.64	8.92 ± 1.33	71.83 ± 3.80

Values are expressed as mean ± standard deviation

Table 8. The mean difference of protein content among different types of samples and its significance

Type of flour		Mean Difference (a-b)	Sig.
Composite cereals	Maize	3.10158*	0.000
	Millet	2.82627*	0.000
	Sorghum	2.28072*	0.003
Maize	Composite cereals	-3.10158	0.000
	Millet	-0.27530	0.681
	Sorghum	-0.82086	0.327
Millet	Composite cereals	-2.82627	0.000
	Maize	0.27530	0.681
	Sorghum	-0.54556	0.527
Sorghum	Composite cereals	-2.28072	0.003
	Maize	0.82086	0.327
	Millet	0.54556	0.527

** The mean difference is significant at 0.05, Sig: Significance*

4.3.3. Minerals and phytate

The results of iron and zinc are presented in Table 8**.** Sorghum showed the highest iron content of 8.37 mg/100 g with the lowest value of 0.17 mg/100 g found in millet. A non-significance difference in iron content between types of complementary foods (p = 0.233) was observed. Highest zinc content was found in composite cereals (5.92) and the lowest value was found in millet 0.50 mg/100 g. A significance difference in zinc content between composite cereals and maize (p = 0.010), sorghum and maize (p = 0.044) and sorghum and millet (p = 0.033) were observed. Phytate content in different types of complementary flour (composite cereals, maize, millet and sorghum) is presented in Table 9. The highest phytate content of 1176.8 mg was found in sorghum whereas the composite cereals exhibited the minimum value of 59.47 mg.

Table 9. The mean minerals content of four types of complementary flour [mg/ 100 g]

Type of CF	N	Iron	Range	Zinc	Range
Composite cereal	51	5.14 ± 1.23	3.17 – 8.36	3.34 ± 1.31	0.63 – 5.92
Maize	22	5.3 ± 1.77	0.17 – 8.19	2.4 ± 1.35	0.63 – 5.63
Millet	18	5.02 ± 0.94	3.37 – 6.89	2.3 ± 1.2	0.50 – 4.84
Sorghum	9	6.06 ± 1.45	4.19 – 8.37	3.5 ± 1.05	1.37 – 5.22
Over all	100	5.25 ± 1.35	0.17 – 8.37	2.99 ± 1.36	0.50 – 5.92

Values are expressed as mean ± standard deviation

4.3.4. Minerals bioavailability

The phytate:iron and phytate:zinc molar ratios are presented in Table 10. Three percent of cereals-based complementary foods had molar ratios of phytates:iron below 1 which is within acceptable range whilst 97% (n = 100) of samples were above 1 indicating that the absorption of iron might be significantly inhibited by the presence of phytate. The highest phytate:iron molar ratio was found in maize 69.22 followed by millet 18.04, composite cereals (16.13) and sorghum (6.99) respectively. The molar ratio of phytate:zinc, in 55% (n = 100) of the complementary foods was below the cut-off point (< 15) and 45% (n = 100) was above the level which might limit the absorption of zinc. The highest level 109.10 was found in composite cereals as opposed to millet with the lowest level of 57.04. The overall mean of phytate:zinc molar ratio was 11.87 ± 13.87.

Table 10. Mean phytate content of complementary foods [mg/ 100 g] and phytates:minerals molar ratio

Type of CF	N	Phytate	Range	Phytates:Iron molar ratio	Phytates:Zinc molar ration
Composite cereal	51	246.19 ± 11.66	59.47 – 821.03	4.21 ± 2.97	10.13 ± 15.65
Maize	22	211.57 ± 6.82	59.50 – 320.95	6.4 ± 14.05	12.2 ± 9.25
Millet	18	306.34 ± 15.67	126.87 – 716.49	5.6 ± 4.00	18.20 ± 0.4
Sorghum	9	387.42 ± 3.52	163.27 – 1176.8	3.77 ± 1.60	8.20 ± 4.42
Over all	100	262.09 ± 18.18	59.47 – 1176.80	4.93 ± 7.10	11.87 ± 13.87

Values are expressed as mean ± standard deviation

4.3.5. Haemoglobin level

The prevalence of anemia among children was 69% which is considered to be severe and of public health concern according to WHO standards (McLean *et al.*, 2009). Based on hemoglobin levels, 4.6% (n = 18) of children were severely anemic, 36.3% (n = 143) and 28.2% (n = 111) had moderate and mild anemia respectively. Significant association between anemia and phytates:iron molar ratio (AOR = 4.2, 95%, 1.2 – 6.9) was observed.

4.4. Discussion

In developing countries cereals are used as the main ingredients in traditional complementary foods for children. They are known to provide reasonable amount of protein, carbohydrate and vitamins (Oghbaei *et al.*, 2016). Analysis of complementary flour in this study indicated that protein content in composite cereals was higher compared to other types of foods. A significant difference ($p \leq 0.001$) in protein composition between composite cereals and other types might be due to inclusion of groundnuts which boosted the overall protein content. Variation on the addition of groundnuts during formulation was observed among households, varying from 0.5 to 2.5 kg depending on the final weight of complementary flour intended to be formulated. Groundnuts provide reasonable amount of protein and other essential nutrients

(Mandal, 2016). Despite composite cereals having higher protein content, it did not meet infant's RDI of protein. The proportion of protein in all other types of complementary foods were inadequate, observed to be below RDI for infants which is 16 g per day (Shiriki *et al*., 2015). The actual protein intake was slightly lower than RDI, indicating the intake of protein was inadequate. This might be due to lack of dietary diversity, less consumption of animal food sources and dependence on cereals-based complementary foods as the main source of protein. The consumption of the dairy product and animal food source were noted to be less consumed by 11% and 18% respectively. Similarly, Anigo *et al.* (2009) reported very low level of protein (5.23 ± 0.03 g/100 g) in maize based complementary foods which did not meet the RDI for infants (Anigo *el al*., 2009). The adequacy of protein is essential for meeting the growing demand of children especially during this critical stage of growth. Growing children require a constant supply of protein for growth, building up new tissues and body maintenance (Michaelsen and Greer, 2014). Inadequate intake of protein for a long time may lead to protein deficiency which may interfere the growth process. Therefore, ensuring the adequate supply of protein during infancy and childhood will facilitate proper growth. However, improvement of protein content in cereals-based complementary food after enrichment with protein dense foods have been reported by Steve and Babatunde (2013).

Energy from the diet is recommended to be adequate to meet the physiological requirement of the body. The energy requirement is expressed as energy intake from the food that will balance energy expenditure (Butte, 2005). Carbohydrate serves as a primary source of energy in the body. Complementary flour were characterized by having adequate carbohydrate content, according to FAO/WHO (1991) (FAO/WHO, 1991). A 100 g of flour is required to provide 64 g of carbohydrate, which is considered to be sufficient to meet the energy requirement. Furthermore, WHO recommended energy density in complementary food should be in a range of 0.8 – 1.0 kcal/g (WHO, 2009). The present study found that the mean carbohydrate value (71.8 ± 3.80 g/100 g) was above the reference point, indicating the adequacy on providing energy that is needed by the body. Additionally, the minimum and maximum carbohydrate content was able to provide 2.3 kcal/g and 3.19 kcal/g respectively, which was above WHO recommendation signifying the provisional of optimal energy in complementary foods to meet energy needs of infants of the age group of 6 - 23 months. Nevertheless, attainment of daily recommended energy intake depends on the frequency of the meal, amount of the food consumed, and energy density of the food (Adimasu *et al*., 2016). Therefore, adequate intake of energy promotes optimal growth and development.

Fat content in cereals-based complementary foods was observed to be very low due to the fact that cereals contain little fat. Dietary fat is essential for the supply of energy in the body, facilitate absorption of fat soluble vitamins and provide essential fatty acids that are required for normal brain development (Aranceta and Pérez-Rodrigo, 2012). All types of complementary flour was not able to provide the RDI of fat which is 30 g per day for infants of the age group of 7 – 12 months (USDA, 2009). From this study, fat content observed to provide only 19% of energy whereas dietary fat is required to contribute 35% of total energy required by the body (Adimasu *et al*., 2016; Lutter and Dewey, 2003). The results suggest that inadequate fat intake might be due to consumption of cereal-based complementary food which is known to contain little fat. Friel *et al*. (2010) found fat intake of 5.9 g/day from complementary foods which did not meet recommended level (Friel *et al*., 2010). The limited supply of dietary fat to infant prevent the body from receiving adequate energy needed for growth and other body function hence adversely affect the growth pattern.

On the other hand, fiber content observed in this study (Table 7) was slightly higher than values reported by Udensi *et al.* (2012). The mean fiber content in maize based complementary flour (5.09 ± 1.50 g/100 g) was reported to be similar with the recommended value of 5 g/100 g (Gibson *et al*., 2010; Onomi *et al*., 2004). The practice of using whole (unrefined) cereals in formulation of complementary flour contributed to the increase in fiber content, especially in maize-based food. The physiological role of fiber is to maintain an internal distension for peristaltic movement of the intestine (Alexy *et al.*, 2006). Infant diet with high fiber content is not advisable as it tends to reduce nutrient digestibility as well as increase malabsorption of micronutrient, under this condition growth retardation may occur (Edwards and Parrett, 2003). Therefore, it is important to note that fiber content of infant's diets should not exceed the recommended level.

Iron and zinc are important micronutrients required by infants and young children to facilitate optimal growth. Low iron content to meet the daily nutritional requirement for children belonging to the age group of 7 – 12 months was found in analyzed cereal-based complementary foods. Infants in this age group require 11 mg per day (RDI) (Domellöf *et al*., 2014). None of the complementary foods was able to meet the daily iron needs of this group. Iron requirement tends to decline as the age of a child increase. The RDI of age group 13 – 24 months is 7 mg per day (Beard, 2008) thus only 6% of infants for this age group their iron requirement was met. Inadequate intake of iron may interfere with the biochemical function of the body including impairment of cognitive development (Beard, 2008). Zinc content was adequate in 55% (n=100) of collected samples, were able to meet the requirement of infants (7 – 24 months) which is 3 mg per day (USDA, 2009). Regardless of higher zinc content in foods, their availability in the body might be interfered by the presence of phytate which tend to inhibit the bioavailability of minerals in the body (Lazarte *et al*., 2015).

Cereals and legumes are naturally containing anti-nutritional factors including phytate which limits the accessibility of minerals (Abdel-Gawad *et al*., 2013). The phytate content in all samples was found to exceed the tolerable level for human consumption. According to Onomi *et al.* (2004) the tolerable level for phytate is 25 mg/100 g. Nonetheless, below this level the micronutrient losses are minimized (Maseta *et al*., 2016). The range of phytate found in this study was 59.47 – 1176.80 g/100 g, Gibson *et al.* (2010) reported phytate content of 600 mg/100 g in unrefined cereals and legumes-based complementary foods. Since most of the complementary foods in this study were cereal-based naturally known to contain phytate, this further supports the fact that minerals bioavailability can be impeded. Attainment of adequate bioavailable minerals in the body is affected by the presence of phytate in the diet which tends to form complex compounds which are not absorbed in the gastro intestinal tract (Kana Sop *et al.*, 2012; Roohani *et al*., 2013). Phytate compromises the bioavailability of minerals which are very important for growth promotion and development of a child (Lazarte *et al*., 2015). The inhibitory effect of phytate on mineral bioavailability however depends on the molar ratio of phytate:minerals. As the molar ratios increase, also the inhibitory effect of minerals absorption increases. From the study, the molar ratio of phytate:iron and phytate:zinc were all above the tolerable limit by 97% and 45% of analyzed samples respectively. The intake of phytate through complementary foods increased the odds of infants being anemic by 4.2 times higher to children who were using cereals based-complementary foods (p = 0.005). Inadequate supply of iron due to dietary related factors may lead to iron deficiency which is the primary cause of anemia (Rao and Parikh, 2015). Similarly, this study reported that, the prevalence of anaemia was 69%, implying low hemoglobin level which might be contributed by inadequate intake of iron. According to Warthn-Medina *et al.* (2015) micronutrients needs in the first two years of child’s life is very high to meet physiological requirements (Warthon-Medina *et al*., 2015). Micronutrients deficiency contribute to impairment of child growth, cognitive and motor development and affect immunological functioning (Rohner *et al* 2013; Wieser *et al.*, 2013; Wong *et al*., 2014). Thus, infants should receive the most nutrient-rich foods to meet their optimal nutrient needs per each age group.

Preliminary food processing methods such as germination, soaking, milling fermentation, heat treatment have been reported to reduce phytate levels in plant-based food (Fabbri and Crosby, 2015; Oghbaei *et al*., 2016). However, results from this study have shown that 87% of cereal-based food products were not subjected to preliminary food preparation methods. Additionally, only 8% of complementary foods were refined maize flour though persistence of phytate was observed at the low level of 59.47 mg/100 g. Henceforth, it is recommended that the use of preliminary food processing methods on cereal-based foods is important for the purpose of enhancing minerals bioavailability. Likewise, Lestienne *et al*. (2005) reported a reduction of phytate level in cereals (millet) by 29% after soaking and 39% after soaking the dehulling. Some of the food processing methods tend to increase nutrient digestibility and bioavailability of minerals. Therefore, application of food processing method in reducing anti-nutritional factors is essential to enhance minerals bioavailability.

4.5. Conclusions

The cereal-based complementary foods were found to meet the adequacy of the nutritional requirement of children (6 – 23 months) in terms of carbohydrate, fiber, and a little bit of zinc and as opposed to protein, fat and iron. Similarly, high levels of phytate above tolerable limits were detected which tend to compromise minerals (zinc and iron) bioavailability and therefore increases the odds of developing micronutrient deficiency. Therefore, diversification of the complementary diets would increase the chances of offering various nutrients, by increasing the likelihood of attaining nutritional goal to infants and young children. In this case promotion of dietary diversification among mothers and care-givers to enhance the attainment of adequate nutrients is required so that infants and young children would be able to achieve the optimal growth potentials.

CHAPTER FIVE

Inadequate Management of Complementary Foods Contributes to Aflatoxin Exposure and Low Nutrition Status in Infants and Young Children[4]

Abstract:

Early exposure of aflatoxins through complementary food is linked to impaired growth in childhood. The current study assessed the extent inadequate management of complementary food increase the risk of aflatoxin exposure and poor nutritional status of infants and young children in Tanzania. A cross-sectional study of complementary feeding practices, aflatoxin exposure and nutritional status was conducted in 101 infants and young children aged between 6 – 23 months in Dodoma region of Tanzania. The intake of complementary food was estimated by using repeated 24 h dietary recall. Flour used as complementary food was sampled from each of the 101 families and aflatoxins were analyzed using high-performance liquid chromatography. Deterministic approach was used to estimate dietary exposure of aflatoxins in the complementary foods. Anthropometric measurements were taken and rates of stunting, underweight and wasting estimated according to the WHO standard procedures. Multivariate logistic regression analysis was used to assess the association between feeding practices and aflatoxin exposure or the growth performance among subjects. The average consumption of complementary flour was 118 g per child per day and 52% of the flours contained groundnuts. AFB_1 was detected in 42.5% of the flour and levels ranged from 0.3 to 2128.01 µg/kg, (mean 228.11± 49.84 µg/kg). Dietary exposures of aflatoxin B_1 ranged from 0.1 to 23,172.81 ng kg^{-1} body weight per day (mean 1337 ± 392.52 ng kg^{-1}). Of the subjects, 40.4% (95% CI; 29.8; 50.9) were stunted and significant association was found between stunted growth and dietary exposure of AFB_1 (AOR = 5.9; 95% CI: 0.019 - 0.028). Early introduction of cereal-and groundnut-based complementary foods in Tanzania is associated with high risk of aflatoxin exposure and impaired growth in children. There is need to integrate aflatoxin management measures in the guidelines for Infant and Young Children Feeding of Tanzania.

---

[4] *Submitted for peer review in the World Mycotoxin Journal (wmj-2018-05-2354).*

Key words: Aflatoxins, complementary food, dietary exposure, infants and young children, nutrition status

5.1. Introduction

The incidence of stunted growth in children under five years of age is at increasing rate, in developing countries (Prendergast and Humphrey, 2014). The prevalence of stunted, underweight and wasted for children in Tanzania is 34.4%, 13.7% and 4.5% respectively (NBS, 2016) . The high rate of under-nutrition is mainly attributed to food insecurity, the burden of diseases, inadequate care and feeding practices (Asfaw *et al.*, 2015). The period of complementary feeding has been observed to be the critical window for growth faltering, nutritional deficiency and high rate of infectious diseases (Krebs, 2015). Ensuring adequate nutrition during the period of complementary feeding for normal child growth is a challenge since it requires adequate knowledge on Infant and Young Child Feeding (IYCF) (Dewey and Vitta, 2013). For many years, choices of complementary foods have been influenced by traditional and cultural practices, without focusing on the nutritive value of the food. Additionally, food safety has not been inculcated alongside food choices and preparation at the household level, especially during the period of complementary feeding.

Majority of households in Tanzania depend on formulated cereal-based complementary foods made either as a single cereal or a mixture of more than one cereals with inclusion of groundnuts in some cases (Muhimbula and Issa-zacharia, 2010). Most of the composite cereal complementary flours are homemade and may include up to seven types of cereals such as maize, millet, sorghum, rice and finger millet as influenced by household food accessibility and purchasing power. Most of the mixtures contain, in addition to cereals, groundnuts which are very susceptible to aflatoxins contamination. The decision on the combination and proportions of each ingredient to use is done by mothers or caregivers who are the main actors of IYCF. Indeed maize (which is also prone to aflatoxins contamination) and groundnuts have been found to be the dominant ingredients in formulations of composite flour used as complementary food (Shirima *et al.*, 2015). This feeding practices may account for the high levels of aflatoxin exposure reported for Tanzania children (Kimanya, 2015; Magoha *et al.*, 2014; Shirima *et al.*, 2015).

It is well documented that infants are also exposed to unacceptable high levels of aflatoxins at the very young age of less than six months (Lombard, 2014; Shirima *et al.*, 2014), suggesting that the foods are introduced before 6 months of age contrary to the WHO recommendations on complementary feeding. Tanzania Demographic Health Survey (TDHS, 2016) reported that 16% of infants below the age of 2 months, 41% of infants at the age of 2 – 3 months and 73% of infants at the age of 4 – 5 months were introduced to complementary food (National Bureau of Statistics, 2016). This justifies that, early introduction of complementary food may accelerate the rate of infanthood exposure to nutritionally inadequate and mycotoxins-containing diet early in life; the time during which the physiological requirement is very high (Moran and Dewey, 2011). Children are more vulnerable to toxicants compared to adults following their lower body weight, less developed metabolites and inability to detoxify (Lombard, 2014). Therefore, exposure to aflatoxin affects child health, limit utilization of intellectual and physical potentials due to its associated effects on immune system, growth kinetics, tissues and organs functions and on susceptibility to diseases in adulthood (Kimanya, 2015; Magoha *et al.*, 2014).

Different pre- and post-harvest handling practices such as sorting, dehulling and washing of cereals have been reported to be useful in the reduction of mycotoxins in food (Chauhan *et al.*, 2015). Dehulling, particularly for maize, has been observed to significantly reduce aflatoxin by up to 92% (Siwela *et al.*, 2005). On the other hand, physical sorting tends to remove damaged, broken and discolored kernels which contain visible moulds, and can lead to significant reduction of mycotoxins from cereals (Kabak *et al.*, 2006). Furthermore, washing of maize with distilled water three times have been reported to reduce mycotoxins specifically deoxynivalenol level by 65 – 69% (Karlovsky *et al.*, 2016).

The effects associated with childhood exposure to aflatoxin are not well known by parents who are primary caretakers of infants. Ngoma *et al*. (2017) reported that there is very little knowledge of aflatoxin contamination in cereals and their associated harmful health effects to human and animals at the household level (Ngoma *et al.*, 2017). Therefore, the aim of this study was to investigate the households management of complementary foods as related to aflatoxin exposure and nutritional outcomes of infants and young children between the age of 6 – 23 months in Dodoma region of Tanzania.

5.2. Methods

5.2.1. Study site, design and sampling

Dodoma region located in the central zone of Tanzania was chosen for this study because of its high prevalence (56%) of stunting in children of under five years of age (NBS, 2011). A cross-sectional study was designed to collect information on complementary feeding practices, aflatoxin exposure and nutritional status to 101 infants and young children. Simple random sampling technique was used to select two districts; namely Dodoma Municipality and Chamwino, out of seven districts of the region. Five wards were randomly selected whereby three wards (Chigongwe, Makole and Mahoma Makulu) were from Dodoma Municipality and two wards (Ikowa and Mlowa), from Chamwino district. Furthermore, one village was randomly sampled from each of the selected wards. Selection of households was done randomly following the criteria of having a child of age between 6 – 23 months and who had been introduced to complementary food. Stratified sampling technique was used to determine the study population, whereby all infants who met inclusion criteria were grouped into three strata (6 – 8 months, 9 – 11 months and 12 -23 months) based on their age. In each stratum, the representative sample was obtained by applying the sampling fraction of ¼ to the total population. Therefore a total of 101 children (48 male and 52 female) qualified for the study. Sick children were not involved in the study since they had illnesses which might impaired their dietary intake.

5.2.2. Food intake interviews

Twenty four hours dietary recall method was used to assess food intake of each indexed child. Each mother was asked to recall all food items and drinks consumed by her child in the past 24 h. The information was collected in two non-consecutive visits. Amount of food reported to be consumed in 24 h dietary recall was estimated by using household utensils such as cups, bowls and spoons. The estimated portion was weighed using a kitchen scale (CAMRY, model EK3131) and the weight was recorded. The respective levels of protein and energy were quantified for each type and amount of food consumed. These were calculated based on the established levels in Tanzania food composition table (TFCT) per 100 g of the similar recipe (MUHAS *et al.*, 2008). The actual intake of nutrients was compared to recommended dietary intake (RDI) reference value in order to observe the dietary adequacy of complementary foods on attaining infant's daily nutritional requirements (WHO/FAO/UNU, 2007). The amount of raw flour consumed per day was estimated from 24 h dietary recall data by considering the amount of thin and stiff porridges reported to be consumed by a child on a respective day. Quantities of flour consumed were calculated by using recipes of thin and stiff porridge whereby in each 100 g of thin porridge, there is 10 g of raw flour and in each 100 g of stiff porridge there is 32.1 g of flour (MUHAS *et al.*, 2008). The average daily flour intake (g/day) estimated during two visits for each individual child was used for dietary exposure assessment.

5.2.3. Anthropometric measurements

Anthropometric measurements were taken for each indexed child. Body weight and length was taken using calibrated instruments according to World Health Organization (WHO) standards. The length was measured using a length board (210; Seca, Hamburg, Germany) to the nearest accuracy of 0.1 cm. The child's weight was measured using a weighing scale (HD-386-BK;Tanita, Tokyo, Japan) accurate to 0.1 kg. Growth indices of height for age z-scores (HAZ), weight for age z-score (WAZ) and height for weight (WHZ) were computed by using ENA for smart. Children whose HAZ, WAZ and WHZ z-score were below -2 standard deviation (SD) of the median values of the reference population were categorized as stunted, underweight or wasted respectively (Schwarz *et al*., 2008).

5.2.4. Management of complementary flours

A structured questionnaire was used to collect information on management and handling of complementary flour for the purpose of understanding household practices. Mothers were asked to provide detailed information on sources, post-harvest handling practices of cereals, preparation and storage of raw ingredients used in the preparation of the flours for complementary food.

5.2.5. Sampling of complementary flour samples

Complementary flour samples were collected in all households recruited in the study during the food intake interviews. All samples collected from household were home-made complementary flour and were grouped into four categories; (i) composite cereals with groundnuts (n = 52), (ii) sorghum (n = 9), (iii) maize (n = 22), (iv) millet (n = 18). Approximately 150 g of home-made complementary flour was collected at the household level. Samples were packed and well-sealed in a paper bag (khaki in colour) and then transported to Nelson Mandela African Institution of Science and Technology laboratory for analysis of AFB_1 and total aflatoxins.

5.2.6. Analysis of aflatoxins in complementary flour

Aflatoxins were analyzed based on analytical methods described by Association of Official Analytical Chemists (AOAC) method number 999.07 (AOAC, 2000). Briefly, 10 +/- 0.1 g of each complementary flour sample was weighed in a blender. Then 1 g of sodium chloride (Sigma-Aldrich), 40 mL of extraction solution (80% of methanol (Sigma-Aldrich, South Africa) in water) followed by 20 mL of n-hexane (Lab Chemicals, India) were added and homogenized at high speed for 2 minutes. The extract was filtered immediately through Whatman filter paper no. 1 (Sigma-Aldrich, South Africa). Then, 10 mL of the filtrate was mixed with 70 mL of phosphate buffer saline (PBS) at pH 7.3. The mixture was then filtered using syringe filter of 0.45 µm (Sigma-Aldrich, South Africa). The immuno-affinity column was mounted on a vacuum manifold, and preconditioned with 10 mL of PBS. Then a total of 80 mL of filtrate was passed through the column at a flow rate of 1mL/min. The column was then washed with 15 mL of distilled water at a flow rate of 1 mL/min. Air was forced through the column to clear excess water. Bound aflatoxins were eluted using 3 mL of a mixture of methanol and acetic acid at a ratio of 99: 1 v/v into a test tube at a flow rate of 0.8 mL/minute. The eluent was evaporated to dryness under a nitrogen sample concentrator.

Derivatization: To the residue, 200 µL of hexane and 50 µl of trifluoroacetic acid (ThermoScientific, England) were added. Test tube was tightly capped and then the content was vortexed for 30 seconds. The mixture was incubated at 40 °C in a sample concentrator for 10 minutes, then vortexed for 10 seconds and then re-incubated for another 10 min to ensure complete derivatization. Solvent was evaporated to dryness under nitrogen gas immediately after incubation. Then from the residue 200 µL of 0.1% acetic acid in HPLC mobile phase was added, the content was mixed well. The solution was filtered through 0.20 µm syringe filter (Sigma-Aldrich, South Africa) and weas transferred to vial prior to analysis of aflatoxins by HPLC.

Detection of aflatoxins: HPLC (Shimadzu, Tokyo, Japan) coupled with fluorescent detector (RF – 20 a, Japan) set at the wavelength of 360 nm excitation and 440 nm emission was used for the analysis of aflatoxins. An aliquot of 50 µL of the filtrate was injected into a HPLC column (Luna 5 µm C 18, 4.6 mm x 250 mm) at a temperature of 25 °C. The mobile phase composed of a mixture of water, acetonitrile and methanol at a ratio of 57: 20: 23 v/v/v respectively, was set at the flow rate of 1 mL/min.

Prior to analysis of aflatoxins to the sample, the analytical method was validated for recovery and precision. The mean recovery of aflatoxins was determined by spiking the sample with known concentration of standard solution of aflatoxin B_1, B_2, G_1 and G_2 at three different levels of 5 µg/kg, 10 µg/kg and 15 µg/kg. The levels was chosen in order to observe the ability of the method to recover and correspond to maximum limits set in Tanzania. The mean recovery for aflatoxin G_1 was 89%, 91% and 117%, for aflatoxin G_2 was 90%, 94% and 105%, for aflatoxin B_2 was 95%, 100% and 101% and for aflatoxin B_1 was 89.2%, 83% and 97.2% respectively. Limit of detection (LOD) and limit of quantification (LOQ) were found by spiking aflatoxin standard in a sample at decreasing concentration. Extraction and quantification were undergone upon lowest detectable concentration (LOD) and lowest quantifiable concentration (LOQ) was found. The LOD for aflatoxin B_1, B_2, G_1 and G_2 was 0.2 µg/kg, 0.25 µg/kg, 0.1 µg/kg and 0.25 µg/kg and LOQ was 0.3 µg/kg, 0.3 µg/kg, 0.2 µg/kg and 0.4 µg/kg respectively. Retention times for aflatoxin G_2, G_1, B_2 and B_1 were 11.5 min, 12.5 min, 16 min and 17.5 min respectively. Relative standard deviation (RSD) was calculated from results obtained under reproducibility and repeatability. The method showed good repeatability, RSD for aflatoxin B_1, B_2, G_1 and G_2 was below 9.5%, 5.8%, 19% and 20% respectively.

The calibration curve was prepared by using intermediate standard solution of concentration ranged from 3.57 ng/ml to 50 ng/ml of aflatoxins B_1, B_2, G_1 and G_2. The standard curve showed the linearity and correlation coefficient (R^2) was 0.9994, used as indicator of the straight line as shown.

5.2.7. Dietary exposure of aflatoxin B_1 and exposure assessment

Exposure assessment was performed by considering AFB_1 which is the most potent aflatoxin. The contamination data determined in this study was used in calculating the dietary exposure. For all undetected samples the value of LOD/2 was used as contamination data (Reeve and Cressey, 2016). Information on individual consumption data, contamination level in food and body weight were used on finding the dietary aflatoxin exposure of infants.

$$\text{Dietary exposure (ng/kg bw/day)} = \frac{\text{Consumption (g day}^{-1}\text{) x concentration (ng g}^{-1}\text{)}}{\text{Body weight of the subject (kg)}}$$

Margin of Exposure (MoE) was used to quantify the level of risk per individual infant. MoE was calculated as a ratio of benchmark dose (0.305 ng per kg body weight) to the dietary exposure assessment data (Shephard, 2008). Infants who had MOE lower than 10,000 represented the risk of public health concern and the risk was categorized as low when MOE is above 10,000 (Barlow *et al*., 2006; EFSA, 2007).

5.2.8. Data analysis

All data were coded and entered into an Epidata version 3.1. ENA for SMART (2011) was used to analyse anthropometric data. Categorization of stunting, underweight and wasting was done by referring to WHO growth standards (Schwarz *et al.*, 2008). Data were then exported to SPSS for Windows software (IBM version 21) for analysis of information collected at household and aflatoxin data. Multivariate logistic regression was used to assess the association between stunting with feeding practices, age groups, types of complementary flour, dietary exposure to aflatoxin and preparation method of complementary flour. Odds Ratio (OR) with 95% Confidence Interval (CI) was used to assess the strength of association. A statistical significance was considered if $p \leq 0.05$.

5.2.9. Ethical Clearance

Ethical approval for conducting this study was obtained from the National Health Research Ethics Sub-Committee (NatHREC) of the National Institute for Medical Research (NIMR) of Tanzania, reference number NIMR/HQ/R.8a/Vol.IX/1973. A parent or caretaker of each child signed an informed consent prior to commencement of surveys. Confidentiality was observed throughout the study.

5.3. Results

5.3.1. Characteristics of subjects

A total of 101 infants and young children (48 male and 52 female) were recruited in the study. Of the subjects, 27% were aged 6 – 8 months, 32% aged 9 – 11 months and 41% aged 12 -23 months. All infants and young children enrolled in the current study were still being breastfed at the time of the survey.

5.3.2. Management of complementary flour

Agriculture was the main economic activity among 78.9% of parents, and was reported to be the source of produce for preparing complementary flour intended for feeding children. After harvesting, 58% of parent's stored cereal in the house and 42% utilized traditional storage facilities. Additionally, during data collection, 62.5% of households reported to use last year stored cereals. About 97% of parents had not been introduced to guidelines for infant and young child feeding. The percentage of parents who soaked ingredients for complementary food was 0.01, dehulled maize 8.99, washed ingredients before use 42.5 and sorted out visibly bad quality ingredients 48.5. These methods were considered as the old methods of preparing cereals.

5.3.3. Time of complementary food introduction

All infants and young children had been introduced to complementary foods at the time of the survey. Forty nine percent of infants were introduced to complementary food before the age of six months. The mean age of introduction of complementary food was 4.88 ± 1.32 months and the lowest age was 1 month.

5.3.4. Types of complementary flour

Different types of complementary flour were collected at household level, this included composite cereals with groundnuts (n = 52), sorghum (n = 9), maize (n = 22) and millet (n = 18). The composition of composite cereal complementary flour varied among households and comprised of 2 to 7 types of cereals, namely maize, millet, sorghum, finger millet, rice and wheat. Maize and groundnuts were the main ingredients in formulation of composite cereal flours.

5.3.5. Complementary food intakes

The overall mean and median intakes of flour per day were 118 ± 17 g/day and 135 g/day respectively. The intakes among infants of 6 – 11 months and 12 – 23 months ranged between 69 – 98 g/day and 101 – 179 g/day respectively. The mean protein intake among infants of 6 – 11 months was 6.09 ± 1.3 g/day and for 12 – 23 months was 9.6 ± 2.1 g/day. About 35.6% of infants aged 6 – 11 months met the recommended dietary intake (RDI) for protein which is 9 g/day and 59% of infants aged 12 – 23 months had the adequate intake of protein which met the RDI of 16 g/day (Shiriki *et al.*, 2015). The average intake of energy corresponded to RDI, the actual intake among infants of age groups of 6 – 11 months and 12 – 23 months was 632 ± 76 kcal/day and 746 ± 102 kcal/day, respectively.

5.3.6. Nutritional status

The average weight of all the children was 8.72 ± 1.51 kg, and the actual weight ranged from 6 to 12.5 kg. The minimum and maximum height of infants was 58.50 cm and 87.59 cm respectively, and the mean values was 78.72 ± 5.56 cm. The overall prevalence of stunting, wasting and underweight was 40.4% (95% CI; 29.8 - 50.9), 4.1% (95% CI; 1.9 – 8.3) and 14.5% (95% CI; 10.3 – 20.0) respectively. According to WHO classification, the prevalence of stunting was categorized as very high, wasting was low and underweight was medium (Schwarz *et al.*, 2008). There was a significant association between stunting and age groups ($p \leq 0.001$, 95% C; 0.00 - 0.17), children of age range between 12 – 23 months had higher rate of stunted growth (62%) compared to 6 – 11 months.

5.3.7. Occurrence of Aflatoxin in complementary flour

Aflatoxins B_1 contamination was detected in 42.5% of home-made complementary flours and the levels ranged from 0.25 to 2,128.1 µg/kg (Table 11). Total aflatoxins ($B_1 + B_2 + G_1 + G_2$) were found at level ranging from 0.40 to 2,129 µg/kg (Table 11) and levels in 30.6% of samples were above the limit of 10 µg/kg set for total aflatoxin in Tanzania. About 30.6% of the samples exceeded the maximum limit of 5 µg/kg set for AFB_1 in Tanzania (TFDA, 2012), whereas 33.6% exceeded the limit of 2.0 µg/kg set by European Union (EU) for processed cereals (EC, 2010). Moreover, 42.5% exceeded the maximum level of 0.1 µg/kg set by EU for baby foods (EC, 2010). Table 12 shows the types of complementary flours that exceeded the regulatory limits. The highest AFB_1 content up to 2,128.09 µg/kg was found in composite cereal and the lowest level of 0.3 µg/kg was found in sorghum. Maize-based flour had higher mean value of AFB_1 (262.96 ± 13.08 µg/kg) compared to the mean value of other types of complementary flour. The lowest mean value of 127.58 ± 18.93 µg/kg was found in sorghum. The overall mean of AFB_1 in homemade complementary flour was 228.11 µg/kg.

Table 11. Occurrence of AFB_1 and total aflatoxins in different types of complementary flour

Type of food	Number of samples	Positive samples (%)	AFB1 ((µg/kg)		Total Aflatoxin (µg/kg)	
			Median value	Range	Median Value	Range
Composite cereal based	52	26(25.7)	243.15 ± 38.15	(0.30 – 2128.09)	294.8 ± 55.2	(0.40 – 2129.1)
Maize based	22	5(4.9)	262.94 ± 13.08	(32.45 – 997.73)	200.8 ± 14.5	(56.8 – 427.84)
Millet based	18	5(4.9)	251.67 ± 25.85	(2.28 – 557.84)	485.9 ± 50.0	(4.83 – 1266.5)
Sorghum based	9	7(6.9)	127.58 ± 18.93	(0.52 – 540.82)	83.7 ± 73.0	(0.63 – 779.90)

Table 12. Prevalence of samples containing aflatoxins levels above regulatory limits

Type of CF	EU Limits			Tanzania Limits	
	>0.1[b] **µg/kg for** baby food	>2[a] **µg/kg for** B_1	>4[a] **µg/kg for** Total aflatoxin	>5 **µg/kg for** B_1	>10 **µg/kg for** Total aflatoxin
Composite cereal	26	20	19	19	19
Maize	5	4	4	4	4
Millet	5	5	5	3	3
Sorghum	7	5	5	5	5

a level set for all cereal and products derived from cereals
b level set for cereal-based foods and baby foods for infants and young children

5.3.8. Exposure Assessment

Dietary exposure of AFB_1 and total aflatoxins ranged from 0.1 to 23,172.81 ng kg^{-1} body weight (bw) per day and 0.28 to 24537.13 ng kg^{-1}/kg bw/day, respectively. The mean dietary exposures were found to be 1337 ± 392.52 ng kg^{-1} body weight per day and 1635.51 ± 437.74 ng kg^{-1} body weight per day for AFB_1 and total aflatoxins respectively. The mean dietary exposure of AFB_1 for infants who were using composite cereal flour was higher $p = 0.001$ (4977.35 ± 487.35 ng kg^{-1} body weight per day) compared to mean dietary exposure for infants who were using maize flour (788.09 ± 217.66 ng kg^{-1} body weight per day), millet (954.65 ± 98.05 ng kg^{-1} body weight per day) or sorghum 1678.2 ± 264.98 ng kg^{-1} body weight per day. There was a significant difference ($p < 0.001$) between dietary exposure of AFB_1 between infants who were using composite cereals compared to infants who were using maize, millet or sorghum.

Margins of Exposure (MoEs) for all the children (n = 101) was lower than 10 000 indicating aflatoxin exposure is of public health concern and requiring high priority for risk management actions.

5.3.9. Association between feeding practices and nutritional status

Awareness on IYCF practices was observed to be very low among parents and caregivers. Only 35% of parents were aware of good practices for infants and young children feeding. There was no significant association between feeding practices and nutritional status (p = 0.646). The time of introduction of complementary food was associated with stunted growth p = 0.006 (AOR = 3.6; 95% CI: 0.004 – 0.007) among infants and young children in the study area.

5.3.10. Association between complementary food handling practices and contamination levels

There was no significant association between preparation method of complementary flour and aflatoxin contamination level (p = 0.81). The odds of detecting AFB_1 in complementary flour was 3.2 times higher in cereals stored in the houses compared to those stored using traditional methods.

5.3.11. Association between aflatoxin exposure and nutritional status

A significant association was observed between the use of composite cereals containing groundnuts and stunted growth (p = 0.023). The odds of stunted growth was 19.4 times higher for children who were using composite cereals flours with groundnuts as compared to those who were using single cereal flours as complementary food. In multivariate regression analysis association between stunting and dietary exposure to AFB_1 was found to be significant p = 0.05 (AOR = 5.9; 95% CI: 0.019 - 0.028) (Table 13).

Table 13. Multivariate analysis of the association between dietary exposure to aflatoxin and Nutritional status

Variable	%	COR	AOR	P - value
Stunting				
Below - 2 SD	56	0.09 (0.001 – 0.02)	5.9 (0.019 – 0.028)*	0.05
Above -2 SD	44	1		
Underweight				
Below - 2 SD	15.7	1.8 (1.6 – 3.9)	0.5 (0.2 – 2.3)	0.6
Above -2 SD	84.3	1		
Wasting				
Below - 2 SD	4.3	0.2 (0.3 – 0.9)	0.6 (0.2 – 5.1)	0.98
Above -2 SD	95.7	1		

*1 Reference, * Significant at p = 0.05, COR Crude Odd Ratio, AOR Adjusted Odd Ratio*

5.4. Discussion

Composite cereals with groundnuts complementary flour meant for improving infants nutritional status in Tanzania was contaminated with AFB_1 above the levels of health concern. Results of this study revealed that the dietary exposure to AFB_1 through complementary food was very high, for infants who were using composite cereals flour their dietary exposure was significantly higher ($p < 0.001$) compared to infants who were using single cereal complementary flour. This is an indication that inclusion of groundnuts and maize in composite cereal flour might contribute to high dietary exposure to aflatoxins. Also, the use of unsafe complementary flour observed to be contaminated with aflatoxins and dependence on the same type of food might expose infants to the increased risk of health concern. Complementary foods are therefore considered the most likely source of aflatoxin exposure in infants and young children during the period of complementary feeding. Consequently, the dietary exposure of infants to AFB_1 was associated with impairment of linear growth, as there was a significant association between dietary exposure to AFB_1 and stunting ($p = 0.05$). These findings are similar to those reported by Gong *et al.* (2004), on the association between aflatoxins exposure and growth impairment, despite the methodologies employed for designing the study were different. Furthermore, stunting has been found to be associated with age ($p \leq 0.001$), indicating that the risk of exposure to aflatoxin increase with age. These findings are in line with the report of Shirima *et al.* (2015), on the association between aflatoxin exposure through maize intake and age. The quantity of food intake increases as children grow and consequently increases the likelihood of being exposed especially if the diet is contaminated with aflatoxins. Additionally, the dietary exposure of the studied children to aflatoxin B_1 is of public health concern, all infants had MoE below 10,000 for AFB_1 and total aflatoxins (Barlow *et al.*, 2006; EFSA, 2007). Appropriate strategies that intending to minimize aflatoxin exposure during the period of complementary feeding is needed in order for infants to attain optimal growth.

Aflatoxins exposure during the period of complementary feeding may be a critical factor for affecting child health and nutritional status. This study also observed high prevalence rate of stunting (40.4%), which is widely used as the indicator of chronic malnutrition. The decline in linear growth has been commonly observed during the period of complementary feeding, especially when a child is approaching the second year of life (Onyango *et al.*, 2014). Homemade complementary flours intended for feeding infant and young children was significantly contaminated with aflatoxins exceeding maximum levels to be of public health concern. The findings of this study revealed that 30.6% of infants consumed contaminated complimentary food above the Maximum Limits (ML) of 5 µg/kg set in Tanzania for AFB_1. Likewise, other studies have found that 11% and 14% of infants consumed food observed to be contaminated with AFB_1 above the ML (Geary *et al.*, 2016; Kimanya *et al.*, 2008). Given that AFB_1 is very potent form of aflatoxin, EU has set a limit of 0.1 µg/kg for baby foods for AFB_1, the findings of this study revealed that 42.50% of complementary flours exceeded the limit (European Commission, 2010). Exposure to AFB_1 observed to pose a negative health effect by impairing child growth and development since aflatoxins are associated with immune suppression (Owaga *et al.*, 2011; Zain, 2011). The mechanism by which aflatoxins causes impairment of growth in human is unclear. It is proposed that aflatoxin exposure impair gut permeability thus increasing susceptibility to infectious diseases and finally affecting growth. In Gambia, aflatoxin exposure was significantly associated with reduced levels of immunoglobulin A (IgA) secretion in children (Turner *et al.*, 2003). Retarded growth is the irreversible process, and a child who was stunted at 2 years of age is likely to grow up to a stunted adult (Adair *et al.*, 2013). In developing countries, exposure to aflatoxins and their toxicological effects on Immunity, nutrition and cellular function affects human health negatively and has been reported to be the cause of 40% of diseases burden as well as increase the risk of chronic disease in adulthood (de Onis and Branca, 2016; Williams *et al.*, 2004).

Formulation of complementary food for infants and young children has gained popularity in both rural and urban households. Currently, there is number of local formulations being known as nutritious flour intended for feeding infants and young children during the period of complementary feeding. Despite being prone to mycotoxins contamination, the available formulations are predominantly cereal-based. Cereals have been observed to be the major ingredients in formulations of complementary food in Tanzania. Lack of appropriate knowledge on formulation could be another factor that exposes infants and young children to dietary exposure of AFB_1. Chances of contamination are subjected to individual cereal and vary from one cereal to another depending on the crop susceptibility and extent of exposure to favorable conditions for fungal growth and mycotoxin production. According to Kamika *et al.* (2016), Kimanya *et al.* (2014) and Shirima *et al.* (2015) maize is more susceptible to aflatoxins contamination. The susceptibility is subjected at different stages of the agricultural chain especially during pre- and post-harvest handling conditions (Chauhan *et al.*, 2016). Additionally, studies observed the occurrence of aflatoxins in sorghum and millet at levels higher than regulatory limits (Chala *et al.*, 2014; Sirma *et al.*, 2016). Furthermore, groundnuts are very good source of oil, protein and other essential nutrients but are mostly contaminated with aflatoxins due to geocarpic nature of the pods which increases vulnerability to fungal growth (Kachapulula *et al.*, 2017; Waliyar *et al.*, 2015). The practice of mixing several cereals in formulation of composite flour may possibly increase the risk for aflatoxins exposure to infants and young children. Majority of complementary flours collected at the field in the present study were composite cereal with groundnuts (52%) and were highly contaminated with aflatoxins. Additionally, a significant association was observed between stunting and use of complementary food made from composite cereal flour (p = 0.023). Furthermore, infants who were using composite cereal with groundnuts had higher risk (19.4 times) of becoming stunted than those who were using complementary food made from single cereal flour. The higher level of AFB_1 in composite flours might be due to the practice of using non-dehulled maize and the inclusion of groundnuts as ingredient in the formulation. The removal of the bran and some part of the outer layer which is more susceptible to aflatoxin accumulation and fungal attack has been shown to decrease the level of aflatoxin in cereals. Siwela *et al.* (2005) reported 92% reduction of aflatoxin levels in maize when dehulled. Similarly, aflatoxins contamination in maize based foods in Tanzania were also reported by Geary *et al.* (2016), Kimanya *et al.* (2014) and Magoha *et al.* (2014) the levels were much lower (0.53 to 364 µg/kg) than the levels reported in the current study (0.25 to 2,128 µg/kg).

Aflatoxins contaminate a wide range of agriculture produce include maize, millet, sorghum, soybeans, rice, finger millet, groundnuts and many other agriculture produce at a favourable exposure of pre- and post-harvesting conditions such as humidity and temperature (Yazdanpanah and Eslamizad, 2015). Improper management of cereals after harvesting is a predisposing factor to post-harvest aflatoxin contamination (Waliyar *et al.*, 2015). Storage of agriculture produce for a long period of time and poor storage facilities are among factors that supporting the growth of aflatoxins producing fungus (Torres *et al.*, 2014). Lack of proper storage facilities at household level was observed in this study whereby the odds of detecting AFB_1 in complementary flour was 3.2 times higher in cereals which were stored in the houses compared to those stored in traditional storage facilities. Damaged kernels increase the vulnerability of invasion of moulds during storage, and aggravate to aflatoxins accumulation (Torres *et al.*, 2014; Waliyar *et al.*, 2015). Dharmaputra *et al.* (2015) found high level of aflatoxins contamination in damaged kernels during the postharvest storage. It has been reported that, duration of storage increases the risk of post-harvest mycotoxins fungal infection in stored grains (WHO & FAO, 2015). Waliyar *et al.* (2015) observed an increase in aflatoxins contamination with the increase in duration of storage. Hell and Mutegi (2011) reported an increase in contamination up to 10 folds within 3 days when agriculture produces were stored at high moisture content (Hell and Mutegi, 2011). Additionally, poor households lack access to proper post-harvest technologies to ensure that cereals are fully dried and stored (Hoffmann *et al.*, 2015).

Lack of dietary diversity could be another factor which increased the risk of infant's exposure to aflatoxins through diet. All infants (100%) were already introduced to cereals based complementary food at the time of the survey. High consumption rate based on quantity was observed, the mean consumption of complementary flour was 118 ± 17 g/day, noted to be higher above the estimation of 106 g/day of maize flour for adults (WHO, 2003). This high rate of consumption of cereal-based foods could be driven by household's poor economic status. Additionally, lack of nutrition-sensitive agriculture and food choices may have contributed to the monotonous diet which is nutritionally inadequate and susceptible to mycotoxins contamination (Ruel, 2003). Dietary diversity approach worked well in China, whereby the exposure to aflatoxins has been lowered by shifting from consuming maize-based food to rice and other food stuff (Chen *et al.*, 2014). Access to varieties of foods provides an opportunity to lower the risk of exposure to mycotoxins by replacing the high risk foods with less risk ones.

5.5. Conclusion

Complementary flours which were intended for feeding infants are widely contaminated with aflatoxins to unacceptable level that may contribute to detrimental health effects. Poor growth rate among studied population might be contributed by high exposure to aflatoxins through complementary food. The use of whole cereals in the formulation of complementary flour contributed to exposure since dehulling reduce mycotoxin contamination in maize. Addressing aflatoxins contamination of agriculture produce is as well critical and challenged especially at household level where food insecurity is prominent. Households particularly in rural setting are not aware of mycotoxins contamination of agriculture produce and its related effect on human health. Strategies for mycotoxins mitigation should begin at household level through education on Good Agricultural Practices (GAP), nutrition-sensitive agriculture and food choices.

CHAPTER SIX

Designing an optimized food-based formula for ensuring adequate nutrition for infants and young children (6 – 23 months)

Abstract:

Age specific food-based interventions have proven to be useful in combating under-nutrition. The objective of this study was to formulate an optimal model formula that will reduce the intake of aflatoxins and anti-nutritional factors and can provide adequate amount of essential nutrients for infants and young children aged 6 – 23 month in rural and semi-urban setting of central zone of Tanzania. Dietary intake of 100 infants and young children was collected using 24 h dietary recall. Dietary data were used to define linear programming models parameters. Input data were set in Microsoft excel 2010 and the linear programming analysis was performed using LINDO software version 6.1. None of the nutrients in the habitual diet met the recommended nutrient intake (RNI) for infants and young children between the age groups of 6-11 and 12-23 months. The nutritional adequacy of the optimized diet attained 71% and 85% of the RNI out of fourteen selected nutrients for infants aged 6 – 11 months and 12 – 23 months respectively. The RNI for iron and zinc increased from 28.2% to 47% and 26.4 to 52% for infants aged 6 – 11 months and 28% to 59% and 24 to 73% for children aged 12 – 23 months respectively after optimization, indicating that they are still the limiting nutrients in the study population. The intake of calcium and thiamine among infants of 6 – 11 months increased from 11.9 to 89% and 51 to 89.7% respectively. In addition, the level of phytate in the optimized diets ranged from 11 to 40 mg/100 g which had been reduced by 89% and 82.5% for infants and young children aged 6 – 11 months and 12 - 23 months respectively. Furthermore, the dietary exposure of infants and young children to aflatoxins in the optimized model formula were reduced by shifting from using cereals as staple food to root, tubers and banana food group. The findings of this study showed that, the optimized model formula can improve nutritional value of complementary diets. However, the RNI for iron and zinc could not be attained by the modeled diet. These findings suggest the need for designing effective food-based intervention that can achieve the nutrient recommendations for all problem nutrients. Fortifying the modeled formula with micronutrient powder can be one of the effective approaches to meet the dietary requirements of iron and zinc.

Key words: Linear programming, complementary feeding recommendation, problem nutrient, optimal diet, food-based approach.

6.1. Introduction

Complementary food is introduced to infants at the age of six months when breast milk is not sufficient to meet the infant's nutritional requirements (Tang *et al*., 2015). World Health Organization (WHO) recommends that infants should be exclusively breast fed for the first six months of their life, followed by the introduction of safe, appropriate and nutrient dense complementary foods that meet infant's nutritional requirements (WHO, 2009). The targeted age for complementary feeding starts at the age of six months and end up at 23 months (Dewey, 2013). The purpose of introducing complementary food is to meet the increased nutritional needs of growing infants that cannot be met by breast milk alone. Optimal growth and development of a child can be achieved in the first 1000 days of life, which is considered as a "critical window" for preventing childhood under nutrition. Consumption of nutritionally adequate and appropriate complementary food provides infants with sufficient nutrients in addition to breast milk (Bhutta *et al*., 2008).

Linear growth is essential in assessing child growth and it is a marker of a good health and nutritional status (de Onis and Branca, 2016). In developing countries, linear growth tends to falter dramatically during the period of complementary feeding (Moran and Dewey, 2011). Inadequate dietary intake and sub optimal health conditions have been observed to be the major factors that hinder children from attaining optimal growth potentials in their first two years of life. Studies have shown that attainment of minimum acceptable diet is a challenge, the proportional of infants aged between 6 – 23 months who received minimum acceptable diet was 43% in America, 26% in Asia, 16% in Africa and 10% in Tanzania (NBS, 2016; Onyango *et al*., 2014). Dietary diversity and meal frequency is a proxy indicator for the attainment of minimum acceptable diet. Consumption of food from at least four food groups increase the likelihood of consuming animal food source, fruit or vegetables and staples (MoHCDGEC, 2015). In resource constrained countries, complementary foods are often low in iron, zinc, calcium and vitamin A (Black *et al*., 2013; Dewey and Vitta, 2013). Failure to achieve minimum acceptable diet among infants have been significantly associated with factors such as poor feeding practices, lack of dietary diversity and poor nutritional quality of complementary foods (Ogbo *et al*., 2016; Patel *et al*., 2012). Currently there are a number of local formulations being traded as nutritious food for infants and young children in Tanzania. The available formulations are predominantly cereals, and contain high levels of phytate and aflatoxins (Maseta *et al*., 2016). Cereal-based complementary foods are frequently lacks essential nutrients required for proper growth and development of children (Santika *et al*., 2009). A study done by Raymond *et al.* (2017) on the nutritional adequacy of local food showed that there are possibilities of improving the quality of complementary diets using nutrient-dense foods in resource-poor settings. Ensuring adequate nutrient intake among infants and young children is important and the promising approach that facilitate optimal growth and development. Therefore, the primary objective of this study was to design an optimal model formula that meet the nutritional goal of children aged 6 – 23 months with acceptable levels of phytate and aflatoxins.

6.2. Methods

6.2.1. Study area and sampling procedure

The study was conducted in Dodoma region located in the central zone of Tanzania. The region was purposively selected based on high prevalence of stunting among infants and young children under five years of age (56%) (NBS, 2011). Simple random sampling technique was used to select two districts out of seven districts whereby Dodoma municipality and Chamwino were chosen. Two wards from Chamwino district and three wards from Dodoma municipality were randomly selected and in each selected ward one village was selected to be a study area.

6.2.2. Study design

A cross sectional study was carried out to assess dietary intake of 100 infants and young children (48% male and 52% female) aged between 6 – 23 months using twenty-four hour dietary recall. Dietary data were used to define linear programming parameters for optimization. Analysis was performed using MS Excel 2010 and LINDO software version 6.1. Sick infants and young children were excluded from the study as their food consumption pattern might have been affected.

6.2.3. Dietary pattern

A 24-hour dietary recall was used to identify all foods consumed by infants and young children (Korkalo *et al*., 2015). Mothers were asked to recall all foods and drinks consumed by their children for the past 24-hour. The 24-hour dietary recall information was collected for two non-consecutive days. Portion size was estimated by deducting the amount of food served with the amount left after feeding the child. Children were categorized into two groups (6 – 11 months and 12 – 23 months) and then the lists of all foods consumed were recorded accordingly. The diversity of the diets were assessed by using a scale of twelve food groups as per Food and Agriculture Organization (FAO) (FAO, 2008). The twelve food groups include; cereals based foods, roots and tubers, legumes, fruits, vegetables, fish, eggs, meat, milk and milk products, sugar, oil and fats, spices and condiments. For each food group consumed in the habitual diet a point was given, the dietary diversity score was calculated by summing total point scored. Three categories were developed from twelve points; 1-3 points were categorized as low dietary diversity, 4 – 6 points medium and 7 – 12 high.

6.2.4. Food record database

Nutrient content of all identified foods was established based on the Tanzania Food Composition Table (TFCT) (MUHAS *et al*., 2008). The dietary records revealed twenty five foods which were then categorized into seven major food groups based on TFCT food groups. The seven food groups included cereals and cereal product; root, tubers and banana; pulses, nuts and seeds; meat, poultry (including eggs) and fish; oils and fats; fruits and vegetables; and miscellaneous, as it is presented in Table 14. All 25 food items were included in the linear programming, and there was no additional of new food item into the model, apart from those consumed by study population. The nutrient profiles were estimated from median portion of each consumed food item. Nutrients losses during cooking or washing were estimated by assigning losses factors to each food according to TFCT (MUHAS *et al*., 2008). Nutrient profile for each food item was calculated individually for each age group and was considered as input data in the linear programming models. The problem nutrients were defined using WHO recommended nutrient intake (RNI) for all nutrients (FAO/WHO, 1985, 2004; WHO/FAO/UNU, 2007). All nutrients which did not achieve 100% RNI from the diet were categorized as constrained nutrient.

6.2.5. Objective function

The objective function was to maximize nutrients content in complementary diets while minimizing the levels of anti-nutritional (phytate) and aflatoxins in the diet. Food was given names as X_1 …………..X_n depending on the list of the food consumed by each specific age group and nutrients were named as N_1………..N_n. The linear equations were expressed as:

$$N_1X_1 + N_2X_2 + N_3X_3 \ldots\ldots\ldots N_nX_n \geq = RNI$$

Each food subgroup was linked to the nutrient profiles database established in this study. The model estimated the nutrient intakes of all food subgroups and ensured that nutritional constraints were achieved.

6.2.6. Nutritional constraints

Linear constraints were used to establish the margins of the optimization process. In the linear programming, model constraints were set by considering factors such as food safety (level of aflatoxins), anti-nutritional factors and essential nutrients that are inadequate in children's diets. The focus was to make sure that the solution provided by the model meets specified constraints.

Nutritional constraints were set to ensure that the nutritional content of desirable nutrients of each optimized food was equal or greater than reference values for infants and young children. Constraints were set based on RNI of selected nutrients such as protein, zinc, iron, calcium, thiamine, riboflavin, niacin, folate, pantothenic acid vitamin A, C, E, $B_{6,}$ B_{12}, and energy for each age group as presented in table 14. In each food subgroup the dietary intake of all foods was constrained within 10^{th} and 90^{th} percentile of intake in each age group. The linear programming assumed high bioavailability of iron and zinc due to controlled level of phytate in the model (it was set as constraints) and adequate supply of vitamin C as an enhancer of iron absorption. The nutrient contribution from the breast milk was taken into account by ensuring selection of realistic diets. WHO average breast milk intake of 660 g/day, 616 g/day and 549 g/day for children of age group 6 – 8 months, 9 – 11 months and 12 – 23 months respectively, was used in linear programming.

In addition, safety constraint was set to ensure the level of aflatoxins B_1 in optimized cereal-based foods was less than 5 µg/kg (TFDA, 2012). Additionally, the anti-nutritional factors constraint was set to the level not exceeding 25 mg/100 g of phytate, which is a recommended threshold (Onomi *et al*., 2004). The recommended model diet for each specific age groups was then tested to assess its adequacy in terms of nutrient content in relation to RNI, percentage achieved for each nutrient was calculated. LINDO software was used to generate recommendations for optimized complementary food to meet nutrient requirement for infants aged between 6 – 11 months and 12 – 23 months by considering constrained nutrients.

6.3. Results

6.3.1. Characteristic of subjects

The study involved a total of 100 children, 48% males and 52% females. Children were categorized into two groups based on their age, 59% were infants aged between 6 – 11 months and 41% were young children aged 12 – 23 months. All children were still being breastfed at the time of data collection and were already introduced to complementary foods.

6.3.2. Dietary patterns of observed diet

Habitual diets were dominated by cereal-based foods, the most consumed foods across the two groups were porridge (100%) made from different types of cereals including maize 22%, millet 18%, sorghum 9% and composite cereals 52%. Additionally, meat and milk were consumed by 18% and 11% of infants respectively. The dietary pattern of observed diet was not diversified, whereby 47.7% of infants ranked to have low dietary diversity score. From the list of foods, a total of 14 and 22 food items were consumed by infants of 6 – 11 months and 12 – 23 months respectively. The percentage of RNI attained was from 0 up to 68% for habitual diet for infants aged between 6 – 11 months, and from 0 up to 76% for infants aged between 12 – 23 months.

6.3.3. Nutritional profile of observed and optimal diet

Infants of 6 – 11 months achieved the nutritional goal of 71% (n = 14) nutrients, the intake of calcium and thiamine were 89% of RNI. On the other hand, the intake of iron and zinc were still constrained in optimized diet, however they were achieved by 47% and 52% of RNI respectively as it is presented in Table 16. Optimized diet satisfied the nutritional requirement of 85% (n = 14) of nutrients for 12 – 23 months children. Iron and zinc were dominantly constrained, they were achieved by 59% and 73% respectively and the intake increased by two folds as compared to observed diet. Two linear programmed model diets were formulated, for infants aged between 6 – 11 months, which consisted of five food groups such as roots, tubers and banana (196.5 g), vegetables and fruits (99 g), pulses and seeds (30 g), milk, meat and fish (155.5 mls); and for young children aged between 12 – 23 months, composed of food groups such as root, tubers and banana (338.8 g), vegetables and fruits (160.2), milk, meat and fish (208.3) g, pulses and seeds (60 g). The nutrient content of the optimized diet was assessed for its nutritional adequacy based on nutritional composition table. The dietary diversity score for optimized model diets was observed to be high for both age groups.

6.3.4. Levels of phytate and aflatoxins in the observed and optimal model diet

Levels of phytate in the observed diet ranged from 59.47 to 1176.8 mg/100 g. The phytate content in the optimized diet were reduced by 89% and 82.8% for infants aged 6 – 11 months and young children 12 – 23 months respectively. Additionally, the levels of aflatoxins B_1 in the observed diet ranged from 0.3 - 2128.01 μg/kg. The dietary exposure of infants to aflatoxins in the optimized model formula were reduced by shifting from using cereals to root, tubers and banana food group as staple food.

Table 13. Food groups (g/day) included in a linear programming optimization with lower and upper limit constraints

		6 - 11 months		12 - 23 months	
		Minimum	**Maximum**	**Minimum**	**Maximum**
Food group	**Sub group**	**P 10**[a]	**P 90**[b]	**P 10**	**P 90**
Cereals	Maize	84	198	86	222
	Millet	66	156	68	167
	Sorghum	79	163	83	231
	Mixed cereals	89	121	94	189
	Rice	--	--	63	88
	Sweet potatoes	15	52	42	89
Root, tubers, banana	Irish potatoes	15	45	66	90
	Banana	45	76	75	118
Pulses, nuts and seeds	Beans	15	27	26	44
	Cowpeas	--	--	34	48
	Groundnuts	--	--	11	42
Vegetables and fruits	Amaranth	15	24	13	32
	Potato leaves	23	39	29	48
	Pumpkin leaves	--	--	59	87
	Spinach	15	15	23	52
	Orange	29	52	34	62
	Grapes	32	45	31	72
	Banana	25	49	43	64
Milk, meat and fish	Fish	26	47	18	67
	Meat	16	42	26	49
	Milk	60	77	75	120
	Egg	--	--	15	50
Fats and oil	Fats and oil	13	29	32	48
Miscellaneous	Tea with milk	25	72	45	115
	Tea without milk	30	83	42	92

a 10 percentile, represent minimum value: *b* 90 percentile, represent maximum value

Table 14. Recommended Dietary Intake reference values

Nutrient	**RDI**	
	7 – 12 months	**1 – 2 Years**
Energy	450 kcal/day	746 kcal/day
Protein	16 g/day	9.6 g/day
Iron	11 mg/day	7 mg/day
Zinc	3 mg/day	3 mg/day
Calcium [a]	400 mg/day	500 mg/day
Thiamine [a]	0.3 mg/day	0.5 mg/day
Riboflavin [a]	0.4 mg/day	0.5 mg/day
Niacin [a]	4 mg/day	6 mg/day
Folate [a]	80 µg/day	150 µg/day
Vitamin A [a]	400 µg/day	400 µg/day
Vitamin C a	30 mg/day	30 mg/day
Vitamin B_6 [a]	0.3 mg/day	0.5 mg/day
Vitamin B_{12} [a]	0.7 µg/day	0.9 µg/day
Vitamin E [a]	2 mg/day	2 mg/day

a WHO and FAO, 2004

Table 15. Percentage of RNI attained between observed and optimized diet for infants of age group 6 – 11 months and 12 -23 months.

Nutrient	6 – 11 months		12 – 23 months	
	Baseline diet (%)	**Optimized (%)**	**Baseline (%)**	**Optimized (%)**
Energy	35	101	76	105
Protein	35.32	110	59	154
Iron	28.24	47	28	59
Zinc	26.4	52	24	73
Calcium	11.96	89	28	100
Thiamine	51	89.7	76.6	103
Riboflavin	68.25	101	63.6	101
Niacin	11	100	66.79	229
Folate	2	129	70.15	244
Vitamin A	7.22	231	28	111
Vitamin C	17.49	105	26.48	203
Vitamin B_6	24	132	75.6	141
Vitamin B_{12}	0	120	0	109.7
Vitamin E	0	124	0	141

6.4. Discussion

We successfully developed a model formula that improved the dietary adequacy of essential nutrients among infants and young children aged 6 – 23 months. The developed age specific diet achieved 100% of RDI for twelve nutrients, about 50% of RDI for iron and zinc and 85% for calcium. Linear programming approach has been useful in optimization of nutrient intake during the period of complementary feeding. The inability of habitual diet to ensure nutrient adequacy for more than fourteen nutrients identified as constrained nutrients reflected lacks of dietary diversity with a tremendous limited intake of animal food sources. The complementary food consumed by the study population was mainly cereal-based, exposing infants and young children to inadequate dietary intake of nutrients required for proper growth and development. In the observed diet the intake of iron, zinc, calcium, folate, niacin, vitamin A and C were below 30% of RNI, the intake of vitamin B_{12} and E were inadequate in both age groups. The efficiency of using linear programming approach has been reported to be advantageous on addressing constrained nutrients by utilizing locally available nutrient dense foods. The developed model diet is diversified and provides the greater opportunity for children to obtain several nutrients in the same meal. The optimized diet intended for infants of 12 – 23 months met 85% of the RNI for nutrients including energy, protein, calcium, thiamine, riboflavin, niacin, vitamin A, C, B_6, B_{12} and E. Fahmida *et al.* (2014) found that, the intake of calcium did not attain 100% of RNI when complementary feeding recommendations were developed, this could be due to the fact that, the recommended nutrient dense food were poor source of calcium. Additionally, Skau *et al.* (2014), reported iron, folate and thiamine remained as problem nutrients in the modeled diets. Findings from this study recommend adequate intake of folate and thiamine while iron and zinc intake is still a challenge in both age groups. The deficit of iron and zinc among infants and young children can be partially complemented with breast milk, which was not included in the linear programming though all infants enrolled in the study were breastfed. In addition, the bioavailability of the attained iron and zinc is expected to be relatively high due to the fact that the modeled diet reduced the level of phytate by 89%, as it was set as a constraint in the linear programming. Therefore, the opted foods have very low phytate content, thereby reduced absorption interferences. It has been reported that, the effect of phytate on minerals absorption is associated with phytate to mineral molar ratio (Lazarte *et al*., 2015). On the other hand the optimized diet composed of meat which is a very good source of heme iron, with 20 – 30% and its absorption is less influenced by dietary factors (Beck *et al*., 2014). Additionally, vitamin C enhance the absorption of iron by reducing ferric to ferrous iron and maintaining the reduced form (Fe^{2+}) for absorption (Walczyk *et al*., 2014). The optimized diet is characterized by adequate intake of vitamin C that meet the RNI by 100%. Therefore, by considering the above facts the absorption of iron and zinc from the optimized diet might have been highly enhanced. The study done by Vossenaar *et al.* (2016) assumed low bioavailability of zinc and iron because phytate was not kept under control when developing complementary feeding recommendations. Studies have also indicated that, some optimized diet did not attain 100% RNI of iron and zinc despite the use of iron-dense foods (Fahmida *et al*., 2014; Santika *et al*., 2009). Additionally, fortified food products have been observed to improve iron, and zinc intake by 27% to 37% of RNI (Fahmida, 2013). This calls for alternative strategies that will ensure adequate intake of iron and zinc for optimal growth and development for infants and young children. Fortification at household level by using micronutrient powder supplementation might be a promising approach on meeting the RNI for micronutrients intake (iron and zinc).

This is the first study in Tanzania that considered phytate and aflatoxins levels in modelling of diets for young children by using linear programming technique. Observed diet found to have high levels of aflatoxin B_1 ranged from 0.3 to 2128.01 µg/kg, the unacceptable level might cause detrimental health effect to infants and young children. Cereals are more susceptible to aflatoxins contamination under exposure to pre and post-harvest conditions. In addressing the problem of aflatoxin, linear programming optimized the use of root, tubers and bananas food group. This food group is less susceptible to aflatoxin contamination compared to cereals. This food group includes food such as raw banana, Irish potatoes, orange flashed sweet potatoes, pumpkins, breadfruit, taro and yams. They are less likely to be contaminated with mycotoxins and therefore might reduce the risk of infants and young children exposure to aflatoxin during the complementary feeding. Additionally, orange fleshed sweet potatoes are observed to be very nutritious and also it contain substantial amount of vitamin A. Studies have been reported that sweet potatoes-based complementary foods are the best alternative (Amagloh and Coad, 2014; Amagloh *et al.*, 2012). Therefore, shifting from using cereals as staple food in complementary feeding to roots, tubers and bananas food group may lead to significantly reduction in the dietary intake of aflatoxin by infants and young children (Atongbiik *et al*., 2017). Jonathan *et al.* (2012) found that fresh sweet potatoes were not contaminated with aflatoxins. However, due to poor shelf life of foods belonging to root and tubers foods group, processing could be a better option in order to increase their availability throughout the year.

However, the developed diet is site specific which may hinder its adoption beyond the study area. Also, 25 food items were reported to be consumed by the study population, which might have impacted the identification of nutrient dense foods, and hence the likelihood of leaving potential nutrient dense food is very high. Hlaing *et al.* (2016) reported consumption of 153 food items among infants and young children in Indonesia.

In conclusion, the study population reported a total of 25 food items being habitually consumed by infants and young children between 6 to 23 months of age. The food items were dominated by cereal and cereals-based products, with very little consumption of legume and animal food sources. Application of linear programming improved dietary adequacy of essential nutrients in complementary foods, lowered susceptibility to phytate and aflatoxins exposure. Though, linear programming approach was limited by constrained nutrients such as iron, zinc, and calcium, calling for additional interventions such as fortification using micronutrient powder to meet the RNI is ideal. Optimization of diet by using linear programming can effectively meet the nutritional goals of infants and young children during complementary feeding. Adequate nutrient intake among infants and young children would be one of the strategies to reduce effects associated with malnutrition during the first 1000 days of life. The implementation of complementary feeding recommendations from this study will break the intergenerational cycle of malnutrition and improve infant’s health, though feasibility study is needed to ascertain the performance of the model in the real population.

CHAPTER SEVEN

General Discussion, Conclusion and Recommendations

7.1. General discussion

The aim of this study was to develop an optimal model diet that ensure the adequate nutrient intake for infants and young children of 6 – 23 months by using linear programming approach. The nutritional status of the study population was assessed, and factors associated with under-nutrition were identified. Furthermore, the quality of complementary food consumed by children was evaluated in terms of its nutrient composition, the presence of anti-nutritional factors and its safety (aflatoxins). The major findings of this study, limitations and recommendations are summarized in this chapter.

Chapter one reviewed the current trend of stunting globally, regionally and at national level. Despite several nutrition interventions programs that have been invested, stunting is still a public health concern affecting low-income countries. It continues to undermine growth, survival and development of infants and young children (Bhutta and Salam, 2012). The causes of under-nutrition are multifactorial and interrelated, the degree of influence varies among culture, communities and geographical location. Underlying factors associated with under-nutrition need to be addressed accordingly in order to break the cycle in a sustainable manner.

Chapter two reviewed the dietary related factors on cereal-based complementary foods that that tend to inhibit nutrient absorption and its implications to child nutrition and health. Plant-based foods (cereals and legumes) are mostly used as complementary foods for infants and young children, in spite the fact that they have been reported to contain high levels of anti-nutritional factors. The nutritional benefits of cereal-based complementary foods are reduced due to the presence of ant-nutritional factors such as phytate, trypsin inhibitors, tannins, oxalate and polyphenols. Phytate is an anti-nutritional factor which hinders absorption of minerals by forming phytate:minerals complexes in the gastrointestinal track (Mahfuz *et al.*, 2016). Lack of intestinal phytase enzymes is the only reason why human beings cannot hydrolyze phytate.

Presence of phytate above the threshold tends to inhibit the absorption of minerals such as iron and zinc. Poor absorption of minerals may contribute to micronutrients deficiency which is a global health concern. Children are most vulnerable population group due to high nutritional requirement to sustain normal growth and development. Deficiency of micronutrients has effect on child nutrition and is associated with impairment of child growth, cognitive development, immune functioning and physiological effects that can be life threatening to infants and young children.

Chapter three explored factors associated with stunting in Chamwino District and Dodoma Municipality. The prevalence of stunting was very high 40.4% according to WHO categorization (Schwarz *et al*., 2008). Children in the studied area failed to acquire normal growth pattern (height for age) due to exposure to associated factors that is contributing to chronic under-nutrition for a long time. Nutrition has a central influence on growth during the first years of life. Child growth tends to be compromised at the age of 6 to 23 months, whereas this period is a critical time for body formation including brain development and other different organs, therefore adequate nutrition during the period of complementary feeding is very crucial. Normal growth pattern has significance effect both within that age (childhood) and in adulthood (Bhutta *et al*., 2013), the consequences of malnutrition are profound and the prolonged deficit acquired during childhood is difficult to compensate in later life. Inadequate nutrient intake is a contributing factor to under-nutrition. It's effect is associated with growth faltering, poor cognitive development and poor school performance (de Onis and Branca, 2016). Complex interaction between socio-economic status, feeding practices, health care and cultural influence increases exposure of children to stunted growth. Factors associated with stunting are many, however this study found that maternal education, age of introduction of complementary food and district of residence were independent factors to stunting (refer Chapter two). There was no significant association between DDS and stunting, but diet play a key role in achieving optimal growth. Low dietary DDS (48%) was observed among the diet of study population, indicating less food groups were consumed. Diversified diet provide a greater opportunity of consuming more food groups and increases the likelihood of acquiring diverse nutrients. Lack of dietary diversity reduces the odds of attaining adequate nutrients in the diet. Being the strong determinant, economic status of the household has a greater influence on type of food to be consumed. The foods with higher rate of consumption were reported to be accessible in terms of availability, affordability and acceptability. Affordability ranked higher among other factors due to financial implication. Income has influence on the diet of an individual and what people desire to eat. The findings of this study demonstrated that majority of household's income depended on agriculture. In addition 82.3% of households were observed to spend less than 1 USD per day. This indicates that majority of households lived below the poverty line. Children from low income households were observed to have inadequate intake of macro and micro-nutrients (Bucholz *et al*., 2011). Therefore, diversified diet is the key element of attaining adequate nutrients that are required to support optimal growth. Conceptualization and capitalization of factors associated with stunting in designing nutrition intervention program would contribute to the improvement of child nutrition status.

Chapter four assessed the nutrient composition and phytate content of home-made complementary foods as well as parent's knowledge and practices on preparation of complementary foods. The period of complementary feeding usually starts when infants are six months old and continue up to twenty-three months. This is the most critical period to child's life as they are transferred to family diet which has less likelihood of meeting their nutritional requirements (if not properly planned), while their nutritional demands are very high. Various food preparation methods such as fermentation, germination, soaking and roasting were observed to reduce the levels of anti-nutritional factors and to increase bio-availability of nutrients (Getenesh *et al*., 2014). Results indicated that only 0.01% of parents applied soaking and 8.99% dehulling respectively. High levels of phytate above the tolerable limit ($\leq$ 25 mg/100 g) in all complementary foods might be due to underutilization of preliminary food preparation methods. Attainment of adequate bioavailable micronutrients to facilitate growth and development of infants may be compromised due to the presence of phytate. The findings of this study observed higher molar ratio of phytate:molar ratio above tolerable limit, signifying hindrance to minerals absorption.

Lack of appropriate knowledge on preparation and formulation may affect the quality of complementary foods in terms of nutrient bioavailability. Preparation method and mixing techniques were observed to be a gap in the studied area whereby 97.2% of parents claimed not to have adequate knowledge on preparation and formulation of complementary food. However, there was variation on mixing ratio and inclusion of cereals together with groundnuts in formulation. Nutrient content of complementary food was not taken as a priority and was not considered as important factor during formulation. Results of the present study demonstrated that majority of parents (98.8%) were preparing complementary foods on their own while they don't have appropriate knowledge. Adequate nutrition during infancy and early childhood is fundamental to the development of the child's full potential.

Chapter five explores the dietary exposure to aflatoxins and its associated effects on impairment of growth and poor nutritional status of infants. Mycotoxins have been associated with under-nutrition, however the mechanism by which aflatoxins may affect child growth is unknown. The possibility is linked with immune suppression which could increase the susceptibility to infectious diseases and thus may affect growth (Yun *et al*., 2016). Aflatoxin B_1 is the most prevalent and potent toxin found in a wide range of cereals. Exposure of infants and young children to aflatoxin B_1 in early years of life increases the risk of poor nutritional status due to its effect on growth impairment, immune suppression and burden of diseases. The toxicological effect on immunity and cellular function affect human health. Its effect tends to prolong in adulthood by increasing the risk of chronic diseases (de Onis and Branca, 2016). The period of complementary feeding is observed to be a critical window for growth faltering due to the fact that majority of infants are introduced to unsafe complementary foods. Most of the complementary foods used in Tanzania are cereal-based, and they are produced locally. The basic ingredients used on formulation of complementary food were observed to be maize and groundnuts, both of them being susceptible to aflatoxin exposure (Kimanya *et al*., 2014). Food and Agriculture (FAO) estimated that 25% of the world's food crops are contaminated with mycotoxins (WHO, 1999). Mycotoxins are secondary metabolites of fungi that possess a significant adverse health effect to humans, its exposure is mostly by inhalation and ingestion (Zain, 2011). Infants and young children in the studied area were exposed through consumption of contaminated complementary foods whereby all children were exposed to the levels which are of public health concern. Frequency of exposure, level of contamination and duration determines the magnitude of exposure. The rate of aflatoxins exposure observed to be very high, this might be contributed by dependency on cereal-based complementary foods as the main dietary staple.

This could be driven by the economic status of households which allow them to afford cereal-based food. Economic status has greater influence on the dietary pattern of an individual (Kell *et al.*, 2015). Dietary diversity could be the only option on minimizing the effect associated with aflatoxin, this can be achieved by replacing the food which is having high risk of aflatoxin contamination with those having lesser risk.

Chapter six demonstrated the benefit of using linear programming to formulate an optimized model diet from the nutrient dense foods which were locally available in a respective setting. In the process of developing optimized diet, linear programming approach takes into account the multiple factors such as cultural, utilization of nutrient dense foods, minimizing the levels of phytate and aflatoxins and maximization of the objective function. The design of food-based optimized diet is subjective to nutritional adequacy, which is based on mathematical optimization in order to overcome constrained nutrients which could not attain 100% of RNI in habitual diet. Optimized diet is practical and desirable, the intention is to minimize the gap in habitual diet. The study identified nutrient dense foods to cover the adequacy of those nutrients which were identified as constrained nutrients. Complementary feeding practices can be improved by utilization of model diet, intended to ensure adequate nutrient intake that meet RNI. However, the optimal model diet serves as a tool on ensuring the adequate nutrient intake among infants and young children.

7.2. Conclusion

The period of complementary feeding covers about half of the "1000 days", the period from conception to child second birthday. Infants and young children are particularly vulnerable to inadequate nutrient intakes during complementary feeding period. The age between 6 – 23 months is of high nutrient demands due to rapid growth rate. The diets of infants and young children in the studied area were mainly cereal-based complementary foods, found to contain inadequate amounts of essential nutrients such as iron, protein and zinc. Additionally, cereal-based food consumed by the studied children faced dual burdens in a sense that they contained high levels of phytate and was also contaminated with aflatoxins above regulatory limits. High level of phytate and phytate:molar ratio in food is an indication of poor minerals bioavailability and utilization in the body. The exposure of infants and young children to aflatoxins during the period of complementary feeding might be the underlying determinant of growth impairment and increases the risk of suffering from health effects such as immune suppression and cancers. High prevalence of stunting (40.4%) have been observed among the study population, indicating a long term effect of chronic nutrient deprivation. Infants need to be protected in terms of their growth, health and future. This can be achieved by making sure that infants are fed safe and nutrient dense foods which will meet their nutritional requirements. The optimized diet achieved a nutritional goal of more than 85% of RNI and two-fold increase for intake of iron and zinc which were identified as constrained nutrients. The modeled diet fulfills the nutritional requirements of the studied population while the levels of phytate and aflatoxins have been reduced. Furthermore, the model proposed the use of roots, tubers and bananas food groups as a staple foods instead of cereals for the purpose of decreasing the likelihood of child exposure to aflatoxins through diet. Therefore, the model can serve as a tool for ensuring adequate nutrient intake among infants and young children during the period of complementary feeding.

7.3. Recommendations

i. Identified factors associated with stunting serves as highlight in prioritization for better planning of nutrition intervention programs by government, private sectors and different actors who intends to promote optimal child growth.

ii. Cereal-based complementary food that intended to meet the nutritional requirement for infants and young children during complementary feeding was significantly contaminated with aflatoxins to unacceptable high levels that would impose adverse health effect to growing children. Therefore, from the findings of this study, we recommend that mycotoxins analysis should be part of TDHS. This approach will enable the country to have a national data and being able to track the progress towards effort that has been invested in mycotoxins mitigation strategies. Also, the government will be in a position to identify areas that are having higher exposure rate of mycotoxins contamination and develop mitigation plans according to the specific context.

iii. The findings of this study highlighted the effectiveness of using Linear programming on optimizing model diet to ensure adequate nutrient intake among infants and young children. The approach can be scaled up in areas whereby meeting children nutritional goal is a problem.

iv. The model needs to be tested in the real population to ascertain its efficacy. If successful, the model can be integrated into nutrition interventions programs, especially in developing countries.

v. To continue promoting and supporting mothers to practice exclusive breast feeding for the first 6 months of infant life and appropriate introduction of complementary foods which are safe and nutrients dense.

vi. To adhere to the good agriculture practices (GAP) and improve post-harvest management practices by ensuring that agriculture produces are harvested on time, properly dried, handled and stored in conditions that does fever growth of fungus that produce mycotoxins.

vii. The government should continue encouraging households to utilize preliminary food processing methods such as dehulling, sorting, winnowing and washing/soaking for the purpose of reducing the levels of anti-nutritional factors and mycotoxins as well as in cereals that are intended for human consumption.

REFERENCES

Abbaspour, N., Hurrell, R., & Kelishadi, R. (2015). Review on iron and its importance for human health Review on iron and its importance for human health. *Journal of Research in Medical Science*. **19**, 164 – 74.

Abdel-Gawad, AS, Ramadhani, B.R and Oraby, R. E. A. (2013). Legume phytases: characteristics and changes in activity during germination. *International Journal of Agriculture Policy and Research*. **1**(4), 93–102. Retrieved from http://www.cabdirect.org/abstracts/20143411899.html

Abdul Gaffer, John Afflitto, S.-H. L. (1992, May 6). Anticalculus oral composition (EP 1989010). Retrieved from http://www.google.com/patents/EP0329069B1?cl=un. Accessed on 26th June, 2016.

Abubakar, A., Uriyo, J., Msuya, S. E., & Swai, M. (2012). Prevalence and Risk Factors for Poor Nutritional Status among Children in the Kilimanjaro Region of Tanzania. *International Journal of Environmental Research and Public Health*. **9**, 3506–3518. https://doi.org/10.3390/ ijerph9103506

Abuya, B. A., Ciera, J., & Kimani-Murage, E. (2012). Effect of mother's education on child's nutritional status in the slums of Nairobi. *BMC Pediatrics*. **12**(1), 80. https://doi.org/10.1186/1471-2431-12-80

Adair, L. S., Fall, C. H. D., Osmond, C., Stein, A. D., Martorell, R., Ramirez-Zea, M., … Victora, C. G. (2013). Associations of linear growth and relative weight gain during early life with adult health and human capital in countries of low and middle income: Findings from five birth cohort studies. *The Lancet*. **382**(9891), 525–534. https://doi.org/10.1016/S0140-6736(13)60103-8

Adimasu, M., Lelisa, A., & Geleta, B. (2016). Complementary Feeding: Review of

Recommendations, Feeding Practices, and Adequacy of Homemade Complementary Food Preparations in Developing Countries – Lessons from ethiopia. *Frontiers in Nutrition*. **3**(3), 413389–41. https://doi.org/10.3389/fnut.2016.00041

Ahmed, T., Hossain, M., & Sanin, K. I. (2013). Global burden of maternal and child undernutrition and micronutrient deficiencies. *Annals of Nutrition and Metabolism*. **61**(suppl 1), 8–17. https://doi.org/10.1159/000345165

Alexy, U., Kersting, M., & Sichert-Hellert, W. (2006). Evaluation of dietary fibre intake from infancy to adolescence against various references - results of the DONALD Study. *European Journal Of Clinical Nutrition*. **60**(7), 909–914. https://doi.org/10.1038/sj.ejcn.1602400

Algarin, C., Nelson, C. A., Peirano, P., Westerlund, A., Reyes, S., & Lozoff, B. (2013). Iron-deficiency anemia in infancy and poorer cognitive inhibitory control at age 10 years. *Developmental Medicine and Child Neurology*. **55**(5), 453–458. https://doi.org/10.1111/dmcn.12118

Ali, S., Chaudry, T., & Naqvi, Q. (2011). Effect of maternal literacy on child health: Myth or reality. *Annals of. Pakistan Institute of Medical Sci*ence. **7**(2), 100–103. Retrieved from http://apims.net/Volumes/Vol7-2/Effect of Maternal Literacy on Child Health-Myth or Reality.pdf

Allen, L. H. (2012). Global dietary patterns and diets in childhood: Implications for health outcomes. *Annals of Nutrition and Metabolism*. **61**(1), 29–37. https://doi.org/10.1159/000346185

Amagloh, F. K., & Coad, J. (2014). Orange-fleshed sweet potato-based infant food is a better source of dietary vitamin A than a maize – legume blend as complementary food. *Food & Nutrition Research*. **35**(1), 51–59.

Amagloh, F. K., Hardacre, A., Mutukumira, A. N., Weber, J. L., Brough, L., & Coad, J. (2012). Sweet potato-based complementary food for infants in low-income countries. *Food & Nutrition Research*. **33**(1), 3–10.

Amare, E., Mouquet-Rivier, C., Rochette, I., Picq, C., Adish, A., & Desse, G. (2015). Adequacy of Iron and Zinc Contents and Estimated Bioavailability in Commercially Available Complementary Foods in Ethiopia. *European Journal of Nutrition and Food Safety*. **5**(5), 438–439. https://doi.org/10.9734/EJNFS/2015/20899

Amare Zelellw, D. (2011). Prevalence and Associated Factors of Stunting Among Schoolchildren, in Debre Markos Town and Gozamen Woreda, East Gojjam Zone, Amhara Regional State, Ethiopia, 2013. *Journal of Nutrition and Food Sciences*. **8**(007), 1 – 5. https://doi.org/10.4172/2155-9600.S8-007

Anigo, K. M., Ameh, D. a, Ibrahim, S., & Danbauchi, S. S. (2009). Nutrient composition of commonly used complementary foods in North western Nigeria. *African Journal of Biotechnology*. **8**(17), 4211–4216. https://doi.org/10.4314/ajb.v8i17.62358

Anwar, S., Nasreen, S., Batool, Z., & Husain, Z. (2013). Maternal education and child nutritional status in Bangladesh: Evidence from demographic and health survey data. *Pakistan Journal of Life and Social Sciences*. **11**(October), 77–84.

Aranceta, J., & Pérez-Rodrigo, C. (2012). Recommended dietary reference intakes, nutritional goals and dietary guidelines for fat and fatty acids: a systematic review. *British Journal of Nutrition*. **107**(S2), S8–S22. https://doi.org/10.1017/S0007114512001444

Asfaw, M., Wondaferash, M., Taha, M., & Dube, L. (2015). Prevalence of undernutrition and associated factors among children aged between six to fifty nine months in Bule Hora district, South Ethiopia. *BMC Public Health*. **15**(1), 41. https://doi.org/10.1186/s12889-015-1370-9

Association of Official Analytical Chemists (AOAC). (2000a). *AOAC Official Method 999.07 Aflatoxin B1 and Total Aflatoxins in Peanut Butter, Pistochio Paste, Fig Paste, and Paprika Powder.*

Association of Official Analytical Chemists (AOAC). (2000b). *Determination of Lead, Cadmium, Copper, Iron and Zinc in Foods Using Atomic Spectrophotometer After Ashing. Officil Method No. 999.11*.

Association of Official Analytical Chemists (AOAC). (2005). .*Determination of Moisture content, Fat content, Ash content, Protein content and Fibre content in Foods using. Official Method no. 925.10, 2003.05, 923.03, 960.52 and 962.09 respectively*.

Atongbiik, M., Opoku, N., & Kweku, F. (2017). A flatoxin contamination in cereals and legumes to reconsider usage as complementary food ingredients for Ghanaian infants : A review. *Journal of Nutrition & Intermediary Metabolism*. **10**, 1–7. https://doi.org/10.1016/j.jnim.2017.09.001

Bailey, R. L., West Jr., K. P., & Black, R. E. (2015). The Epidemiology of Global Micronutrient Deficiencies. *Annals of Nutrition and Metabolism*. **66**(2), 22–33. https://doi.org/10.1159/000371618

Barlow, S., Renwick, A. G., Kleiner, J., Bridges, J. W., Busk, L., Dybing, E., & Edler, L. (2006). Risk assessment of substances that are both genotoxic and carcinogenic Report of an International Conference organized by EFSA and WHO with support of ILSI Europe q. *Food and Chemical Toxicology*. **44**, 1636–1650. https://doi.org/10.1016/j.fct.2006.06.020

Bbaale, E. (2014). Maternal education and child nutritional status: evidence from Uganda. *African Journal of Economic and Management Studies*. **5**(1), 52–74. https://doi.org/10.1108/AJEMS-06-2012-0040

Bbosa, G. S., Kitya, D., Odda, J., & Ogwal-okeng, J. (2013). Aflatoxins metabolism , effects on epigenetic mechanisms and their role in carcinogenesis. *Health*. **5**(10), 14–34.

Beard, J. L. (2008). Why iron deficiency is important in infant development. *The Journal of Nutrition*. **138**(12), 2534–2536. https://doi.org/138/12/2534 [pii]

Beck, K. L., Conlon, C. A., Kruger, R., & Coad, J. (2014). Dietary determinants of and possible solutions to iron deficiency for young women living in industrialized countries: A review. *Nutrients*. **6**(9), 3747–3776. https://doi.org/10.3390/nu6093747

Beck, K. L., & Heath, A.-L. M. (2013). Dietary approaches to assessing iron-related nutrition. *Current Opinion in Clinical Nutrition and Metabolic Care*. **16**(6), 712–8. https://doi.org/10.1097/MCO. 0b013e328364f382

Bhat, R., Rai, R. V, & Karim, A. A. (2010). Mycotoxins in Food and Feed : Present Status and Future Concerns. *Comprehensive Reviews in Food Science and Food Safety*. **9**, 57–81.

Bhutta, Z. A., Ahmed, T., Black, R. E., Cousens, S., Dewey, K., Giugliani, E., … Shekar, M. (2008). What works? Interventions for maternal and child undernutrition and survival. *The Lancet*. **371**(9610), 417–440. https://doi.org/10.1016/S0140-6736(07)61693-6

Bhutta, Z. A., & Salam, R. A. (2012). Global Nutrition Epidemiology and Trends. *Annals of Nutrition and Metabolism*. **61**(s1), 19–27. https://doi.org/10.1159/000345167

Bhutta, Z. A., Salam, R. A., & Das, J. K. (2013). Meeting the challenges of micronutrient malnutrition in the developing world. *British Medical Bulletin*. **106**(1), 7–17. https://doi.org/10.1093/bmb/ldt015

Black, M. M. (2012). Integrated strategies needed to prevent iron deficiency and to promote early child development. *Journal of Trace Elements in Medicine and Biology*. **26**(2-3), 120–123. https://doi.org/10.1016/j.jtemb.2012.04.020

Black, R. E., Allen, L. H., Bhutta, Z. A., Caulfield, L. E., de Onis, M., Ezzati, M., … Rivera, J. (2008). Maternal and child undernutrition: global and regional exposures and health consequences. *The Lancet*. **371**(9608),243–260. https://doi.org/10.1016/S0140-6736(07)61690-0

Black, R. E., Victora, C. G., Walker, S. P., Bhutta, Z. A., Christian, P., de Onis, M., … Uauy, R. (2013). Maternal and child undernutrition and overweight in low-income and middle-income countries. *The Lancet*. **382**(9890), 427–451. https://doi.org/10.1016/S0140-6736(13)60937-X

Bohn, L., Meyer, A. S., & Rasmussen, S. K. (2008). Phytate: impact on environment and human nutrition. A challenge for molecular breeding. *Journal of Zhejiang University SCIENCE B*. **9**(3), 165–191. https://doi.org/10.1631/jzus.B0710640

Brown, K. ., Peerson, J., Baker, S. K., & Hess, S. . (2009). Preventive zinc supplementation among infants,preschoolers, and older prepubertal children. *Food and Nutrition Bulletin*. **30**(1), 12–40.

Brown, K. H., Peerson, J. M., Rivera, J., & Allen, L. H. (2002). Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. *The American Journal of Clinical Nutrition*. **75**(6), 1062–1071.

Bucholz, E. M., Desai, M. M., & Rosenthal, M. S. (2011). Dietary intake in head start vs non-head start preschool-aged children: Results from the 1999-2004 national health and nutrition examination survey. *Journal of the American Dietetic Association*. **111**(7), 1021–1030. https://doi.org/10.1016/j.jada.2011.04.009.

Butte, N. F. (2005). Energy requirements of infants. *Public Health Nutrition*. **8**(7a), 957 – 967. https://doi.org/10.1079/PHN2005790.

Carter, A. R. C., & Jacobson, J. L. (2010). Iron Deficiency Anemia and Cognitive Function in Infancy. *Pediatrics*. **126**(2), e427 – e434. https://doi.org/10.1542/peds.2009-2097

Casale, D., Desmond, C., & Richter, L. (2014). The association between stunting and psychosocial development among preschool children: A study using the South African Birth to Twenty cohort data. *Child: Care, Health and Development*. **40**(6), 900–910. https://doi.org/10.1111/cch.12143

Chala, A., Taye, W., Ayalew, A., Krska, R., Sulyok, M., & Logrieco, A. (2014). Multimycotoxin analysis of sorghum (Sorghum bicolor L . Moench) and fi nger millet (Eleusine coracana L . Garten) from Ethiopia. *Food Control*. **45,** 29–35.https://doi.org/10.1016/j.foodcont.2014.04.018

Champ, M. (2002). Non-nutrient bioactive substances of pulses. *British Journal of Nutrition*. **88**(3), 307 – 319. Retrieved from http://journals.cambridge.org/abstract_S000711450200260X. Accessed on 17th June, 2016.

Chang, S., Zeng, L., Brouwer, I. D., Kok, F. J., & Yan, H. (2013). Effect of iron deficiency anemia in pregnancy on child mental development in rural China. *Pediatrics*. **131**(3), e755–63. https://doi.org/10.1542/peds.2011-3513

Chauhan, Y., Tatnell, J., Krosch, S., Karanja, J., Gnonlonfin, B., Wanjuki, I., Harvey, J. (2015). An improved simulation model to predict pre-harvest aflatoxin risk in maize. *Field Crops Research*. **178**, 91–99. https://doi.org/10.1016/j.fcr.2015.03.024

Chen, J., Egner, P. a, Ng, D., Jacobson, L. P., Muñoz, A., Zhu, Y., Kensler, T. W. (2014). Reduced Aflatoxin Exposure Presages Decline in Liver Cancer Mortality in an Endemic Region of China. *Cancer Prevention Res*earch. **6**(10), 1038–1045. https://doi.org/10.1158/1940-6207.CAPR-13-0168.

Chen, Y., & Li, H. (2009). Mother’s education and child health: Is there a nurturing effect? *Journal of Health Economics*. **28**(2), 413–426. https://doi.org/10.1016/j.jhealeco.2008.10.005

Coulombe, R. A., Huie, J. M., Sharma, R. W., & Wilson, D. W. (1991). Pharmacokinetics of Intratracheally Administered Aflatoxin B1. *Toxicology and Applied Pharmacology*. **109**, 196 – 206.

Darwish, W. S., Ikenaka, Y., Nakayama, S. M. M., & Ishizuka, M. (2014). An Overview on Mycotoxin Contamination of Foods in Africa. *Toxicology*. **76**(6), 789 – 797. https://doi.org/10.1292/jvms.13-0563

Davies, N. T., & Reid, H. (1979). An evaluation of the phytate, zinc, copper, iron and manganese contents of, and zn availability from, soya-based textured-vegetable-protein meat-substitutes or meat-extenders. *British Journal of Nutrition*. **41**(3), 579–89. https://doi.org/10.1079/ BJN19790073

de Onis, M., & Branca, F. (2016). Childhood stunting: A global perspective. *Maternal and Child Nutrition*. **12**, 12–26. https://doi.org/10.1111/mcn.12231

Dellavalle, D. M. (2013). Iron Supplementation for Female Athletes : Effects on Iron Status and Performance Outcomes. *Nutrition and Ergogenic AIDS*. **12**(4), 2–7.

Deshpande, J., Joshi, M., & Giri, P. (2013). Zinc: The trace element of major importance in human nutrition and health. *International Journal of Medical Science and Public Health*. **2**(1), 1. https://doi.org/10.5455/ijmsph.2013.2.1-6

Dewey, K. (2013). The challenge of meeting nutrient needs of infants and young children during the period of complementary feeding: an evolutionary perspective. *The Journal of Nutrition*. **143**, 2050 – 2054. https://doi.org/10.39.45/jn.113.182527

Dewey, K., and Vitta, B. S. (2013). Strategies for ensuring adequate nutrient intake for infants and young children during the period of complementary feeding. A and T Techinical Brief. *Washington: Alive & Thrive* - FHI 360. Dharmaputra, O. S., Ambarwati, S., & Retnowati, I. N. A. (2015). Fungal infection and aflatoxin contamination in stored nutmeg (Myristica fragrans) Kernels at various stages of delivery chain in north sulawesi province. *Biotropia*. **22**(2), 129–139. https://doi.org/10.11598/btb.2015.22.458

Domellöf, M., Braegger, C., Campoy, C., Colomb, V., Decsi, T., Fewtrell, M., van Goudoever, J. (2014). Iron requirements of infants and toddlers. *Journal of Pediatric Gastroenterology and Nutrition*. **58**(1), 119–29. https://doi.org/10.1097/MPG.0000000000000206

European Commision (EC) (2006). Commission regulation No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Journal of European Union*, (1881), 5–24. https://doi.org/10.2203/dose-response.06-012.

Edwards, C., & Parrett, M. (2003). Dietary fibre in infancy and childhood. *The Proceedings of the Nutrition Society*. **62**, 17–23. https://doi.org/10.1079/PNS2002231

Erdman, & Poneros-Schneier, A. (2013). *Mineral Absorption in the Monogastric GI Tract*. *Mineral Absorption in the Monogastric GI Tract*. Retrieved from https://books.google.com/books?isbn= 1468491113. Accessed on 5th April, 2016

European Commission. (2010). Commission Regulation No. 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. *Official Journal of the European Union*. L **50/8**(2009), 5.

European Food Safety Authority (EFSA). (2007). Opinion of the scientific panel on contaminants in the food chain on a request from the commission related to the potential increase of consumer health risk by a possible increase of the existing maximum levels for aflatoxins in almonds, hazelnuts and pistachios and derived products. *The European*

Food Safety Authority Journal. **446**, 1–127.

European Food Safety Authority (EFSA). (2009). Scientific Opinion on the appropriate age for introduction of complementary feeding of infants. *The European Food Safety Authority Journal*. **7**(12), 1–38. https://doi.org/10.2903/j.efsa.2009.1423.

Fabbri, A. D. T., & Crosby, G. a. (2015). A review of the impact of preparation and cooking on the nutritional quality of vegetables and legumes. *International Journal of Gastronomy and Food Science,* 1–10. https://doi.org/10.1016/j.ijgfs.2015.11.001

Fahmida, U. (2013). *Use of fortified foods for Indonesian infants. In: Preedy VR, Srirajaskanthan R, Patel VB, eds. Hand-book of food fortification and health: from concepts to publications health application.* New York: pp 383 - 93.

Fahmida, U., Santika, O., Kolopaking, R., & Ferguson, E. (2014). Complementary feeding recommendations based on locally available foods in Indonesia. *Food and Nutrition Bulletin*. **35**(4), 174–179.

FAO/WHO. (1985). Energy and Protein Requirement. Energy and Protein Requirement Vol. 724. https://doi.org/92 5 105212 3

FAO/WHO. (1991). Guidelines on Formulated Supplementary Foods For Older Infants and Young Children. Joint Publication. Geneva Vol. 4. FAO/WHO. (2004). Vitamin and mineral requirements in human nutrition Second edition.

Fekadu, Y., Mesfin, A., Haile, D., & Stoecker, B. J. (2015). Factors associated with nutritional status of infants and young children in Somali Region , Ethiopia : a cross- sectional study. *BMC Public Health*. **15**, 1–9. https://doi.org/10.1186/s12889-015-2190-7

Fischer Walker, C. L., Ezzati, M., & Black, R. E. (2009). Global and regional child mortality and burden of disease attributable to zinc deficiency. *European Journal of Clinical Nutrition*.

63(5), 591–597. https://doi.org/10.1038/ejcn.2008.9

Food and Agriculture Organization, F. (2008). *Guidelines for measuring household and individual dietary diversity*.

Friel, J. K., Hanning, R. M., Isaak, C. a, Prowse, D., & Miller, A. C. (2010). Canadian infants' nutrient intakes from complementary foods during the first year of life. *BMC Pediatrics*. **10**, 43. https://doi.org/10.1186/1471-2431-10-43

Frost, M. B., Forste, R., & Haas, D. W. (2005). Maternal education and child nutritional status in Bolivia: Finding the links. *Social Science and Medicine*. **60**(2), 395–407. https://doi.org/10.1016/ j.socscimed.2004.05.010

Fuqua, B. K., Vulpe, C. D., & Anderson, G. J. (2012). Intestinal iron absorption. *Journal of Trace Elements in Medicine and Biology*. **26**(2-3), 115–119. https://doi.org/10.1016/j.jtemb.2012.03.015

Gatahun, E. A., & Direseling M. Abyu. (2015). Nutrition & Food Sciences Dietary Diversity Feeding Practice and Determinants among Children Aged 6-23 Months in Kemba Woreda , Southern Ethiopia Implication for Public Health Intervention. *Nutrition and Food Sciences*. **13003**(13), 1 – 9. https://doi.org/10.4172/2155-9600.1000S13003.

Geary, P. A., Chen, G., Kimanya, M. E., Shirima, C. P., Oplatowska-Stachowiak, M., Elliott, C. T., Gong, Y. Y. (2016). Determination of multi-mycotoxin occurrence in maize based porridges from selected regions of Tanzania by liquid chromatography tandem mass spectrometry (LC-MS/MS), a longitudinal study. *Food Control*. **68**, 337–343. https://doi.org/10.1016/ j.foodcont.2016.04.018

Gemede, H. (2014). Potential Health Benefits and Adverse Effects Associated with Phytate in Foods. *Food Science and Quality Management*. **27**, 45–55.

Getenesh, B., Addisalem, M., Afework, K., Susan, J. W., & Carol, J. H. (2014). Household food processing methods to enhance iron and zinc bioavailability in formulated haricot bean and maize complementary food. *African Journal of Food Science*. **8**(4), 190–195. https://doi.org/10.5897/AJFS2014.1141.

Gibson, R. S., Bailey, K. B., Gibbs, M., & Ferguson, E. L. (2010). A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. *Food and Nutrition Bull*etin. **31**(2), S134–46. https://doi.org/doi:10.1007/s00394-006-0637-4.

Gibson, R. S., Perlas, L., & Hotz, C. (2006). Improving the bioavailability of nutrients in plant foods at the household level. *The Proceedings of the Nutrition Society*. ***65***(2), 160–168. https://doi.org/10.1079/PNS2006489.

Global Panel. (2016). The Cost of Malnutrition : Why Policy Action is Urgent. London, UK: Global Panel on Agriculture and Food Systems for Nutrition, (3).

Golden, M. H. (2009). Proposed recommended nutrient densities for moderately malnourished children. *Food and Nutrition Bulletin*. **30**(3), S267–342. Retrieved from http://www.ncbi.nlm.nih.gov/ pubmed/19998863.

Gong, Y., Hounsa, A., Egal, S., Turner, P. C., Sutcliffe, A. E., Hall, A. J., Wild, C. P. (2004). Postweaning exposure to aflatoxin results in impaired child growth: A longitudinal study in Benin, West Africa. *Environmental Health Perspectives*. **112**(13), 1334–1338. https://doi.org/10.1289/ehp.6954.

Gopalsamy, G. L., Alpers, D. H., Binder, H. J., Tran, C. D., Ramakrishna, B. S., Brown, I., Young, G. P. (2015). The relevance of the colon to zinc nutrition. *Nutrients*. **7**(1), 572–83. https://doi.org/10.3390/nu7010572.

Grantham-McGregor, S., Cheung, Y. B., Cueto, S., Glewwe, P., Richter, L., & Strupp, B. (2007).

Developmental potential in the first 5 years for children in developing countries. *Lancet*. **369**(9555), 60–70. https://doi.org/10.1016/S0140-6736(07)60032-4

Greiner.R., K. ., & Jany.K-D. (2006). Phytate - An undesirable constituent of plant-based foods? *Journal Fur Ernahrungsmedizin*. **8**(3), 18–28.

Grimshaw, K. E. C., Maskell, J., Oliver, E. M., Morris, R. C. G., Foote, K. D., Mills, E. N. C., Margetts, B. M. (2013). Introduction of complementary foods and the relationship to food allergy. *Pediatrics*. **132**(6), e1529–38. https://doi.org/10.1542/peds.2012-3692

Gulec, S., Anderson, G. J., & Collins, J. F. (2014). Mechanistic and regulatory aspects of intestinal iron absorption. *AJP: Gastrointestinal and Liver Physiology*. **307**(4), G397–G409. https://doi.org/10.1152/ajpgi.00348.2013.

Haas, J. D., & Iv, T. B. (2001). Iron-Deficiency Anemia : Reexamining the Nature and Magnitude of the Public Health Problem Iron Deficiency and Reduced Work Capacity : A Critical Review of the Research to Determine a Causal Relationship.. *Journal of Nutrition*. 676–690.

Hamza, R. T., Hamed, A. I., & Sallam, M. T. (2012). Effect of zinc supplementation on growth hormone-insulin growth factor axis in short Egyptian children with zinc deficiency. *Italian Journal of Pediatrics*. **38**(1), 21. https://doi.org/10.1186/1824-7288-38-21.

Hell, K., & Mutegi, C. (2011). Aflatoxin control and prevention strategies in key crops of Sub-Saharan Africa. *African Journal of Microbiology Research*. **5**(5), 459–466. https://doi.org/10.5897/AJMR10.009.

Hlaing, L. M., Fahmida, U., Htet, M. K., Utomo, B., Firmansyah, A., & Ferguson, E. L. (2016). Local food-based complementary feeding recommendations developed by the linear programming approach to improve the intake of problem nutrients among 12–23-month-old Myanmar children. *British Journal of Nutrition*. **116**(S1), S16–S26.

https://doi.org/10.1017/S000711451500481X.

Hoffmann, V., Jones, K., & Leroy, J. (2015). Mitigating aflatoxin exposure to improve child growth in Eastern Kenya: study protocol for a randomized controlled trial. *BMC*. **16**(1), 552. https://doi.org/10.1186/s13063-015-1064-8.

Horton, S., & Ross, J. (2003). The economics of iron deficiency. *Food Policy*. **28**, 51–75. https://doi.org/10.1016/S0306-9192(02)00070-2.

Horton, S., & Steckel, R. H. (2011). Malnutrition: Global economic losses attributed to malnutrition 190 - 2000 and projection to 2050. pp 1 - 36.

Hotz, C., & Gibson, R. S. (2007). Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plant-based diets. *The Journal of Nutrition*. **137**(4), 1097–1100.

Huang, J., Simcox, J., Mitchell, T. C., Jones, D., Cox, J., Luo, B., … McClain, D. A. (2013). Iron regulates glucose homeostasis in liver and muscle via AMP-activated protein kinase in mice. *FASEB Journal*. **27**(7), 2845–54. https://doi.org/10.1096/fj.12-216929

Hurrell, R., & Egli, I. (2010). Iron bioavailability and dietary reference values. *The American Journal of Clinical Nutrition*. **91**(5), 1461S–1467S. https://doi.org/10.3945/ajcn.2010.28674F.Am.

Huskisson, E., Maggini, S., & Ruf, M. (2007). The influence of micronutrients on cognitive function and performance. *The Journal of International Medical Research*. **35**, 1–19. https://doi.org/10.1177/147323000703500101.

Islam, M. A., Ahmed, T., Faruque, A. S. G., Rahman, S., Das, S. K., Ahmed, D., … Cravioto, A. (2012). Microbiological quality of complementary foods and its association with diarrhoeal morbidity and nutritional status of Bangladeshi children. *European Journal of*

Clinical Nutrition. **66**(11), 1242–1246. https://doi.org/10.1038/ejcn.2012.94.

Jonathan, S., Abdul-Lateef, M., Olawuyu, O., & Oyelakin, A. (2012). Studies on bio-deterioration, aflatoxin contamination and food values of fermrnted, dried and stored ipomoea chips. *Nature and Science*. **10**(11), 123 – 128.

Jonsdottir, Olof Thorsdottir, I., Hibberd, P. L., Fewtrell, M. S., Wells, C. J., Palsson, G. I., Lucas, Alan Geir, G., & Kleinman, R. E. (2012). Timing of the Introduction of Complementary Foods in Infancy : A Randomized Controlled Trial. *Pediatrics*. **130**, 1038–1045.

Kabak, B., Dobson, A. D. W., & Var, I. (2006). Strategies to prevent mycotoxin contamination of food and animal feed: A review. *Critical Reviews in Food Science and Nutrition.* **46**(8), 593–619. https://doi.org/10.1080/10408390500436185.

Kachapulula, P. W., Akello, J., & Bandyopadhyay, R. (2017). Aflatoxin contamination of groundnut and maize in Zambia : observed and potential concentrations. *Journal of Applied Microbiology*. **122**, 1471–1482. https://doi.org/10.1111/jam.13448.

Kamika, I., Ngbolua, K.-N., & Tekere, M. (2016). Occurrence of aflatoxin contamination in maize throughout the supply chain in the Democratic Republic of Congo. *Food Control*. **69**, 292–296. https://doi.org/10.1016/j.foodcont.2016.05.014.

Kana Sop, M. M., Gouado, I., Mananga, M. J., Djeukeu Asongni, W., Amvam Zollo, P. H., Oberleas, D., & Tetanye, E. (2012). Trace elements in foods of children from Cameroon: A focus on zinc and phytate content. *Journal of Trace Elements in Medicine and Biology*. **26**(2-3), 201–204. https://doi.org/10.1016/j.jtemb.2012.03.018.

Karlovsky, P., Suman, M., Berthiller, F., Meester, J. De, Eisenbrand, G., Perrin, I., … Speijers, G. (2016). Impact of food processing and detoxification treatments on mycotoxin contamination. *Mycotoxin Research*. **32**, 179–205. https://doi.org/10.1007/s12550-016-0257-7.

Kayaalti, Z., Akyuzlu, D. K., & Soylemezoglu, T. (2015). Evaluation of the effect of divalent metal transporter 1 gene polymorphism on blood iron, lead and cadmium levels. *Environmental Research*. **137**, 8–13. https://doi.org/10.1016/j.envres.2014.11.008.

Kell K. P., Judd S. E, Pearson K. E., S. J. M. and F. J. R. (2015). Associations between socio-economic status and dietary patterns in US black and white adults. *British Journal of Nutrition*. **113**(11), 1792–1799. https://doi.org/10.1017/S0007114515000938 Associations.

Kevin, C. J. and P. (2014). Iron deficiency and iron deficiency anemia. *Journal of Clinical and Laboratory Investigation.* **74**(244), 82–89. https://doi.org/10.3109/00365513.2014.936694.

Khan Mohamed Shoaib, Shah Farid Ullah, A. Z. and S. A. (2015). Biochemistry & Pharmacology : Open Access Hidden Deficiency of Micronutrients in Apparently Healthy Children of. *Biochemistry and Pharmacology*. **4**(3), 1 – 6. https://doi.org/10.4172/2167-0501.1000172.

Khlangwiset, P., Shephard, G. S., & Wu, F. (2011). Aflatoxins and growth impairment : A review. *Critical Reviews in Toxicology*. **41**(January), 740–755. https://doi.org/10.3109/10408444.2011. 575766.

Kimanya, M. E. (2015). The health impacts of mycotoxins in the eastern Africa region. *Current Opinion in Food Science*. **6**, 7–11. https://doi.org/10.1016/j.cofs.2015.11.005.

Kimanya, M. E., De Meulenaer, B., Roberfroid, D., Lachat, C., & Kolsteren, P. (2010). Fumonisin exposure through maize in complementary foods is inversely associated with linear growth of infants in Tanzania. *Molecular Nutrition and Food Research*. **54**(11), 1659–1667. https://doi.org/10.1002/mnfr.200900483.

Kimanya, M. E., De Meulenaer, B., Tiisekwa, B., Ndomondo-Sigonda, M., Devlieghere, F., Van Camp, J., & Kolsteren, P. (2008). Co-occurrence of fumonisins with aflatoxins in home-stored maize for human consumption in rural villages of Tanzania. *Food Additives & Contaminants*. **25**(11), 1353–1364. https://doi.org/10.1080/02652030802112601.

Kimanya, M. E., Meulenaer, B. De, Tiisekwa, B., Ugullum, C., Devlieghere, F., & Camp, J. Van. (2009). Fumonisins exposure from freshly harvested and stored maize and its relationship with traditional agronomic practices in Rombo district, Tanzania. *Food Additives & Contaminants*. **26**(8), 1199–1208. https://doi.org/10.1080/02652030902922784.

Kimanya, M. E., Shirima, C. P., Magoha, H., Shewiyo, D. H. D. H., Meulenaer, B., Kolsteren, P., … Gong, Y. Y. (2014). C0-exposure of aflatoxin with deoxynivalenol and fumonisins from maize based complementary foods in Rombo, Northern Tanzania. *Food Control*. **41**(1), 76–81. https://doi.org/10.1016/j.foodcont.2013.12.034.

Kirksey, A., Wachs, T. D., Yunis, F., Srinath, U., Rahmanifar, A., McCabe, G. P., … Jerome, N. W. (1994). Relation of maternal zinc nutriture to pregnancy outcome and infant development in an Egyptian village. *The American Journal of Clinical Nutrition*. **60**(5), 782–92. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7942587.

Korkalo, L., Freese, R., Alfthan, G., Fidalgo, L., & Mutanen, M. (2015). Poor micronutrient intake and status is a public health problem among adolescent Mozambican girls. *Nutrition Research*. **35**(8), 664–673. https://doi.org/10.1016/j.nutres.2015.05.013.

Krebs, N. F. (2014). Food-Based Complementary Feeding Strategies for Breast-Fed Infants. *Nutrition Today*. **49**(6), 271–277. https://doi.org/10.1097/NT.0000000000000064.

Kulkarni, H., Mamtani, M., & Patel, A. (2012). Roles of zinc in the pathophysiology of acute diarrhea. *Current Infectious Disease Reports*. **14**(1), 24–32. https://doi.org/10.1007/s11908-011-0222-8.

Kulwa, K. B. M., Mamiro, P. S., Kimanya, M. E., Mziray, R., & Kolsteren, P. W. (2015). Feeding practices and nutrient content of complementary meals in rural central Tanzania: implications for dietary adequacy and nutritional status. *BMC Pediatrics*. **15**(1), 171. https://doi.org/10.1186/s12887-015-0489-2.

Kumar, P., Mahato, D. K., Kamle, M., & Mohanta, T. K. (2017). Aflatoxins : A Global Concern for Food Safety , Human Health and Their Management. *Frontiers in Microbiology*. **7**(January), 1–10. https://doi.org/10.3389/fmicb.2016.02170.

Kumar, V., Sinha, A. K., Makkar, H. P. S., & Becker, K. (2010). Dietary roles of phytate and phytase in human nutrition: A review. *Food Chemistry*. **120**(4), 945–959. https://doi.org/10.1016/ j.foodchem.2009.11.052.

Kumssa, D. B., Joy, E. J. M., Ander, E. L., Watts, M. J., Young, S. D., Walker, S., & Broadley, M. R. (2015). Dietary calcium and zinc deficiency risks are decreasing but remain prevalent. *Scientific Reports*. **5**, 1 – 11. https://doi.org/10.1038/srep10974.

Labadarios, D., Steyn, N. P., & Nel, J. (2011). How diverse is the diet of adult South Africans ? *Nutrition Journal*. **10**(1), 33. https://doi.org/10.1186/1475-2891-10-33.

Laswai, H. S., & Tetens, I. (2000). Nutritional composition and micronutrient status of home made and commercial weaning foods consumed in Tanzania.

Lazarte, C. E., Carlsson, N.-G., Almgren, A., Sandberg, A.-S., & Granfeldt, Y. (2015). Phytate, zinc, iron and calcium content of common Bolivian food, and implications for mineral bioavailability. *Journal of Food Composition and Analysis*. **39**, 111–119. https://doi.org/10.1016/ j.jfca.2014.11.015.

Lestienne, I., Mouquet-Rivier, C., Icard-Vernière, C., Rochette, I., & Trèche, S. (2005). The effects of soaking of whole, dehulled and ground millet and soybean seeds on phytate degradation and Phy/Fe and Phy/Zn molar ratios. *International Journal of Food Science* *and Technology*. **40**(4), 391–399. https://doi.org/10.1111/j.1365-2621.2004.00941.x.

Liang, J., Han, B., Nout, M. J. R., & Hamer, R. J. (2008). Effects of soaking , germination and fermentation on phytic acid , total and in vitro soluble zinc in brown rice. *Food Chemistry*. **110**, 821–828. https://doi.org/10.1016/j.foodchem.2008.02.064.

Linert, W., & Kozlowski, H. (2012). Metal Ions in Neurological Systems. *Springer Science & Business Media*. Retrieved from https://books.google.com/books?id=5Hhgc_eQAMgC&pgis=1. Accessed on November, 2016.

Lombard, M. J. (2014). Mycotoxin exposure and infant and young child growth in Africa: what do we know? *Annals of Nutrition & Metabolism*. **64**(2), 42–52. https://doi.org/10.1159/000365126

Lozoff, B., Jimenez, E., Hagen, J., Mollen, E., & Wolf, A. W. (2000). After treatment for iron deficiency in infancy. *Pediatrics*. **105**(4), E51.

Lozoff, B., Smith, J. B., Kaciroti, N., Clark, K. M., Guevara, S., & Jimenez, E. (2013). Functional significance of early-life iron deficiency: Outcomes at 25 years. *Journal of Pediatrics*. **163**(5), 1260–1266. https://doi.org/10.1016/j.jpeds.2013.05.015.

Lukacik, M., Thomas, R. L., & Aranda, J. V. (2008). A meta-analysis of the effects of oral zinc in the treatment of acute and persistent diarrhea. *Pediatrics*, *121*(2), 326–336. https://doi.org/10.1542/peds.2007-0921 Lundström, U. (1994). Iron deficiency anaemia. *British Society of Gastroenterology*. **35**(6), 864–865. https://doi.org/10.1136/pgmj.2009.089987.

Lutter, C. K., & Dewey, K. G. (2003). Nutrient Composition for Fortified Complementary Foods Proposed Nutrient Composition for Fortified Complementary Foods 1. *Journal of Nutrition*. **133**, 2962 – 2972.

Magoha, H., Kimanya, M., De Meulenaer, B., Roberfroid, D., Lachat, C., & Kolsteren, P. (2014). Risk of dietary exposure to aflatoxins and fumonisins in infants less than 6 months of age in Rombo, Northern Tanzania. *Maternal & Child Nutrition*. 1–12. https://doi.org/10.1111/mcn.12155

Mahfuz, S., Hasan, A., Hassan, M., Saha, S., Islam, M., Billah, M., & Islam, S. (2016). Dietary phytate intake inhibits the bioavailability of iron and calcium in the diets of pregnant women in rural Bangladesh : a cross-sectional study. *BMC Nutrition*. 1–10. https://doi.org/10.1186/s40795-016-0064-8

Manary, M. J., Hotz, C., Krebs, N. F., Gibson, R. S., Westcott, J. E., Broadhead, R. L., & Hambidge, K. M. (2002). Zinc homeostasis in Malawian children consuming a high-phytate, maize-based diet. *Am J Clin Nutr*, *75*(6), 1057–1061. Mandal, S. (2016). Phytochemical analysis of groundnut (Arachis hypogea L) Swapan Mandal. *International Journal of Experimental Research and Review*. **6**, 35–38.

Maren Fischer, Ines Egli, Isabelle Aeberli, michael Z. and R. H. (2015). Strategies to Enhance Iron Absorption from Tef-injera in Young Women - ProQuest. *European Journal of Food Research and Review*, *5.5*, 1169 – 1170. Retrieved Maret, W. (2013). Zinc biochemistry: from a single zinc enzyme to a key element of life. *Advances in Nutrition.* **4**(1), 82–91. https://doi.org/10.3945/an.112.003038.

Maseta, E., Mosha, T. C., Laswai, H., & Nyaruhucha, C. N. (2016). Nutritional Quality, Mycotoxins and Antinutritional Factors in Quality Protein Maize-Based Supplementary Foods for Children in Tanzania. *International Journal of Sciences*. **5**(7), 38 – 41. https://doi.org/10.18483/ijSci.1082.

Matanda, D. J., Urke, H. B., & Mittelmark, M. B. (2016). Changes in optimal childcare practices in Kenya: Insights from the 2003, 2008-9 and 2014 demographic and health surveys. *PLoS ONE*. **11**(8), 1–23. https://doi.org/10.1371/journal.pone.0161221.

Mayer, L. S., Uciechowski, P., Meyer, S., Schwerdtle, T., Rink, L., & Haase, H. (2014). Differential impact of zinc deficiency on phagocytosis, oxidative burst, and production of pro-inflammatory cytokines by human monocytes. *Metallomics : Integrated Biometal Science*. **6**, 1288–95. https://doi.org/10.1039/c4mt00051j.

McCann, J. C., Hudes, M., & Ames, B. N. (2006). An overview of evidence for a causal relationship between dietary availability of choline during development and cognitive function in offspring. *Neuroscience and Biobehavioral Reviews*. **30**(5), 696–712. https://doi.org/10.1016/j.neubiorev.2005.12.003.

McLean, E., Cogswell, M., Egli, I., Wojdyla, D., & de Benoist, B. (2009). Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993–2005. *Public Health Nutrition*. **12**(04), 1 – 11. https://doi.org/10.1017/S1368980008002401.

Melese, T. (2013). Nutritional Status of Ethiopian Weaning and Complementary Foods: A Review. *Open Access Scientific Reports, 2*(2), 1–9. https://doi.org/10.4172/scientificreports.

Michaelsen, K. F. (2015). Child growth. *World Review of Nutrition and Dietetics*. **113**, 1–5. https://doi.org/10.1159/000360310.

Michaelsen, K. F., & Greer, F. R. (2014). Protein needs early in life and long-term health 1 – 4. *American Journal of Clinical Nutrition*. **99**, 718–723. https://doi.org/10.3945/ajcn.113.072603.1.

Miletta, M. C., Schöni, M. H., Kernland, K., Mullis, P. E., & Petkovic, V. (2013). The role of zinc dynamics in growth hormone secretion. *Hormone Research in Pædiatrics*. **80**(6), 381–9. https://doi.org/10.1159/000355408

Miller, B. D. D., & Welch, R. M. (2013). Food system strategies for preventing micronutrient malnutrition. *Food Policy*. **42**, 115–128. https://doi.org/10.1016/j.foodpol.2013.06.008.

Ministry of Health, Community Development, Gender, Elderly and Children (MoHCDGEC) [Tanzania mainland], Ministry of Health (MoH) [Zanzibar], National Bureau of Statistic (NBS), Office of the chief Government Statistician (OCGS) and ICF (2016). Tanzania Demographic and Health Survey and Malaria Indicator Survey (TDHS-MIS). Retrieved from https://www.dhsprogram.com/pubs/pdf/FR321/FR321.pdf. Accessed on June, 2016.

Mokori, A., Schonfeldt, H., & Hendriks, S. L. (2016). Child factors associated with complementary feeding practices in Uganda. *South African Journal of Clinical Nutrition*. **0658**(November), 1–8. https://doi.org/10.1080/16070658.2016.1225887.

Morales-Ruán, M. D. C., Villalpando, S., García-Guerra, A., Shamah-Levy, T., Robledo-Pérez, R., Avila-Arcos, M. A., & Rivera, J. A. (2012). Iron, zinc, copper and magnesium nutritional status in Mexican children aged 1 to 11 years. *Salud Pública de México*. **54**(2), 125–34.

Moran, V. H., & Dewey, K. (2011). Maternal & child nutrition:consequences of malnutrition in early life and strategies to improve maternal and chid diets through targeted fortified products. *Maternal and Child NutritionChild Nutrition*. **7**(3), 1 – 4. https://doi.org/10.1111/j.1740-8709.2011.00348.x.

Muhimbili University of Health and Allied Sciences (MUHAS), Tanzania Food and Nutrition Center (TFNC), and Havard School of Public Health (HSPH). (2008). Tanzania Food Composition Tables. Dar es salaam.

Muhimbula, H. S., & Issa-zacharia, A. (2010). Persistent child malnutrition in Tanzania : Risks associated with traditional complementary foods (A review). *African Journal of Food Science*. **4**(November), 679–692. Muhimbula, H. S., Issa-zacharia, A., & Kinabo, J. (2011). Formulation and sensory evaluation of complementary foods from local , cheap and readily available cereals and legumes in Iringa, Tanzania. *African Journal of Science*, *5*(January), 26–31.

Muthayya, S., Rah, J. H., Sugimoto, J. D., Roos, F. F., Kraemer, K., & Black, R. E. (2013). The Global Hidden Hunger Indices and Maps: An Advocacy Tool for Action. *PLoS ONE*. **8**(6), 1–12. https://doi.org/10.1371/journal.pone.0067860.

Nadadur, S. S., Srirama, K., & Mudipalli, A. (2008). Iron transport & homeostasis mechanisms: their role in health & disease. *The Indian Journal of Medical Research*. **128**(4), 533–544. https://doi.org/http://dx.doi.org/10.1108/17506200710779521.

Nagahori, C., Tchuani, J. P., & Yamauchi, T. (2015). Factors associated with nutritional status in children aged 5-24 months in the Republic of Cameroon. *Nursing & Health Sciences*. **17**(2), 229–35. https://doi.org/10.1111/nhs.12176.

National Bureau of Statistics (NBS). (1997). Tanzania Demographic and Health SurveyVol. 53.

National bureau of Statistics (NBS). (2011). Tanzania Demographic and Health Survey 2010. *Tanzania Demographic and Health Survey*. https://doi.org/zania Demographic and Health Survey 2004-05. Dar es Salaam, Tanzania: National Bureau of Statistics and ORC Macro

National Bureau of Statistics, & Macro International Inc. (2000). *Tanzania Reproductive and Child Health Survey 1999*. *Statistics*. Dar es Salaam, Tanzania: National Bureau of Statistics and ORC Macro.

Negash, C., Whiting, S. J., Henry, C. J., Belachew, T., & Hailemariam, T. G. (2015). Association between maternal and child nutritional status in Hula, rural Southern Ethiopia: A cross sectional study. *PLoS ONE*. **10**(11), 1–8. https://doi.org/10.1371/journal.pone.0142301.

Nigeria National Bureau of Statistic. (2014). *Report on The Nutrition and Health Situation of Nigeria Data Collection – 9 th February - 15 th May 2014 Nutrition and Health Survey Using SMART Methods*.

Norhaizan, M. ., & Norfaizadatul, A. A. (2009). Determination of Phytate , Iron , Zinc , Calcium Contents and Their Molar Ratios in Commonly Consumed Raw and Prepared Food in Malaysia. *Malasian Journal of Nutrition.* **15**(2), 213–222.

Nortaa, E., & Sowley, K. (2016). Aflatoxins : A silent threat in developing countries. *African Journal of Biotechnology*. **15**(35), 1864–1870. https://doi.org/10.5897/AJB2016.15305.

Nouri Saeidlou, S., Babaei, F., & Ayremlou, P. (2014). Malnutrition, overweight, and obesity among urban and rural children in north of west azerbijan, Iran. *Journal of Obesity*. **2014**, 1–6. https://doi.org/10.1155/2014/541213.

Nyaradi, A., Li, J., Hickling, S., Foster, J., & Oddy, W. H. (2013). The role of nutrition in children's neurocognitive development, from pregnancy through childhood. *Frontiers in Human Neuroscience*. **7**, 97. https://doi.org/10.3389/fnhum.2013.00097.

Obade, M., Andang'o, P., Obonyo, C., & Lusweti, F. (2015). Exposure of Children 4 to 6 Months of Age to Aflatoxin in Kisumu County, Kenya. *African Journal of Food, Agriculture, Nutrition, and Development*. **15**(2), 9949–9963.

Obade, M. I., Andang, P., Obonyo, C., & Lusweti, F. (2015). Aflatoxin Exposure in Pregnant Women in Kisumu County , Kenya. *Current Research in Nutrition and Food Science*. **3**(2), 140–149.

Ogbo, F. A., Page, A., Idoko, J., Claudio, F., & Agho, K. E. (2016). Have policy responses in Nigeria resulted in improvements in infant and young child feeding practices in Nigeria? *International Breastfeeding Journal*. **12**(1), 9. https://doi.org/10.1186/s13006-017-0101-5

Oghbaei, M., Prakash, J., & Yildiz, F. (2016). Effect of primary processing of cereals and legumes on its nutritional quality: A comprehensive review. *Cogent Food & Agriculture*. **2**(1), 1136015. https://doi.org/10.1080/23311932.2015.1136015.

Okomo, P., Egali, I., Bohwere, P., Cercamondi, I., Zeder, C., Njage, P., & Owino, V. (2016). Estimated Iron and Zinc Bioavailability in Soybean-Maize-Sorghum Ready to Use Foods: Effect of Soy Protein Concentrate and Added Phytase. *Journal of Food Processing & Technology*. **07**(02), 1 –5. https://doi.org/10.4172/2157-7110.1000556.

Okwunodulu, I.N Iwe, M. . (2015). Micronutrient evaluation of fortified soymilk from spouted whole soybean for complementary feeding using response surface methodology. *Journal of Molecular Studies and Medicine Research*. **01**(01), 16–25. https://doi.org/10.18801/ijbmsr.010115.02.

Onoja, U. S., Akubor, P. I., Gernar, D. I., & Chinmma, C. E. (2014). Evaluation of Complementary Food Formulated from Local Staples and Fortified with Calcium, Iron and Zinc. *Journal of Nutrition & Food Sciences*. **4**(6), 1–6. https://doi.org/10.4172/2155-9600.1000326.

Onomi, S., Okazaki, Y., & Katayama, T. (2004). Effect of dietary level of phytic acid on hepatic and serum lipid status in rats fed a high-sucrose diet. *Bioscience, Biotechnology, and Biochemistry*. **66**(6), 1379 – 1381. Retrieved from http://www.tandfonline.com/doi/abs/10.1271/bbb.68.1379.

Onyango, A. W., Borghi, E., de Onis, M., Casanovas Mdel, C., & Garza, C. (2014). Complementary feeding and attained linear growth among 6-23-month-old children. *Public Health Nutrition.* **17**(9), 1975–1983. https://doi.org/10.1017/S1368980013002401.

Owaga, E., Muga, R., Mumbo, H., & Aila, F. (2011). Chronic dietary aflatoxins exposure in Kenya and emerging public health concerns of impaired growth and immune suppression in children. *International Journal of Biological and Chemical Sciences*. **5**(3), 1325–1336. https://doi.org/10.4314/ijbcs.v5i3.72287.

Pallauf, J., & Rimbach, G. (1997). Nutritional significance of phytic acid and phytase. *Archieve* *of Animal Nutr*ition. **50**(May 2013), 301 – 319.

Patel, A., Pusdekar, Y., Badhoniya, N., Borkar, J., Agho, K. E., & Dibley, M. J. (2012). Determinants of inappropriate complementary feeding practices in young children in India: secondary analysis of National Family Health Survey 2005-2006. *Maternal and Child Nutrition*. **8**, 28–44. https://doi.org/10.1111/j.1740-8709.2011.00385.x.

Patwari, A. K. (1999). Diarrhoea and malnutrition interaction. *Indian Journal of Pediatrics*. **66**(1 l), S124–S134. https://doi.org/10.1016/S0140-6736(89)91505-5.

Pelig-Ba, K. B. (2009). Assessment of phytic acid levels in some local cereal grains in two districts in the Upper East Region of Ghana. *Pakistan Journal of Nutrition*. **8**(10), 1540–1547. https://doi.org/10.3923/pjn.2009.1540.1547.

Petry, N., Egli, I., Gahutu, J. B., Tugirimana, P. L., Boy, E., & Hurrell, R. (2014). Phytic Acid Concentration Influences Iron Bioavailability from Biofortified Beans in Rwandese Women with Low Iron Status 1, 2. *The Journal of Nutrition*. **144**, 1681–1687. https://doi.org/10.3945/ jn.114.192989.1681.

Platel, K., & Srinivasan, K. (2015). Bioavailability of Micronutrients from Plant Foods: An Update. *Critical Reviews in Food Science and Nutrition*, 00–00. https://doi.org/10.1080/ 10408398.2013.781011.

Prendergast, A. J., & Humphrey, J. H. (2014). The stunting syndrome in developing countries. *Paediatrics and International Child Health*. **34**(4), 250–65. https://doi.org/10.1179/ 2046905514Y.0000000158.

Przyrembel, H. (2012). Timing of introduction of complementary food: Short- and long-term health consequences. *Annals of Nutrition and Metabolism.* **60**(2), 8–20. https://doi.org/10.1159/ 000336287.

Rah, J. H., Depee, S., Kraemer, K., Steiger, G., Bloem, M. W., Spiegel, P., Bilukha, O. (2012).

The Journal of Nutrition Supplement: Multiple Micronutrient Nutrition—Evidence from History to Science to Effective Programs Program Experience with Micronutrient Powders and Current Evidence 1,2. *The Journal of Nutrition*. **142**, 191–196. https://doi.org/10.3945/jn.111.140004.

Rajan, A., Pai, S. M., & Margaret, E. B. (2015). *Asia Pacific Journal of Research*. **1**(Xxiv), 48–52.

Rao, A., & Parikh, A. (2015). Original article prevalence of anemia in school age children. *Journal of Advanced Medical and Dental Science Resesearch.* **3**(6), 32–35.

Raymond, J., Kassim, N., Rose, J. W., Agaba, M., Raymond, J., Kassim, N., Agaba, M. (2017). Optimal formulations of local foods to achieve nutritional adequacy for 6 – 23-month-old rural Tanzanian children. *Food & Nutrition Research*. **61**(1), 1 – 11. https://doi.org/10.1080/16546628.2017. 1358035.

Reddy, K., Salleh, B., Saad, B., Abbas, K., Abel, C. A., & T, S. W. (2010). An overview of mycotoxin contamination in foods and its implications for human health An overview of mycotoxin contamination in foods and its implications for human health. *Toxin Review*. **29**(1), 3 – 26. https://doi.org/10.3109/15569541003598553.

Reeve, J., & Cressey, P. (2016). *The New Zealand Mycotoxin Surveillance Program 06-14 Report Series* (Vol. 8).

Rohner, F., Northrop-Clewes, C., Tschannen, A. B., Bosso, P. E., Kouassi-Gohou, V., Erhardt, J. G., Mascie-Taylor, C. N. (2013). Prevalence and public health relevance of micronutrient deficiencies and undernutrition in pre-school children and women of reproductive age in Côte d'Ivoire, West Africa. *Public Health Nutrition*. **17**(9), 1–13. https://doi.org/ 10.1017/S136898001300222X.

Romulus-Nieuwelink, J. J. C., Doak, C., Albernaz, E., Victora, C. G., & Haisma, H. (2011).

Breast milk and complementary food intake in Brazilian infants according to socio-economic position. *International Journal of Pediatric Obesity : IJPO : An Official Journal of the International Association for the Study of Obesity*. **6**(2-2), e508–14. https://doi.org/10.3109/ 17477166.2010.512387.

Roohani, N., Hurrell, R., Kelishadi, R., & Schulin, R. (2013). Zinc and its importance for human health: An integrative review. *Journal of Research and Medical Science*. **18**(2), 144–157. Retrieved from. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3724376&tool=pmcentrez&rendertype=abstract.

Roos, N., Sørensen, J. C., Sørensen, H., Rasmussen, S. K., Briend, A., Yang, Z., & Huffman, S. L. (2013). Screening for anti-nutritional compounds in complementary foods and food aid products for infants and young children. *Maternal and Child Nutrition*. **9**(1), 47–71. https://doi.org/10.1111/j.1740-8709.2012.00449.x.

Ruel, M. T. (2003). Animal source foods to improve micronutrient nutrition and human function in developing countries. Proceedings of a conference. June 24-26, 2002. Washington, DC, USA. *The Journal of Nutrition*. **133**(11 Suppl 2), 3875S–4061S. https://doi.org/0022-3166/03.

Sachdev, H., Gera, T., & Nestel, P. (2005). Effect of iron supplementation on mental and motor development in children: systematic review of randomised controlled trials. *Public Health Nutrition*. **8**(02), 117–132. https://doi.org/10.1079/PHN2004677.

Santika, O., Fahmida, U., & Ferguson, E. L. (2009). Development of Food-Based Complementary Feeding Recommendations for 9- to 11-Month-Old Peri-Urban Indonesian Infants Using Linear Programming 1, 2. *The Journal of Nutrition*. **139**(May 2008), 135 – 141. https://doi.org/10.3945/jn.108.092270.Indonesia.

Schwarz, N. G., Grobusch, M. P., Decker, M.-L., Goesch, J., Poetschke, M., Oyakhirome, S., Klipstein-Grobusch, K. (2008). WHO 2006 child growth standards: implications for the prevalence of stunting and underweight-for-age in a birth cohort of Gabonese children in comparison to the Centers for Disease Control and Prevention 2000 growth charts and the National Center for Heal. *Public Health Nutrition*. **11**(7), 714–719. https://doi.org/10.1017/S1368980007001449.

Shephard, G. S. (2008). Part A Risk assessment of aflatoxins in food in Africa Risk assessment of aflatoxins in food in Africa. *Food Additives & Contaminants*. **25**(10), 1246 – 1256. https://doi.org/10.1080/02652030802036222

Shiriki, D., Igyor, M. A., & Gernah, D. I. (2015). Nutritional Evaluation of Complementary Food Formulations from Maize , Soybean and Peanut Fortified with Moringa oleifera Leaf Powder. *Food and Nutrition Sciences*. **6**(April), 494–500. https://doi.org/10.4236/fns.2015.65051.

Shirima, C. P., Kimanta, M. E., Kinabo, J. L., Srey, C., Wild, C. P., & Gong, Y. Y. (2014). Dietary Exposure to aflatoxin and fumonisin among Tanzanian children as determined using biomarkers of exposure. *Molecular Nutrition and Food Research*. **57**(10), 1874–1881. https://doi.org/10.1002/mnfr.201300116.

Shirima, C. P., Kimanya, M. E., Routledge, M. N., Srey, C., & Kinabo, J. L. (2015). A Prospective Study of Growth and Biomarkers of Exposure to Aflatoxin and Fumonism during Early Childhood in Tanzania. *Environmental Health Perspectives*. **123**(2), 173–179.

Sirma, A., Senerwa, D., Grance, D., Makita, K., Mtimet, N., Kang'ethe, E., & and Lindahl, J. (2016). Aflatoxin B1 occurance in millet, sorghum and maize from four agro-ecological zones. *African Journal of Food, Agriculture, Nutrition, and Development*. **16**(3), 10991–11003.

Siwela, A. H., Siwela, M., Matindi, G., Dube, S., & Nziramasanga, N. (2005). Decontamination of aflatoxin-contaminated maize by dehulling. *Journal of the Science of Food and Agriculture*. **85**(15), 2535–2538. https://doi.org/10.1002/jsfa.2288

Skau, J. K. H., Bunthang, T., Chamnan, C., Wieringa, F. T., Dijkhuizen, M. a., Roos, N., & Ferguson, E. L. (2014). The use of linear programming to determine whether a formulated complementary food product can ensure adequate nutrients for 6- to 11-month-old Cambodian infants. *American Journal of Clinical Nutrition*. **99**(1), 130–138. https://doi.org/10.3945/ajcn.113.073700.

Smith, L. E., Prendergast, A. J., Turner, P. C., Humphrey, J. H., & Stoltzfus, R. J. (2017). Review Article Aflatoxin Exposure during Pregnancy, Maternal Anemia, and Adverse Birth Outcomes. *American Journal of Tropical Medicine and Hygiene*. **96**(4), 770–776. https://doi.org/10.4269/ajtmh.16-0730.

Soetan, K. O., & Oyewole, O. E. (2009). The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds : A review. *African Journal of Food Science*. **3**(9), 223–232.

Sokolovic, N., Selvam, S., Srinivasan, K., Thankachan, P., Kurpad, a V, & Thomas, T. (2014). Catch-up growth does not associate with cognitive development in Indian school-age children. *European Journal of Clinical Nutr*ition. **68**(1), 14–8. https://doi.org/10.1038/ejcn.2013.208

Solomons, N. W. (2013). Update on zinc biology. *Annals of Nutrition and Metabolism*. **62**(SUPPL.1), 8–17. https://doi.org/10.1159/000348547

Steiber, A., Hegazi, R., Herrera, M., Landy Zamor, M., Chimanya, K., Pekcan, A. G., … Ojwang, A. A. (2015). Spotlight on Global Malnutrition: A Continuing Challenge in the 21st Century. *Journal of the Academy of Nutrition and Dietetics*. **115**(8), 1335–1341. https://doi.org/10.1016/j.jand.2015.05.015.

Steve, I. O., & Babatunde, O. I. (2013). Chemical Compositions and Nutritional Properties of Popcorn-Based Complementary Foods Supplemented With Moringa oleifera Leaves Flour. *Journal of Food Research*. **2**(6), 117–132. https://doi.org/10.5539/jfr.v2n6p117.

Sudfeld, C. R., Charles McCoy, D., Danaei, G., Fink, G., Ezzati, M., Andrews, K. G., & Fawzi, W. W. (2015). Linear Growth and Child Development in Low- and Middle-Income Countries: A Meta-Analysis. *Pediatrics*. **135**(5), e1266–e1275. https://doi.org/10.1542/peds.2014-3111.

Tang, L., Lee, A. H., & Binns, C. W. (2015). Predictors of early introduction of complementary feeding: Longitudinal study. *Pediatrics International*. **57**(1), 126–130. https://doi.org/10.1111/ped.12421.

Taruvinga, A., Muchenje, V., & Mushunje, A. (2013). Determinants of rural household dietary diversity: The case of Amatole and Nyandeni districts, South Africa. *International Journal of Development and Sustainability*. **2**(4), 1 –15. http://isdsnet.com/ijds-v2n4-4.pdf.

TFDA. (2012). Aflatoxin Contamination and Potential Solutions for Its Control in Tanzania. *Abt Associates Incooperate.* **12**(12), 1898–1903.

Thankachan, P., Walczyk, T., Muthayya, S., Kurpad, A. V., & Hurrell, R. F. (2008). Iron absorption in young Indian women: The interaction of iron status with the influence of tea and ascorbic acid1-3. *American Journal of Clinical Nutrition*. **87**(4), 881–886.

Thavarajah, P., & Thavarajah, D. (2014). Inaccuracies in Phytic Acid Measurement : Implications for Mineral Biofortification and Bioavailability. *American Journal of Plant Sciences*. **2014**(January), 29–34. https://doi.org/http://dx.doi.org/10.4236/ajps.2014.51005

Thejpal, R. (2015). Iron deficiency in children. *S Afr Med J*, *105*(7), 8–10.

https://doi.org/10.7196/ SAMJnew.7781.

Torres, A. M., Barros, G. G., Palacios, S. A., Chulze, S. N., & Battilani, P. (2014). Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination. *Food Research International*. **62**, 11–19. https://doi.org/10.1016/j.foodres.2014.02.023.

Turner, P. C., Moore, S. E., Hall, A. J., Prentice, A. M., & Wild, C. P. (2003). Modification of immune function through exposure to dietary aflatoxin in Gambian children. *Environmental Health Perspectives*. **111**(2), 217–220. https://doi.org/10.1289/ehp.5753.

Udensi, E. A., Odom, T. C., & Ihemanma, O. J. N. R. O. E. C. A. (2012). Production and evaluation of the nutritional quality of weaning food formulation from roasted millet and Mucuna cochinchinesis. *Sky Journal of Food Science*. **1**(1), 1–5.

Unicef. (2013). *Improving child nutrition. The achievable imperative for global progress*. https://doi.org/978-92-806-4686-3.

UNICEF/WHO/World Bank Group. (2017). *L*evels and trends in child malnutrition*: Joint Child Malnutrition Estimates*. Geneva.

United States Department of Agriculture. (2009). Nutritional Needs of Infants Dietary Reference Intakes (DRIs). In *Infant Nutrition and Feeding* (pp. 11–40). Retrieved from http://www.nal.usda.gov/wicworks/Topics/FG/Chapter1_NutritionalNeeds.pdf.

Varkevisser, C. M., Pathmanathan, I., & Brownlee, A. (1991). *Designing and conducting health syatems research projects* (Vol. 16). Geneva, Switzerland. Retrieved from http://www.who.int/iris/ handle/10665/37051.

Vossenaar, M., Knight, F. A., Tumilowicz, A., Hotz, C., Chege, P., & Ferguson, E. L. (2016). Context-speci fi c complementary feeding recommendations developed using Optifood could improve the diets of breast-fed infants and young children from diverse livelihood groups in northern Kenya. **20**(6), 971–983. https://doi.org/10.1017/S1368980016003116

Walczyk, T., Muthayya, S., Wegmuller, R., Thankachan, P., Sierksma, A., Frenken, L. G. J., … Hurrell, R. F. (2014). Inhibition of iron absorption by calcium is modest in an iron-fortified, casein- and whey-based drink in Indian children and is easily compensated for by addition of ascorbic acid. *The Journal of Nutrition*. **144**(11), 1703–1709. https://doi.org/10.3945/jn.114.193417.

Waliyar, F., Osiru, M., Ntare, B. R., Kumar, K. V. K., Sudini, H., Traore, A., & Diarra, B. (2015). Post-harvest management of aflatoxin contamination in groundnut. *World Mycotoxin Journal*. **8(**2), 245–252. https://doi.org/10.3920/WMJ2014.1766

Waliyar, F., Umeh, V. C., Traore, A., Osiru, M., Ntare, B. R., Diarra, B., Sudini, H. (2015). Prevalence and distribution of a fl atoxin contamination in groundnut (Arachis hypogaea L .) in Mali , West Africa. *Crop Protection*. **70**, 1–7. https://doi.org/10.1016/j.cropro.2014.12.007

Walker, S. P., Chang, S. M., Wright, A., Osmond, C., & Grantham-McGregor, S. M. (2015). Early childhood stunting is associated with lower developmental levels in the subsequent generation of children. *The Journal of Nutrition*. **145**(4), 823–8. https://doi.org/10.3945/jn.114.200261

Warthon-Medina, M., Moran, V. H., Stammers, A.-L., Dillon, S., Qualter, P., Nissensohn, M., Lowe, N. M. (2015). Zinc intake, status and indices of cognitive function in adults and children: a systematic review and meta-analysis. *European Journal of Clinical Nutrition*. **69**(6), 649–661. https://doi.org/10.1038/ejcn.2015.60

Ministry of Health and Social Welfare, (2015) *National Nutrition Strategy*. Dar - Es - Salaam, Tanzania.

WHO. (1995). Physical status: the use and interpretation of anthropometry. Report of a WHO

Expert Committee. *World Health Organization Technical Report Series*. https://doi.org/854

WHO. (2003). Regional per Capita Consumption Of Raw and Semi-processed Agricultural Commodities / prepared by the Global Environment Monitoring System/Food Contamination Monitoring and Assessment Programme (GEMS/Food).

WHO. (2008). The new WHO child growth standards. *Paediatria Croatica Supplement*. **52**(SUPP.1), 13–17. https://doi.org/10.4067/S0370-41062009000400012

WHO. (2009). Infant and young child feeding. In *Infant and young child feeding* (Vol. 155, pp. 1 – 79). https://doi.org/10.1111/j.1740-8709.2009.00234.x

WHO. (2011). *Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity*. *Geneva, Switzerland: World Health Organization*. https://doi.org/2011

WHO and FAO. Code of practice for the prevention and reduction of mycotoxin contamination in cereals including annexes on ochratoxin a, zearalenone, fumonisins and trichothecenes and aflatoxins (cac/rcp 51-2003(rev 2014) (2015).

WHO, UNICEF, & WFP. (2007). Preventing and controlling micronutrient deficiencies in populations affected by an emergency. *World Health Organization*. 5–6. Geneva, Switzerland.

WHO/FAO/UNU. (2007). Protein and amino acid requirements requirements requirements in nutrition. *World Health Organization (WHO)*. Geneva, Switzerland.

Wieser, S., Plessow, R., Eichler, K., Malek, O., Capanzana, M. V, Agdeppa, I., & Bruegger, U. (2013). Burden of micronutrient deficiencies by socio-economic strata in children aged 6 months to 5 years in the Philippines. *BMC Public Health*. **13**, 1 – 15. https://doi.org/10.1186/1471-2458-13-1167.

Williams, J. H., Phillips, T. D., Jolly, P. E., Stiles, J. K., Jolly, C. M., & Aggarwal, D. (2004). Human aflatoxin in developing countries: a review of toxicology, exposure, potential health consequences, and interventions. *American Society for Clinical Nutrition*. **80**(June 2015), 1106–1122.

Wong, A. Y. S., Chan, E. W., Chui, C. S. L., Sutcliffe, A. G., & Wong, I. C. K. (2014). The phenomenon of micronutrient deficiency among children in China: a systematic review of the literature. *Public Health Nutrition*. **17**(11), 2605–18. https://doi.org/10.1017/S1368980013002978.

Wyman, S., Simpson, R. J., McKie, A. T., & Sharp, P. a. (2008). Dcytb (Cybrd1) functions as both a ferric and a cupric reductase in vitro. *FEBS Letters*. **582**(13), 1901–1906. https://doi.org/10.1016/j.febslet.2008.05.010.

Yazdanpanah, H., & Eslamizad, S. (2015). Aflatoxins and Their Management. *Biological Toxins and Bioterrorism*. 103 – 119. https://doi.org/10.1007/978-94-007-5869-8

Yun, Y., Watson, S., & Routledge, M. N. (2016). Aflatoxin Exposure and Associated Human Health Effects , a Review of Epidemiological Studies. *Food Safety*. **4**(L), 14 – 15. https://doi.org/10.14252/foodsafetyfscj.2015026.

Zain, M. E. (2011). Impact of mycotoxins on human health in developing countries. *Journal of Saudi Chemical Society*. https://doi.org/10.1080/02652030701567442.

Zimmermann, M. B., Chaouki, N., & Hurrell, R. F. (2005). Iron deficiency due to consumption of a habitual diet low in bioavailable iron: A longitudinal cohort study in Moroccan children. *American Journal of Clinical Nutrition*. **81**(1), 115–121.

Zone, S., Berhanu, B., Abegaz, K., & Kinfe, E. (2015). Assessment of Knowledge and Practices on Complementary Food Preparation and Child Feeding. *International Journal of Food*

Science and Nutritional Engineering. **5**(2), 82–87. https://doi.org/10.5923/j.food.20150502.02.

APPENDICES

Appendix 1. Formula for calculating sample size

Standardized formula for sample size calculation by Varkeviser, 1991.

$$n = \frac{z^2 P\,(100 - P)}{E^2}$$

Where by:

n= Minimum sample size

z = Value corresponding to the confidence level = 1.96

P = Estimated prevalence rate (56% stunting prevalence)

E = Margin of error =10% with confidence interval 90%

The minimum sample size was determined as 378, however a total of 394 households in the villages qualified for the study and were recruited.

Appendix 2. Structured Questionnaire

Questionnaire for assessing parent's knowledge, attitude and practices on preparation of complementary food.

Household number …………………………………………..

Part A: Background Characteristics

Number of questionnaire ……………..

Date of interview ……..……………..

1. Age of the mother …..……………

2. Marital status ( )

1. Single 2. Married 3. Divorced 4. Cohabiting 5. Widow

3. Level of education ( )

1. None 2. Adult education 3. Some primary school 4. Completed std 7 5. Some secondary education 6. Completed form four 7. High school 8. Certificate 9. Diploma 10. Degree 11. Others specify……………….

4. Occupation of the mother

1. House wife 2. Employed 3. Farmer 4. Business (petty) 5. Huge business 6. Others specify…………

5. How many children do you have ……………………

6. Age of the current child …..……………..

7. Is the child continuing breast feeding? ( )

1. Yes 2. No

8. For how long the child is supposed to be exclusively breastfed? ( )

1. 1-3 months 2. 4-5 months 3. 6 months 4. 1 year
5. 1 year and half 6. 2 years 7. I don't know 8. Others ……

9. Weight of a child: ……………… Height:…………………Hb……………….

Part B: Knowledge and Practice of mothers on preparing complementary feeding

10. Do you feed your child food other than breast milk..?

1. Yes 2. No

11. If no why? (skip question 12 and 13)

…………………………………………………………………………………………
…………………………………………………………………………………………
…………………………………………………………………………………………
…………………………………………………………………………………………
…………………………………………………………

12. If yes, which food? Mention

…………………………………………………………………………………………
…………………………………………………………………………………………
…………………………………………………………………………………………
…………………………………………………………………………………………
…………………………………………………………………………………………
…………………………………………………………………………………………
………………..

13. Which methods do you use to prepare CF and why?

…………………………………………………………………………………………
…………………………………………………………………………………………
……………………………………………………………………………….............
....
....
....
.........................?

14. When did you start to give your child complementary foods? (   )

1. One month 2. Two months 3. Three months 4. Four months 5. Five months 6. Six months 7. Seven months 8. Other specify

15. How often in a day and at what quantity (volume)?..

11. Why did you introduce complementary food to your child? ( )

1. Breast milk is no longer enough to a child
2. It was a correct age
3. Mother in law advised me
4. Husband advised me
5. Baby was crying all the time
6. Other specify

12. What is the appropriate age for introduction of complementary food to a child? ( )

5. Below 4 months 2. 4 – 6 month 3. 6 month 4.

6. I don't know 5. Others

13. Do you prepare complementary food on your own? ( )

1. Yes 2. No

14. If yes, where did you get the knowledge on how to prepare complementary food?

15. ( )

1. Training/Seminar by NGO 2. MCH clinic3. Midwife 4. Other (specify)

16. And how do you prepare the food, what are your main ingredients….? (Do you have any formular..?)

……………………………………………………………………………………………
……………………………………………………………………………………………
……………………………………………………………………………………………
……………………………………………………………………………………………
……………………………………………………………………………………………
……………………………………………………………………………………………
……………………………………………………………………………………………
…………………

17. If no in question 18, where do you used to get complementary foods? ( )

1. Shops 2.Marketi 3 Super market 4. To my neighbor 5. Other specify

18. Do you have any guideline or manual which is guiding you on how to prepare complementary foods? (   )

1. Yes   2. No

19. If Yes where did you get it, (who gave you) …………………………….

20. What are suitable ingredients (foods) for making complementary foods?

1. Cereals, tubers and roots 2. Fruits and vegetables 3. Legumes, meat and meat products 4. Fat/oil and sugar 5. I do not know   6. Others

21. How many times a day a child aged 6 - 8months should be given complementary foods? (   )

1. Once 2. Twice 3. Thrice 4. I do not know 5. Others

22. How many times a day a child aged 9 - 11 months should be given complementary foods? (   )

1. Once 2. Twice 3. Thrice 4. I do not know 5. others

23. How many times a day a child aged 12 - 24 months should be given complementary Foods? (   )

1. Once 2. Twice 3. Thrice 4. I do not know 5. others

24. Is it important to add fat/oil/milk in baby's foods? (   )

1. Yes   2. No

25. If yes what are the advantages?   (   )

1. To increase nutrients
2. To increase energy density
3. To add flavor of the food
4. I don't know
5. Others specify …………………………………………………………………………

………………………………………………………………………………………………….

26. What do you add in your baby's foods?   (   )

1. Fats/oils

2. Milk

3. Sugar

4. Salt

5. Nuts

6. Others (Specify)……………………………………………………………………………

27. Apart from complementary foods, what other kinds of food are you giving your child?

1. ……………………………………………………………………………………………

2. ……………………………………………………………………………………………

3. ……………………………………………………………………………………………

4. ……………………………………………………………………………………………

28. Which method do you use to process cereals or legumes?

1. Soaking
2. Germination
3. Roasting
4. Fermentation
5. Milling

6. Others specify………………………………………………………………………...

Part C: Post harvest management of cereals

29. Cereals were harvested when they are dry or wet? (   )

1. Dry 2. Wet

30. If wet which drying methods did you use? (   )

1. Mats 2. Roof 3. Smoke 4. Others specify

31. Type of storage you are using (   )

1. Improved 2. Traditional 3. Other specify

32. Source of stored cereals (   )

1. I have harvested 2. Bought within the village 3. Bought outside the village

33. Did you sort cereals before storage? (   )

1. Yes 2. No

34. If Yes which criteria did you use when sorting? ( )

1. Colour 2. Insect infestation 3.Damaged 4. Shape 5. Size 6. Other specify

35. Length of storage period ---

36. For how many years have you used the store?----------------------

Part D: Storage and Handling Complementary Food

37. How do you store complementary flour?

1. ……………………………………………………………………….

2. ……………………………………………………………………….

42. For how long do complementary flour is stored? (How long does a prepared CF is kept before it finishes?

1……………………………………………………………………………………….

Appendix 3. Twenty four dietary recall form

NELSON MANDELA AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY

Child 24 hours dietary recall form.

Household number ……………………………………….

Time	Types of food and its ingredients	Method of cooking	Total weight of food consumed
Morning			
Mid-morning			
Lunch			
Mid-evening			
Dinner			
Snacks			
If any			

Appendix 4. Informed consent form

NELSON MANDELA AFRICAN INSTITUTION OF SCIENCE AND TECHNOLOGY

CONSENT FORM

Informed consent form for parents/guardian on behalf of their children (6 – 24 month) to participate In the study of developing model formula for nutrient computation on formulation of complementary food in Tanzania.

This Informed Consent Form has two parts:

- Part I: Introduction (to share information about the research with you)
- Part II: Certificate of Consent (for signatures if you agree to take part)

PART I: Introduction

I am Nyabasi Makori a student of Nelson Mandela African Institution of Science and Technology. I am doing research on developing a model formula for nutrients computation on formulation of complementary food in Tanzania. I am going to give you detailed information of the research and invite you to be part of this research. If there are some words or anything that you do not understand, please ask me to stop and I will stop and take time to explain. If you have any question feels free to ask any time as we are going through.

Purpose of the research

Lack of suitable complementary food is a contributing factor to under nutrition, in Tanzania (44% of stunting, TDHS report, 2010). Generalization of nutrient requirement without considering nutritional needs for specific age category (6 - 8, 9 - 11 and 12 - 24 months) can be one of the cause which affects child daily nutrient intake by failing to meet the Recommended Dietary Intake.

In Tanzania, cereals (maize, millet, sorghum) are used as major components of most food, but are report to be major crops contaminated with mycotoxins at the same time are naturally contained anti-nutritional factors which compromise the bioavailability of certain minerals including iron, zinc and calcium. Exposure of infants to CF with high levels of anti-nutritional and mycotoxins can contribute to high levels of malnutrition in which developing countries are most affected.

The aim of the research is to assess the exposure of infants to different levels of aflatoxin and anti-nutritional factors in relation to nutrition status. In achieving this sample of complementary food will be collected at household level for assessing, analyzing and determination of levels of aflatoxin contamination and anti-nutrition factor. Nutritional assessment of children between 6 – 24 months of age will be done for assessing nutritional status of the study population, therefore height and weight of children will be taken. Blood sample (100 microliter minimum and 0.5mls maximum for repeated measure) will be taken from children for analysis of hemoglobin level since anti-nutritional factors tend to compromise absorption and bioavailability of iron. Parents will be asked to recall the consumption Patten for the food the children had consumed for the past 24 hours and will be recorded in a tool that will capture 24 h dietary recall data. The aim of collecting 24 h dietary recall data is to see whether the food (nutrients) consumed by children meet recommended dietary intake. Association between food consumed (nutrient intake) and nutrition status of the population will be determined. Parents will be asked to share/provide information on knowledge, attitude and practice on preparation of complementary food through questions in a structured questionnaire.

After getting all above information will help a researcher to develop a modal formula for computing nutrients on formulation of complementary food for each age category which will facilitate optimal growth and development of a child. Thank you for listening.

Associated Risk and Benefit

For participating in the study you will be expecting the following risk and benefits. The only expected risk that will be faced for participating in the study is the pain due to puncturing the site (the left middle figure at the top) for blood sample collection which will be used for measuring hemoglobin concentration. Precaution will be taken to reduce risk and discomfort that will be associated with sample collection. Trained laboratory technician will be responsible for collection of blood sample from participants.

The following are expected benefits for participating in the study:-

a) Participants will be able to know their nutritional status in relation to their age.
b) Participants will be able to know status of hemoglobin concentration. Results will help to know if they are anemic or not.
c) Participants will be able to know complementary food they are using to feed their children meet the recommended nutritional requirements.
d) Participants will be able to know the status of safety of the complementary food they are using, if contaminated will aflatoxin or not.

PART II: Certificate of Consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Name of Participant ____

Signature of Participant ____________________

Date _____________________________

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Researcher _________________________________

Signature of Researcher ____________________________

Date _____________________________

In case of anything contact:

1. Nyabasi Makori (Principal Investigator),

Nelson Mandela African Institute of Science and Technology, Tel: 0754 770730,

Box 447,

Arusha, Tanzania.

2. National Health Research Ethics Sub-Comitee (NatHREC),

National Institute for Medical Research (NIMR)

Tel: +255-22-2121400,

P. O. Box 9653,

Dar – Es – Salaam, Tanzania.