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Assessment of nutritional status and dietary aflatoxins exposure in Children in Singida District Council

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ASSESSMENT OF NUTRITIONAL STATUS AND DIETARY AFLATOXINS EXPOSURE IN CHILDREN IN SINGIDA DISTRICT COUNCIL

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A Dissertation Submitted in Partial Fulfillment of the requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Feeding practices are the critical determinant of the growth and development of children during the first 1000 days of life with a long time effect on adulthood. This study aimed at assessing the diversity and safety complementary foods in terms of aflatoxins (AF). A total of 290 households with infants aged between 6-24 months were recruited from 10 villages in Singida District. Mothers were interviewed on infant feeding practices and handling of food crops using a structured questionnaire. Anthropometric measurements of index child were taken, followed by collection of 1 to 3 cereals based food samples used in complementary feeding in 180 households. A total of 218 flour samples made from maize, sorghum, millet, composite and fermented-germinated sorghum locally known as *magai* and *magai* drink; from *magai* flour were collected. Food samples were analyzed for aflatoxins using High-Performance Liquid Chromatography (HPLC). Anthropometric measurements showed that 37.7% were stunted; 20.3% underweight and 8.3% wasted. Furthermore, aflatoxin B1 was detected in 80 (36.7%) out of 218 complementary food samples. Aflatoxin B1 levels ranged from 0.33 to 23.75 µg/kg whereas, total aflatoxins were detected in 185 (84.9%) of 218 samples in a range of 0.47 to 289.28 µg/kg. However, mean dietary exposure to all food were; *magai* drink (133.36 ng/kg body weight per day), composite flour (106.0 ng/kg body weight per day), maize flour (111.70 ng/kg body weight per day), sorghum flour (94.68 ng/kg body weight per day) and millet flour (92 ng/kg body weight per day).

DECLARATION

I, Rufina Fredrick do hereby declare to the Senate of 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.

Signature: ..... Date: 04/08/2021

Rufina Fredrick

The above declaration is confirmed

Signature: ..... Date 04/08/2021

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Dr. Haikael Martin

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CERTIFICATION

The undersigned certify that they have read the dissertation titled "Assessment of aflatoxin exposure in children through complementary feeding in Singida District Council, Tanzania" and recommend for examination in fulfillment for the requirements for the degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

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DEDICATION

With great joy, I dedicate this work to my childrens Glory, Grace and Gideon for their prayers, unconditional love and patience during the whole period of my study and beyond.

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LIST OF ABBREVIATIONS AND SYMBOLS

AF	Aflatoxin
AFB1	Aflatoxin B1
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AFM1	Aflatoxin M1
BW	Body Weight
CVD	Cardiovascular diseases
DDS	Dietary Diversity Score
DMO	District Medical Officer
EU	European Union
HAZ	Height for Age z- scores
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
IGF	Insulin-like growth Factor
IYCF	Infant and Young Child Feeding
LOD	Limit of detection
LOQ	Limit of quantification
MOE	Margin of Exposure
NIMR	National Institute for Medical Research
SD	Standard Deviation
SNAP	Singida Nutrition Agroecology Project
PSPSS	Statistical Social Package for Social Science
TDHS	Tanzania Demographic Health Survey
TB	Tuberculosis
TBS	Tanzania Bureau of Standards
WAZ	Weight for Age z-score
WHO	World Health Organisation
WHZ	Weight for Height z-score

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Undernutrition is still a significant health problem in children below the age of five years, whereby stunting is the leading indicator. Globally, about 165 million children under the age of five years are stunted with a prevalence of 26% (Stewart *et al*., 2013). In Africa, the prevalence of stunted children was reported to be 20% (World Health Organization [WHO], 2018), whereby in Tanzania, about 3 million children are stunted with the prevalence of 31.8% (Ministry of Health, Community Development, Gender, Elderly and Children [MoHCDGEC], 2018). Malnutrition remains the main cause of poor growth and development (Nti, 2014) which leads to recurrent infections and diseases. Persistence of undernutrition within the age of 0-2 years becomes irreversible and leads to severe consequences (Sudfeld *et al.,* 2015). Poor cognitive performance in school, low productivity and wages at adulthoods (De Onis & Branca, 2016) and non-communicable diseases are the results of persisted undernutrition (Nti, 2014). Some evidence has shown that stunting is attributed by age and it is rare in infants between 0-6 months due to adequate supply of nutrients if exclusive breastfeeding is well achieved (Stewart *et al.,* 2013). The stunting rate increases gradually with an increase in age from 6 -24 months following the introduction of complementary feeding (Chen, 2016). This increased rate of stunting could be due to introduction of nutritionally inadequate complementary foods and recurrent infections (Stewart *et al.,* 2013). Furthermore, the infections resulted from poor sanitation and personal hygiene due to carelessness like improper disposal of fecal and the use of open latrines linked to environmental, socio-cultural, economic and geographic factors (Chen, 2016; Eroğlu, 2019; Stewart *et al.,* 2013).

Timely introduction of safe, adequate and appropriate complementary foods (WHO, 2003b) after the age of six months alongside breastfeeding is known to promote good nutritional status of children within the first two years of age. Dietary diversity brings in an important synergy to supply the recommended energy density and other nutrients needed for physical growth, brain development and health maintenance (Arimond & Ruel, 2004). Feeding of children with a diversified diet from plant and animal sources right from the introduction of complementary food has demonstrated positive impacts on nutritional status (Traoré *et al.,* 2012). This refers to feeding at least four food groups namely; grains, roots or tubers, legumes or nuts, flesh foods (meat, poultry, fish and seafood), milk and milk products, eggs, vitamin A-rich plant foods and other fruits and

vegetables within a week (Rah *et al.*, 2010). A study conducted in South Africa revealed statistical significance between child growth and dietary diversity (Steyn *et al.*, 2006). Other studies reported a positive correlation between dietary diversity and improved nutritional status (Arimond & Ruel, 2002; Hatløy *et al.*, 2000).

In Tanzania, 41% of the children aged 0-23 months are introduced to complementary foods as early as before the age of six months (United Nations International Children's Emergency Fund [UNCEF], 2015). This is due to social-cultural and economic factors, together with the absence of nutrition education to mothers. Mostly complementary foods are cereals based with limited amount of other food groups necessary to provide essential nutrients for growth and development. Cereal based foods lack essential nutrients and most contain anti- nutritional factors that prevent the absorption of some nutrients. Of a critical disadvantage, cereals especially maize are prone to fungal contamination and may result in mycotoxin exposure among children (Ezekiel *et al.*, 2018).

Mycotoxins are toxic secondary metabolites produced by organisms of the fungus kingdom. Of a wide range of mycotoxins known, aflatoxins rank the first in the list of mycotoxins of public health concern, and are produced by *Aspergillus flavus* and *Aspergillus parasiticus* which contaminate cereal crops (Gong *et al*., 2016). Cereal and legume crops are usually highly susceptible to these contaminants due to the hot and humid climate as well as poor field management and storage practices (Wild & Gong, 2009). There are four types of aflatoxins namely aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) which are commonly found in cereal-based foods and feeds (Gong *et al.*, 2016). Of the four types, aflatoxin B1 is the most toxic and identified as carcinogenic by the International Agency for Research on Cancer (IARC) (Loprieno, 1975).

Nation and international regulatory bodies have set maximum tolerable limits for these toxins in trade and baby foods (Brown *et al*., 1999; Gong *et al.*, 2016) for consumers protection. Nevertheless, it has been a challenge for developing countries to meet these standards due to poor strategies and policies on management practices as well as lack of technological resources for constant monitoring (Strosnider *et al.*, 2006). In addition, subsistence and rain-fed agriculture practiced in rural particularly semi-arid areas with a high risk of food insecurity (Kulwa *et al.*, 2015), are known to contribute to high levels of aflatoxins exposure (Gong *et al.*, 2016). These long terms residential related exposure leads to chronic exposure to more than 5 billion people (including children) worldwide. Developing countries are leading in accumulation of toxins

through contaminated cereal-based foods that are frequently consumed as the main staple and weaning foods (Wu, 2014).

The exposure normally occurs to any individual in different stages of growth including children where it ends up in health problems like hepatitis and cancer to adults and stunting in children (Gong *et al.,* 2016). Stunting in children is a result of immunity suppression which causes susceptibility to recurrent infections whereby the mechanism of this relationship is a bit complicated. Different studies had detected different mechanisms on this like; disruption of insulin-like growth factors (IGF) caused by AF exposure which lead into toxicity in the liver (Castelino *et al.*, 2015), immunosuppressive effect of AF exposure which explores the child into infection susceptibility thereby may decrease appetite and nutrients bioavailability (Gong *et al.*, 2008), and furthermore inhibition of protein synthesis caused by AF exposure may damage the intestinal walls to reduce nutrients absorption (Smith *et al*., 2012) and likely growth impairment.

However, in most cases, these effects are created during weaning as a result of frequent consumption of cereal-based complementary foods (Gong *et al.*, 2003) which are the most suspect of AFB1 and it is most destructive compared to AFM1 from breastmilk (Gong *et al.*, 2016). The study done in Kenya by Okoth and Ohingo (2004), shows the relation between high levels of AF found in maize-based family foods and growth retardation in children associated with low weight for height (wasting). Another study conducted in Tanzania reported a high level of contamination of homegrown maize of up to 158 µg/kg which is above the limit set by Tanzania regulatory of 10 µg/kg (Kimanya *et al.*, 2008) and the cereal was found to be the main ingredient of complementary foods (Shirima *et al.*, 2014) especially in rural areas where dietary diversity would be minimal due to education and economic viewpoint.

Thus, it has a health effect in child growth particularly stunting as indicated in one of the studies done in semi-arid regions characterized by food insecurity and low dietary diversification problems (McKinney, 2006). To date, there are no studies documented the extent of consumption of cereal-based foods in children aged 6-24 months of age in Singida, Tanzania. Moreover, there are no data on the extent of aflatoxins contamination of the cereal-based materials used in the preparation of complementary foods in this location and there resulted in infant exposure to these toxins. Therefore, this study was conducted to estimate consumption of cereal-based complementary food among children aged less than 2 years in Singida region of the central semiarid part of Tanzania. Specifically, the study was determined the levels of aflatoxins contamination of cereal-based

materials used in preparation of complementary foods and a subsequent dietary exposure to aflatoxins among these children.

1.2 Statement of the problem

Aflatoxins contamination is one of the major challenges afflicting human health worldwide. Aflatoxins exposures in children can occur through breastfeeding (Magoha *et al.,* 2014) and complementary foods especially in areas where maize which susceptible to aflatoxin and fumonisins (Kimanya *et al.,* 2010) is used as a basic ingredient in the preparation of complementary foods. Other crops which are prone to aflatoxins and commonly used in the preparation of complementary foods include groundnuts, sorghum, millet and finger millet (Makori *et al.*, 2019). Aflatoxins exposure affects the growth and development of children and makes them more susceptible to infectious diseases and stunting (Gheysens, 2015; Gong *et al*., 2003). Reports show a remarkable association between aflatoxins exposures in children and both stunting and underweight, which are indicators of acute and chronic malnutrition during weaning and family foods (Gong *et al.,* 2002).

However, socioeconomic status and poor knowledge on nutrition, contribute to significant adverse health impact as dietary diversification receive less attention in low-income populations (including Tanzania) by relying heavily on only a few dietary staples such as maize, sorghum, groundnuts, and rice; which are often used in complementary feeding, and are highly susceptible to aflatoxins contamination (Wu *et al.,* 2014).

1.3 Rationale of the study

Despite various nutrition interventions employed to balance diets; little has been done to integrate food safety and mycotoxins contamination in areas where food diversity is hampered by low social-economic status. The Singida Nutrition and Agro-Ecological Project (SNAP) found out high levels of food insecurity and low level of dietary diversity linked to undernutrition mainly stunting (24.7%) (unpublished report). As a follow-up, this study was designed to complement findings from the SNAP project by ascertaining the risk of aflatoxins exposure in children through multiple complementary foods and their contribution to undernutrition to better inform the undernutrition mitigation strategies in Singida District Council.

1.4 Research objectives

1.4.1 General objective

This study was designed to assess the risk of AF exposure in children through complementary foods (including family) foods in selected villages of the Singida District Council.

1.4.2 Specific objectives

(i) To assess the dietary diversity and nutritional status of children aged 6-24 months.

(ii) To assess aflatoxins exposure in children through cereal-based and other food materials that are commonly used as complementary foods.

1.5 Research questions

(i) How does the nutritional status of the children will be associated with their dietary patterns?

(ii) What extent do the complementary foods contaminated by aflatoxins?

1.6 Significance of the study

The study provided and document the information on the magnitude and impacts of exposure to AF in children thus it created room for further studies and interventions for improvement of feeding practices and health to abandon the risks of undernutrition and its related burden diseases.

1.7 Delineation of the study

This study aimed at assessing the diversity and safety of complementary foods in terms of aflatoxins. Generally, this dissertation is divided into five chapters; Chapter one consists of the background information, statement of the research problem, rationale of the study, objectives, research questions and the significance of the study. Chapter two presents the literature review in which major issues on aflatoxin contamination in baby complementary food and its effect in nutritional status are discussed. Chapter three covers the methods and techniques used in the assessment of nutritional status to children of 6 to 24 months, as well as aflatoxins exposure in children through cereal-based and other food materials that are commonly used as complementary foods. Chapter four presents the results and discussion whereby food materials used to feed children are contaminated above the limit, and nutritional status of the children are still poor. Chapter five presents the conclusion and recommendations. Although the results of this study show high levels of contamination in *magai* dink which is used as soft drink to children, however, the

number of *magai* samples were very few. This limitation makes a research gap for future studies on impact of *magai* in AF exposure in children and the whole family.

CHAPTER TWO

LITERATURE REVIEW

2.1 Nutritional status of children during 0-24 months of age

Desirable nutrition during the first 1000 days of life is essential because this is a window period where critical growth and development take place (Dewey, 2003). This period starts from pregnancy to the second birthday of a child, where the child needs good nutrition for brain development, prevention of stillbirth and low birth weight (Khandelwal *et al.*, 2014). During pregnancy, adequate nutrient intake has an impact on the nutritional status of the children as it would strengthen their immunity to disease, prevent premature births and low birthweight.

This will prosper the growth and development of the children in utero, after birth and later in life because essential nutrients were sufficiently supplied. However, malnourished mothers are likely to bear low birth weight babies and in the long run, could be stunted when they receive poor feeding practices up to the age of 23 months. Furthermore, these may be corrected early in between birthdate to 24 months of age by improving the feeding practices to ensure normal growth and development of the child and his/her bright future.

In spite of all public efforts including, vitamin A supplementation, promoting fortification of wheat flour with Fe, Zn, B_{12}, oil with vitamin A and salt with iodine, as well as immunization against preventable diseases in elimination of nutritional problem, but still, impaired growth is of high prevalence in Tanzania (Shirima *et al.*, 2015). This is shown by high rates of poor growth in children below 5 years of age as the proportion of stunting (short for their age), underweight (thin for their age) and wasting (thin for their height) are 34%, 14% and 5% respectively (Tanzania Demographic Health Survey [TDHS], 2015).

2.1.1 Feeding practices and dietary diversity in complementary foods to children

These are practices that are significant in supporting healthy growth rates. The practices include exclusive breastfeeding from birth to six months, timely and safe introduction of adequate complementary feeding that supplying of energy, protein, and other important micronutrients like vit A, Iron and Zinc after six months to 24 months (Dewey *et al.*, 2003). In the provision of these foods, consideration of dietary diversity is important to ensure supply of essential nutrients in right proportion through consumption of various groups of food like cereals, legumes, animal products,

fruits and vegetables as well (De Onis *et al.*, 2013). Diversification will promote healthy growth and development of the child in standard parameters. Through optimal dietary diversity with the inclusion of animal source foods in a good frequency, a significant effect on length gain to an effect size of d=0.21 would be observed (Imdad *et al.*, 2011a).

However, low economic status, food insecurity, poor nutrition education and negligence in most of the mothers in developing countries may lead to low dietary diversity and cause a rapid increase in stunting of about 50% in children aged 6-24months (Victora *et al.*, 1992). Furthermore, in sub-Saharan countries, cereals are commonly used as the main dishes in complementary feeding to young children, and it is prepared in the form of porridge. This explores the children not only into the low quality diet in terms of energy, protein and other essential nutrients but eventually could also cause chronic malnutrition due to AF exposure (Kulwa *et al.*, 2015).

Maize as the main staple in Tanzania communities including Singida, also considered as the main component of complementary food on its own or in mixture with other cereals and legumes (Kulwa *et al.*, 2015). Furthermore, sun-dried leafy vegetables were included in children and family meals throughout the year in the study area and central Tanzania at large (Mulokozi & Svanberg, 2003), thus improvements is needed to ensure more diversification and reduces the risks of AF exposure to the children.

2.1.2 Complementary foods

Complementary foods are additional foods given to the child primarily after six months of exclusive breastfeeding to 23 months of age for nutritional requirements support to breastmilk which is no longer sufficient to provide all nutrients needed for the growth and development of the child (Imdad *et al.*, 2011b). These foods work effectively for positive nutritional status only if are introduced on time, in safety manners, adequate amount and appropriately proportional.

However, daily average energy requirements for healthy children are 615 kcal at 6–8 months, 686 kcal at 9–11 months and 894 kcal at 12–23 months of age (Dewey & Brown, 2003), in which complementary foods contribute about 200, 300 and 500 kcal per day in the respective age categories respectively. Attaining these energy levels, feeding frequency and energy density of complementary foods should be adequate in addition to average breastmilk from the mother. The feeding frequency of two to three meals at 6-8 months and three to four meals at 9-24 months are recommended. But, most of the infants who introduced to complementary feeds are terminated

from breast milk before the age of 24 months, thereby are at risk of malnutrition and growth rates (Dewey & Adu-Afarwuah, 2008; Kent *et al.*, 2006).

In developing countries, the average expected energy intake from complementary foods is approximately 200 kcal at 6–8 months, 300 kcal at 9 –11 months and 550 kcal to 12–23 months (WHO, 2003), wherein most cases, infants fail to meet the recommended energy and nutrient intake during this stage. This is common in developing countries where inadequate dietary intake is observed in Tanzania as they prepare more watery porridge and make it less in energy content than the recommended level in their Food Composition Table (91.0 - 130.3 kcal) (Lukmanji *et al.*, 2008). It also identified that local foods that are normally consumed are low in iron and vitamin A as shown in Tanzania Demographic Health Survey (ICF Macro, 2011). The main cause of these inadequate supply of complementary feeds could be food insecurity in terms of physical, economic and instability inaccessibility of the foods (Kulwa *et al*., 2015).

In most cases, complementary foods are the mixture of cereal and legume-based foods and the same foods consumed by other family members which are suspected to have high levels of AF contaminants (Gong *et al.*, 2016). On the other way round, the contaminated foods are saved with foods like milk which are also contaminated with a metabolized form of AFB1 which is AFM1 (Okoth & Ohingo, 2004). Nonetheless, dietary diversification seems to be a problem in rural populations especially children who are at weaning age (Okoth & Ohingo, 2004) which could be a neutralizing factor of high levels of AF and its toxicity to the child (Wu *et al.*, 2014). Thus this could introduce the children into high risk of infections which are not only caused by low immunity as suppressed by toxicity from AF (Gong *et al.*, 2008), but also nutrient deficiencies caused by low-quality diet. By the way, the results of long term and recurrence infections could drain the stored nutrients in the body, losing the child's appetite and hinders nutrients absorption thereby it could lead the child into low or no weight and height gain and subsequently, poor nutritional status particularly stunting, underweight and wasting (Dewey & Mayers, 2011).

2.2 Aflatoxin contamination and exposure in children through cereal-based foods that are commonly used as complementary foods

Aflatoxin contamination in food is caused by fungal invasion commonly *Aspergillus flavus* and *Aspergillus parasiticus*. They occur naturally in food crops including maize, sorghum, rice, groundnuts and wheat which are also the main ingredients for complementary food in children in Sub Saharan Africa. Among others, environmental factors particularly climatic conditions

associated with these natural toxins through complementary foods have a vital effect in exposing Tanzanian children to mycotoxin particularly aflatoxin (Kimanya *et al.,* 2010). This could be caused by variation in soil fertility in different regions because fungal invasion is common to low fertile and drought land, improper management on farming practices, pre-harvest, harvesting and post-harvest period (Hell *et al.,* 2008).

According to WHO sufficient complementary feeding can be attained through the initiation of indigenous foodstuffs and local foods (WHO & UNICEF, 2003). But the nutrient density of local foods in developing countries is low to meet the recommended nutrients to infants even though it is taken in conjunction of breast milk (Briend *et al.,* 2003; Santika *et al.,* 2009). However, the same foods consumed locally as complementary and family foods especially maize and sorghum could expose the children into aflatoxin (Dicko *et al.,* 2006; Okoth & Ohingo, 2004). This is because their mothers lack awareness on pre and post-harvesting management practices that would reduce the levels of AF into acceptable limits (Alberts *et al.,* 2017). Long term exposure and low nutrient density food can lead the child into poor nutritional status particularly stunting.

2.2.1 Other Routes of aflatoxin exposure in children

Aflatoxin exposure in children may also occur through other routes like utero exposure and breastmilk. In utero and breastmilk exposure, different studies detect some levels of AF in cord's blood from pregnant mothers likewise in breast milk soon after delivery (Mahdavi *et al.,* 2010; Turner *et al.,* 2007). After birth, the children were short to their age, which was suspected to be stunted due to AF exposure through their mother's cord (Mahdavi *et al.,* 2010). The mechanism created by utero exposure to growth impairment was suspected by observations in a given study; thereby white blood cell-DNA of children aged 2 – 8 months shows some changes in different genes including growth and immune functional genes that could have a link to poor growth (Hernandez-Vargas *et al.,* 2015).

Through breastmilk, children are at high risk of exposure provided that their mothers could be exposed through contaminated cereals and legumes (Wild & Gong, 2009) as in many developing countries these are the main staple foods and are highly suspected to AF contamination. However, the two routes; utero and breastmilk exposure could be subjected to chronic effects with complementary foods introduced to the child after 6 months of exclusive breastfeeding. Thus, complementary feeding could remain the major source of children's exposure to AF especially in rural and/or in semi-arid regions due to the favorite climatic conditions for fungal growth and it

also subject the population into food insecurity and low dietary diversity at large (Marroquín-Cardona *et al.*, 2014).

CHAPTER THREE

METHODOLOGY

3.1 Study site, design and recruitment of participants

This cross-sectional study was carried out between May and June, 2018 in Singida District Council within the scope of the SNAP project (Fig. 1) in Singida, Central Tanzania. Ten villages, five from each of the highest and lowest dietary diversity reported by the SNAP project (Fig. 1 & 2) were selected for this follow-up study. From each of the 10 villages namely; Mrama, Kihunadi, Mkimbii, Ikhanoda, Ngamu, Mninguna, Mudida, Mitula Kinyagigi and Mwanyonye. Twenty-nine households with children aged between 6-24 months were randomly selected through an entire villages reconnaissance with the help of community guides and village leaders.

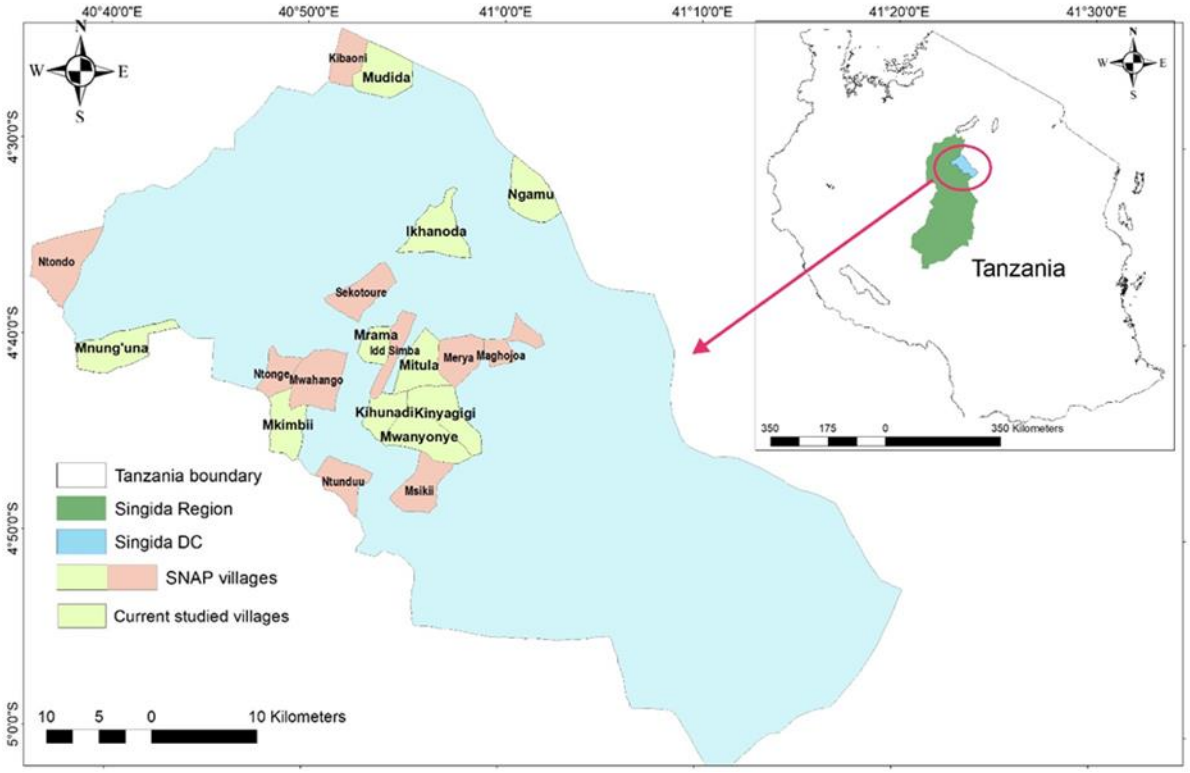

Figure 1: Map of Singida District Council showing 20 villages covered by the SNAP project and a subset of 10 villages covered in the current study

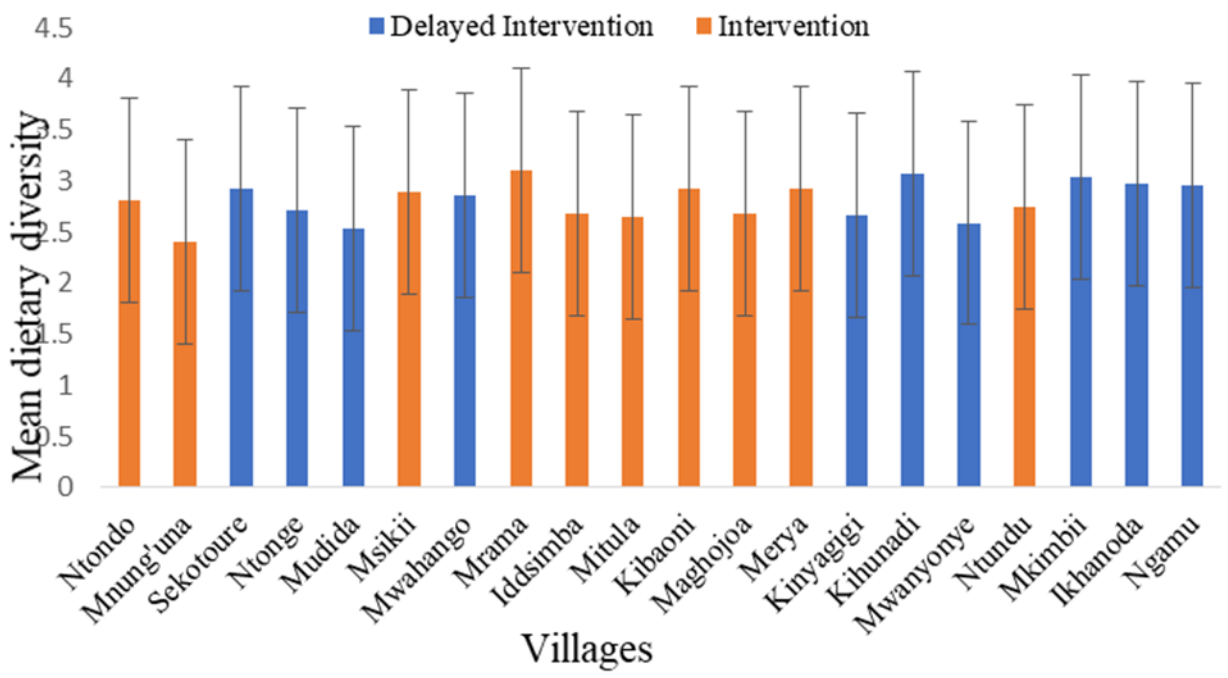

Figure 2: Dietary diversity in 20 villages of SNAP project (SNAP annual progress report, (2018)

Children aged 6 - 24 months and living in one of the selected villages at least from the time of their mother's conception to the date of the survey. Another inclusion criterion was willingness of the mother/caregiver to participate in the study. Disable children and those who have been sick in the past two weeks or suffering from chronic diseases such as Human Immunodeficiency Virus (HIV), tuberculosis (TB), Cardiovascular Diseases (CVD) and the like were excluded from the study. Twin children were excluded from the study as well.

3.2 Data and sample collection

3.2.1 Assessment of feeding practices

In total, 290 households selected for the study were interviewed. Prior to the interview, respondents were informed of the study and their willingness to participate in the study was sought on a written consent form. Structured questionnaires (Appendix 1) were used to gather information on demography, infant and young children feeding practices from the mothers/primary caregivers. Food frequency questionnaires were also used to assess frequency of consumption of most susceptible foods. Mothers/caregivers were asked to recall and report all foods that were repeatedly given to the child as complementary food in a week before the survey (Fig 3).

According to Arimond and Ruel (2004); Brown *et al.* (2002) and WHO (2008) food groups consumed in a week before the interview were grouped into 7; cereal/tubers/roots, flesh foods, eggs, dairy, legumes, vitamin A fruits and vegetables and other fruits and vegetables to ascertain food dietary diversity where the mother was requested to recall intake of these food groups by the child in seven (7) days. In addition to feeding practices, food handling was assessed in the same questionnaire. The mother/caregiver were asked to recall on the storage time and facilities used in keeping cereals after harvest before final consumption. Furthermore, methods used to prepare maize before milling were also recalled.

Figure 3: Household interview with a mother

3.2.2 Anthropometric measurements

Bodyweight and height of the recruited children were measured using calibrated instruments to assess growth indices on height-for-age, weight-for-age and weight-for-length for evaluation of stunting, underweight and wasting respectively. According to WHO, weight (Fig. 4) was taken in light clothing by using weighing scale (874, SECA, Hamburg, Germany) to the nearest accuracy, of 0.1 kg, and height (Fig. 5) was measured by lying a child on a length board (210, Seca, Hamburg, Germany) to the accuracy of 0.1 cm with a flexible end to allow adjustment for each

child.

Figure 4: Measurement of baby's weight on a two-in one weighing scale

Figure 5: Measurement of child's length on a length board

3.2.3 Sample collection

Samples of crop produces commonly used in complementary feeding and which are susceptible to aflatoxins contamination were sought from all 290 interviewed households, however only180 households were found to have at least one of the whole or form of foods used in preparation of complementary food. Therefore, based on availability, up to 3 samples of the susceptible whole or form of food produces were collected from 180 households making a total of 218 food samples. Samples varied from maize and maize flour, sorghum and sorghum flour, millet flour, composite flour, "*magai*" flour (milled germinated sorghum), to "*magai* drink" (fermented drink prepared from *magai* flour). A minimum of 200 g or 100 mL of solids and liquid samples respectively, were collected. Dried samples were packed in khaki bags and kept in a dry place, liquid samples were collected into sterile plastic containers and kept at 4 °C then frozen at -20°C within six hours of collection until analysis.

3.3 Aflatoxin analysis

Prior analysis of samples, calibration of the machine was done by running aflatoxins standards (Aflatoxin analytical mix of B1 B2, G1 and G2 standards) at a concentration of 20, 15 10 and 5 µg/kg. Calibration curve of R= 0.99 was obtained from the levels of standard concentration (20, 15 10 and 5 µg/kg) with retention time of 3.72 min, 4.77 min, 6.02 min and 8.41 min for aflatoxins G2, G1, B2 and B1 respectively. Then the method was validated for accuracy and precision. Blank samples of maize, millet, sorghum and composite flour were spiked with AF standards at three levels concentration of 0.5, 1.0 and 2.0 µg/kg. Thereafter, recovery percentage was calculated by using the following formula, % Recovery = (Detected concentration x 100)/ Spiked/Expected concentration.

The mean recoveries for total aflatoxin in the spiked samples (maize, millet, sorghum and composite) were 114.5 %, 111.25%, 114.36% and 104.41% respectively. The detection and quantification limit were determined using visual method as described by Sengul. (2015). The lowest detectable concentration and Limit of quantification were determined by spiking samples by reducing the concentration of aflatoxins standards (B1, B2, G1 and G2) gradually. Limits of detection (LODs) were obtained as 0.25 µg/kg, 0.27 µg/kg, 0.38 µg/kg and 0.27 µg/kg and limits of quantification (LOQ) were 0.27 µg/kg, 0.32 µg/kg, 0.42 µg/kg and 0.36 µg/kg for AFB1, AFB2, AFG1 and AFG2, respectively.

After calibration and validation, liquid samples were thawed at room temperature (Nemati *et al.*, 2010; Song *et al.*, 2013). Both liquid and solid samples were thoroughly mixed separately before measuring a portion for analysis. Aflatoxins contamination of the collected samples was determined by the method described by Stroka *et al*. (2000) with a slight modification made by Kimanya *et al*. (2008).

3.3.1 Extraction and cleanup

A portion of 25 g or mL of each food sample in 100 mL of 60% methanol (Maker, country) as an extraction solution was blended at high speed for 3 min. The extract obtained was immediately filtered through Whatman filter paper No. 1 (Sigma-Aldrich, Country). Dilution of 4 mL of an extract with 8 mL of phosphate-buffered saline (PBS) (Maker, Country) at pH 6.8. Then, the mixture was passed through the immunoaffinity column (IAC) (Romer Lab, Coring System Diagnostix GmbH, Gernsheim, Germany) placed on a vacuum manifold (24-Port SPE Vacuum Manifold System, ALLTECH Associates, and Lokeren, Belgium) at the flow rate of 3 mL/min. The column was then rinsed with 10 mL of HPLC grade water (Maker, Country) with air forced through the column to ensure runoff.

3.3.2 Elution

Elution of bounded aflatoxins were done by using 1 mL of acetonitrile (Maker, Country) into a glass amber vial. Four hundred microliter of the eluent was mixed with 600 µL of derivatizing reagent (70: 20: 10 v/v/v of water: trifluoroacetic acid: acetic acid) and then heated in a water bath at 65 $^{\circ}$C for 15 min. The mixture was allowed to cool and then injected into the HPLC system.

3.3.3 Quantification of aflatoxins in High-Performance Liquid Chromatography (HPLC)

A High-Performance Liquid Chromatography (Shimadzu, Tokyo, Japan) with pump LC 20AD and fluorescence detector model RF-10AXL (Maker, Country) was set at a wavelength of 365 nm (excitation) and 450 nm (emission). The HPLC column C_{18} (Spherisorb 80-3 ODS-1, 5 µm, 4.6 × 150 mm) (Maker, Country) was used at 40 °C with a mobile phase composed of water: methanol: acetonitrile at a ratio of 60: 30: 10 v/v/v and a flow rate of 1.5 mL/min. The running time was set at 11 min.

3.4 Data analysis

3.4.1 Assessment of nutritional status

This was done by using ENA for the smart software of WHO for the analysis of anthropometric measurements to compute the z-scores and determination of prevalence of stunting, underweight and wasting (Alabi *et al.,* 2016) (*https://smartmethodology.org*). Furthermore, the anthropometric data in forms of z-scores were exported into SPSS version 20 where, classification of the nutritional indices into stunted, underweight, wasted and normal was analyzed. Therefore, children with z-score ≥-2 were grouped as normal and those with <-2 were grouped as stunted, underweight and wasted based on HAZ, WAZ and WHZ respectively (Khlangwiset *et al*., 2011). When a z-score falls below -3 it defines severe levels of each of the indices.

The Chi-square test and frequencies in descriptive statistics were then used for assessing the significance of stunting to different variables. Factors tested for association with nutritional status particularly stunting were demographic characteristics, feeding practices, food handling practices as well as AF exposure. Ninety-five percent confidence interval (CI) was used to assess the strength of association with statistical significance of $p<0.05$.

3.4.2 Dietary diversity score

Dietary diversity scores were computed in Statistical Package for Social Science (SPSS) by counting the frequency of food groups fed to the child in a week as recorded in the food frequency questionnaire. Food groups consumed by a child for 3 or more days in a week were given "1" and those which were not consumed by the same child for 3 or more days in a week were given "0"(Arimond & Ruel, 2004). Finally, food groups that scored 1 were counted to find out dietary diversity scores for every child. In this case, the highest score the child gained would be 7 with a score of 1 for every food group consumed for ≥3 days in a week. Therefore, if the child was fed 4 or more food groups for ≥3 days in a week, the child would have met the minimum Dietary Diversity (DD) (Kennedy *et al.,* 2011). Furthermore, dietary diversity scores were grouped into 2 categories of minimum dietary diversity score and below minimum dietary diversity score. A child who consumed ≥4 and 1-3, different food groups for 3 or more days in a week were grouped as minimum dietary diversity score and below minimum dietary diversity scores respectively.

3.4.3 Ethics and consents

Ethical clearance from the National Institute for Medical Research (NIMR/HQ/R. 8a/Vol. 1X/2739) (Appendix 2) was obtained. The ethical committee approved a written, mother/caregiver consent form. In addition, an introduction letter from the study institution was presented to the Singida District Council for permission to visit villages and households and to the District Medical Officer (DMO) for permission to conduct anthropometric measurements and dietary interviews. Mothers or guardians were made aware of the objectives of the study and clarification on all the procedures for data collections was given. Those who agreed to participate in the study were requested to sign a written informed consent form. For the participants who did not know how to read and write, consent was provided orally and translated to their language by the village leader and then thumbprint signature was used.

3.4.4 Ascertaining the risk of infant's exposure to aflatoxins

Mothers or guardians were asked to estimate using household utensils the amount of the major raw ingredient such as flour used to prepare food for index child in a day, and then the ingredient was measured on a kitchen digital scale and recorded. For each child, exposure to aflatoxin B1 and total aflatoxins was calculated by multiplying aflatoxin contaminations (µg/kg) by the estimated amount of flour consumed (kg) per day and then dividing by the child's body weight (kg) (Magoha *et al.*, 2016). Non-detected samples were assigned the value of aflatoxin B1 contamination level of LOD divided by 2 (Reeve and Cressey, 2016). Therefore, dietary exposure to aflatoxins was computed from:

$$Dietary\ exposure\ \left(ng/kg\ bw/day\right) = \frac{consumption\ \left(g/day\right) \times concentration\ \left(ng/kg\right)}{body\ weight\ \left(kg\right)\ of\ the\ index\ child}$$

The child whose exposure was found to be above the margin of exposure (MOE) of 0.017 ng/kg BW per day was considered at risk of being exposed to unacceptable levels of aflatoxin through complementary feeding, thus indicates public health concern (EFSA 2007). However, demographic information, nutritional status of the children and feeding practices information which were also coded in SPSS, were then associated with levels of dietary exposure to aflatoxin. Therefore, for the child whose exposure found to be above 0.017 ng/kg BW per day or margin of exposure below 10 000, was considered to be at a risk of being exposed to aflatoxin through complementary feeding and it indicates public health concern (European Food Safety Authority [EFSA], 2007). Ninety five

percent confidence interval (CI) was used to assess the strength of association with statistical significance of $p \leq 0.05$.

3.4.5 Dietary exposure to aflatoxin

Concentrations of aflatoxins (in µg/kg) that found in different complementary food, the weight of the children (in kg) from households in which food samples were collected, as well as the estimated amount of foods they consumed (in g); were kept in excel for computation of AF dietary exposure. Furthermore, data that show detectable and undetectable contaminations of aflatoxin in foods were exported from excel to SPSS version 20 to estimate the number of children who were consuming contaminated foods above the regulatory limits of the EU (European Union) and Tanzania. In EU limits, minimum tolerable concentrations considered were 0.1 µg/kg for baby foods in AFB1, 2 µg/kg for processed cereals in AFB1 and 4 µg/kg for all foods in AF total (Makori *et al.*, 2019). In Tanzania limits, 5 µg/kg and 10 µg/kg were considered in cereal foods for AFB1 and AF total respectively.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Demographic information of the participants

Statistics shows that, about 96% of the interviewed mothers were married and live with their husbands, 96.9% are smallholder farmers who depends on rain-fed agriculture as the main occupation to sustain livelihood and 90.7% of the households composed of up to 9 household members. Mother's level of education was mainly standard seven (91%), secondary education (5.5%) and a few percentages did not attend any formal school (2.4%). Out of 290 children recruited in the survey, 52.4% and 47.6% of them were male and female respectively, and classified into three groups of age (6-8, 9-11 and 12-24 months) where, about 63% of the whole population fall in the age of 12-24 months (Table 1).

Table 1: Socio-demographic characteristics of the studied households (n=290)

Variable	N	%
Marital status		
Single	9	3.1
Married	279	96.2
Divorced	1	0.3
Widow	1	0.3
Level of education		
No formal education	7	2.4
Standard 1-7	264	91
Form 1-6	16	5.5
College	3	1
Occupation		
Farming	281	96.9
Farming and livestock keeping	1	0.3
Employed	4	1.4
Self-employed	4	1.4
Family size		
One-nine	263	90.7
Ten-twelve	23	7.9
<twelve	4	1.4
Age of the child		
6-8 months	57	19.7
9-11 months	49	16.9
12-24 months	184	63.4
Sex of the child		
Male	152	52.4
Female	138	47.6

4.1.2 Feeding practices

(i) Breastfeeding and complementary feeding

Majority of the children were still breastfeeding, (85.9%) and few of them were no longer breastfeeding (14.1%), where majority of the children were feeding on family foods, which were stiff porridge (71.1%) with green vegetables particularly jute mallow. More than half of the families (61.4%) reported to normally prepare special flour for baby's porridge, however only few of them had this flour in their households during survey. The special flour was mainly composed of cereals like whole maize, sorghum, millet and finger millet. Majority of the families could offer a maximum of 3 meals per day (70%) to their children (Table 2). without any in-between meals like snacks, as 80% of the respondents were not giving any snacks to the children.

Table 2: Feeding practices of the participants

Variable	N	%
Breastfeeding	249	85.9
Yes	41	14.2
No		
Complementary feeding		
Early introduction	12	4.1
Timely introduction	278	95.9
Special food for children		
Yes	12	61.4
No	278	38.6
Dietary diversity score		
Minimum DDS	17	5.9
Below minimum DDS	273	94.1
Food frequency		
Porridge	174	60.6
Ugali	112	39.0
Milk	1	0.3
Meal frequency		
1	2	0.7
2	30	10.3
3	204	70.3
4	35	12.1
5	15	5.2
>5	4	1.4

(ii) Dietary diversity

This study found out the total mean dietary diversity score of 2.34, where villages with the highest and lowest dietary diversity scores were Ngamu (2.59) and Mitula (2.1) respectively, Fig. 6. The lowest mean dietary diversity indicates that the majority of the children received 1-2 food groups a week before the survey was conducted. However, there was no significance difference in dietary diversity among villages, as all ten villages were feeding their children with only 2 food groups in a week. Figure 7 shows the proportion of the children who met minimum dietary diversity by consuming more than 4 food groups a week before the survey was conducted. Thus, only 5.8% (4.5%, 1.0% and 0.3% were fed 4, 5 and 6 food groups respectively) of the population were feeding their children with at least 4 food groups in a week. WHO recommends 4 food groups in a week as the minimum cut off point for the minimum dietary diversity score (WHO, 2008).

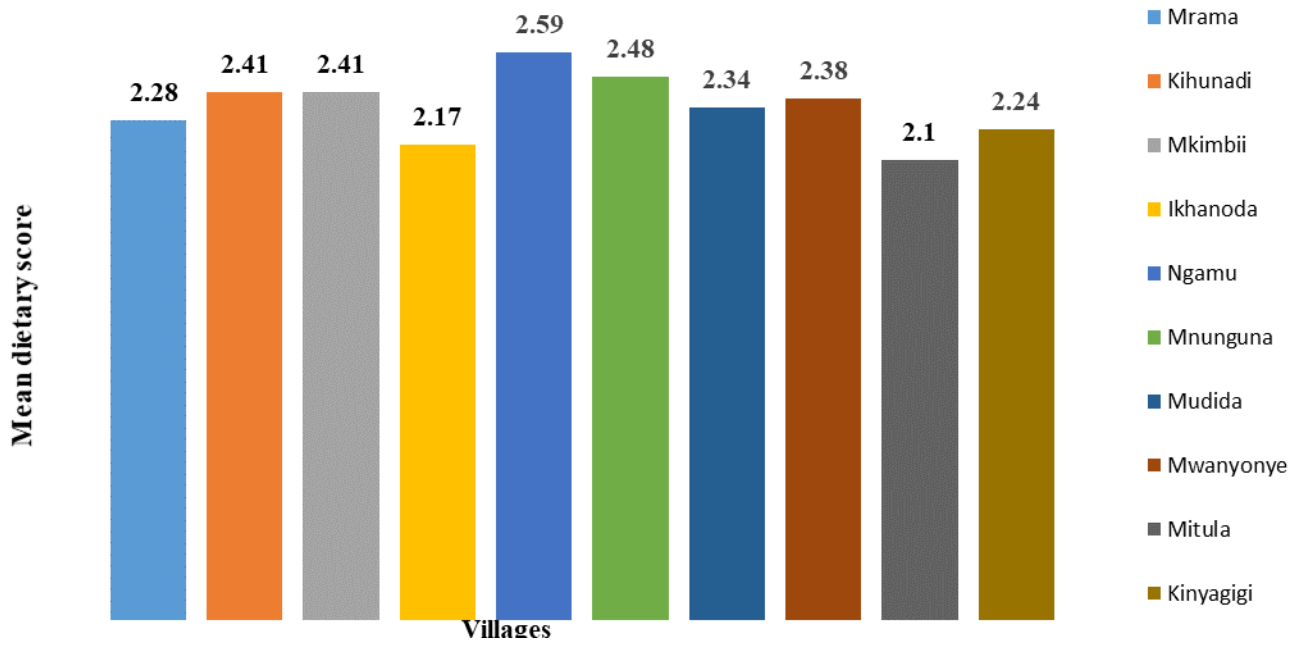

Figure 6: Mean dietary diversity score in 10 villages

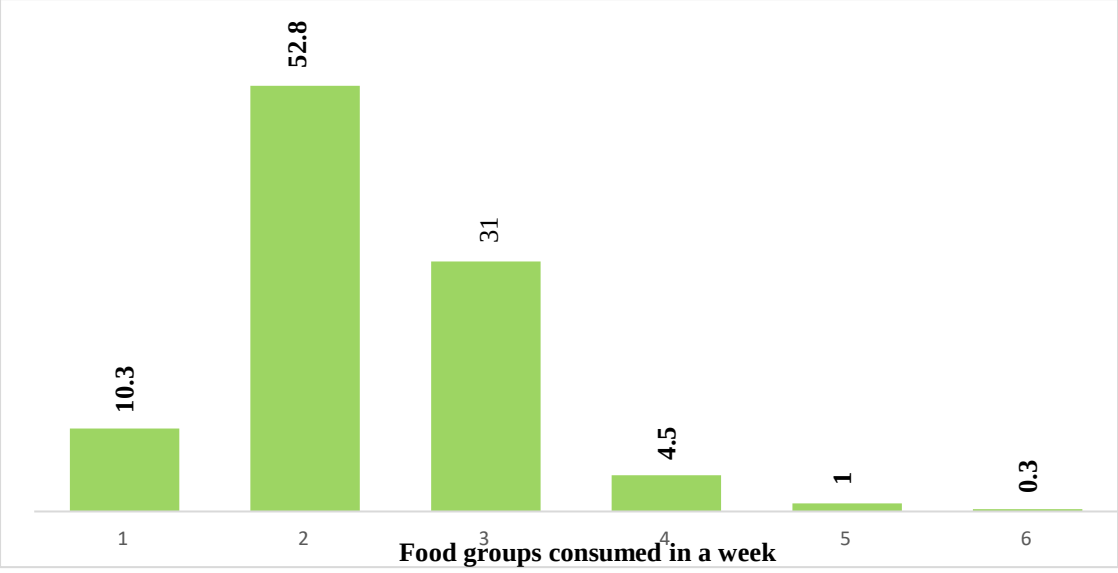

Figure 7: Proportion of the children who met minimum dietary diversity

4.1.3 Nutritional status of participants

Descriptive statistics indicated the prevalence of stunting, underweight and wasting as 37.7%, 20.3% and 8.3% respectively (Fig 8). These result shows high negative deviation (Z-scores <-2) from normal distribution estimated by WHO (Fig. 9), implying poor growth of the index children in terms of height and weight compared to their age. Male children were more stunted than their

counterpart (p=0.000) by 46.7%. Moreover, the age group of 12-24 months were more stunted than the other groups by 46.4% (p=0.001) at 95% CI.

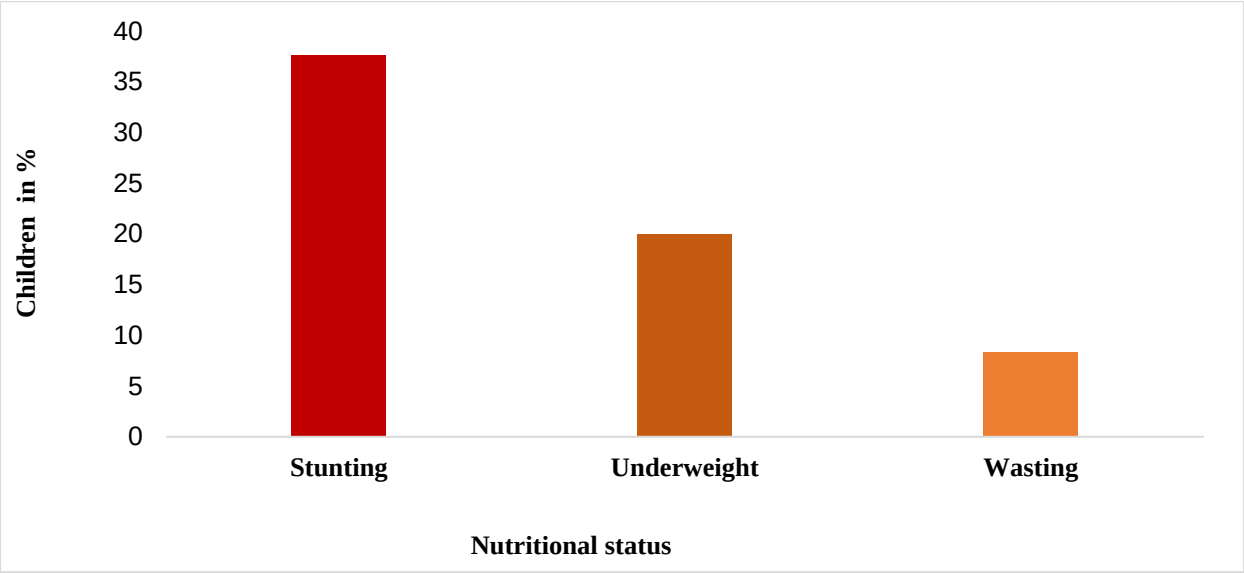

Figure 8: Prevalence of stunting (37.7%), underweight (20.3%) and wasting (8.3%) in children

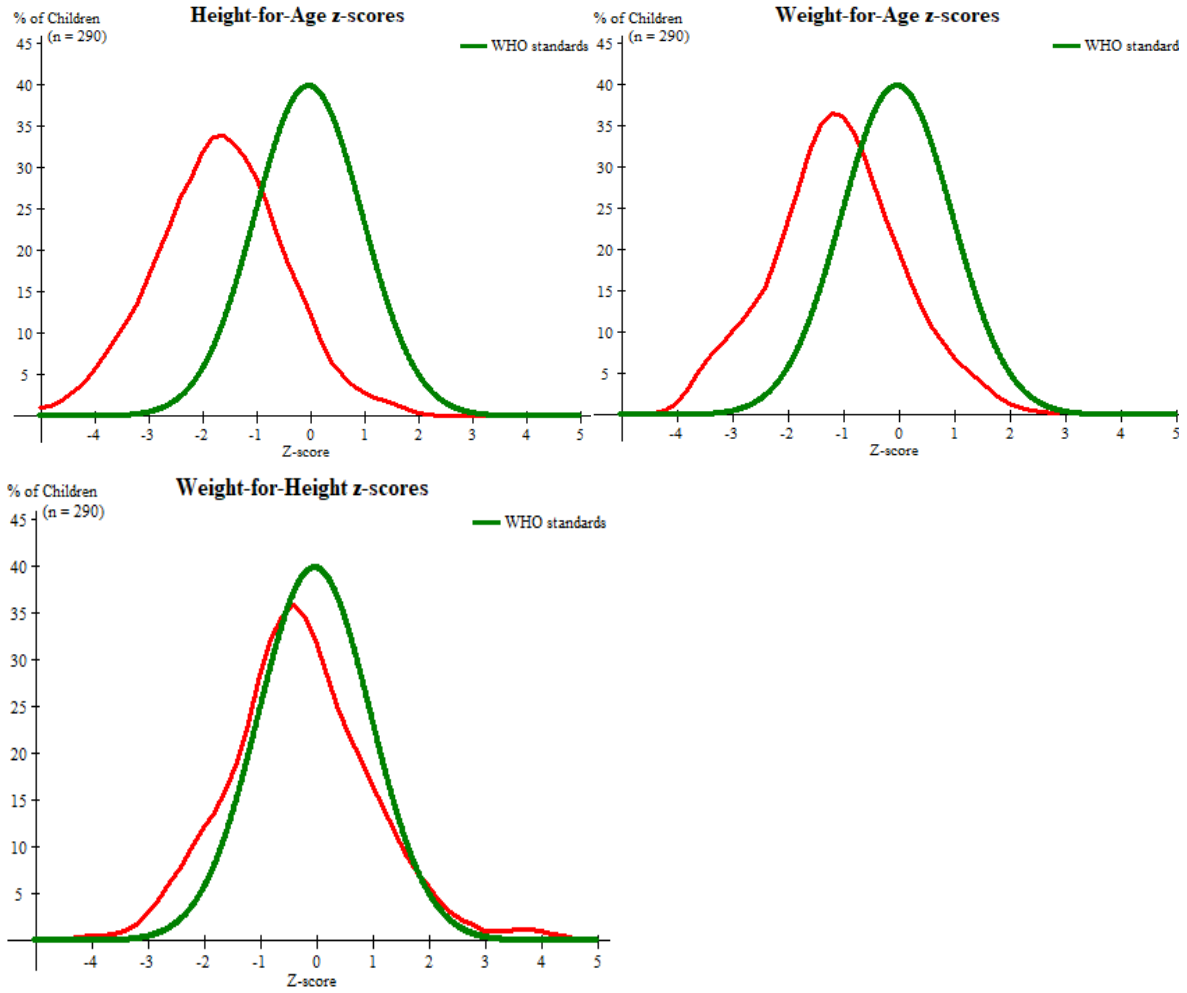

Figure 9: Deviation of Z-scores as obtained in children nutritional status in relation to WHO cut off points

4.1.4 Demographic factors and its contribution to stunting

On the Chi-square test followed by multivariate analysis, of all sociodemographic factors tested, only sex and age of the children show significant contribution to the poor nutritional status of the children particularly stunting for age with (p=0.000); stunting and underweight for sex with (p= 0.001 and p=0.007) respectively. Other factors like education level of the mother, occupation and family size had no statistically significant contribution to either of the two as well as wasting but they could act as confounders in causing stunting in association to age and sex of the children (Table 3).

Table 3: Association between nutritional status and socio-demographic information

Variable	N	%	p-value		
			Stunting	Underweight	Wasting
Age of the children					
6-8mo	57	19.7	**0.000**	**0.244**	**0.958**
9-11mo	49	16.9			
12-24mo	184	63.4			
Sex					
Male	152	52.4	**0.001**	**0.007**	**0.08**
Female	138	47.6			
Marital status					
Single	9	3.1	**0.477**	**0.223**	**0.805**
Married	279	96.2			
Divorced	1	0.3			
Widow	1	0.3			
Occupation					
Farming	281	96.6	**0.770**	**0.518**	**0.849**
Livestock and farming	1	0.3			
Employed	4	1.4			
Self-employed	4	1.4			
Family size					
One-nine	263	90.7	**0.227**	**0.928**	**0.162**
Ten-twelve	23	7.9			
<twelve	4	1.4			
Level of education of education					
No formal education	7	2.4	**0.334**	**0.826**	**0.725**
Standard 1-7	264	91.0			
Form 1-6	16	5.5			
College	3	1.0			

4.1.5 Effects of poor feeding practices in stunting

Among other factors tested in feeding practices, breastfeeding status shows statistical significance in the nutritional status of the children as more than 50% (22 out of 41) of the children who were no longer breastfeeding were stunted. The Chi-square test shows the significance of (p=0.020) in 95% CI. Time of introduction of complementary feeding, meal frequency, commonly consumed foods and dietary diversity shows no statistical significance on contribution to their nutritional status (Fig. 6). However, dietary diversity could have an indirect contribution to the fact that most of the children (94%) were consuming foods below minimum dietary diversity (BMDD) that is <4 food groups in a week.

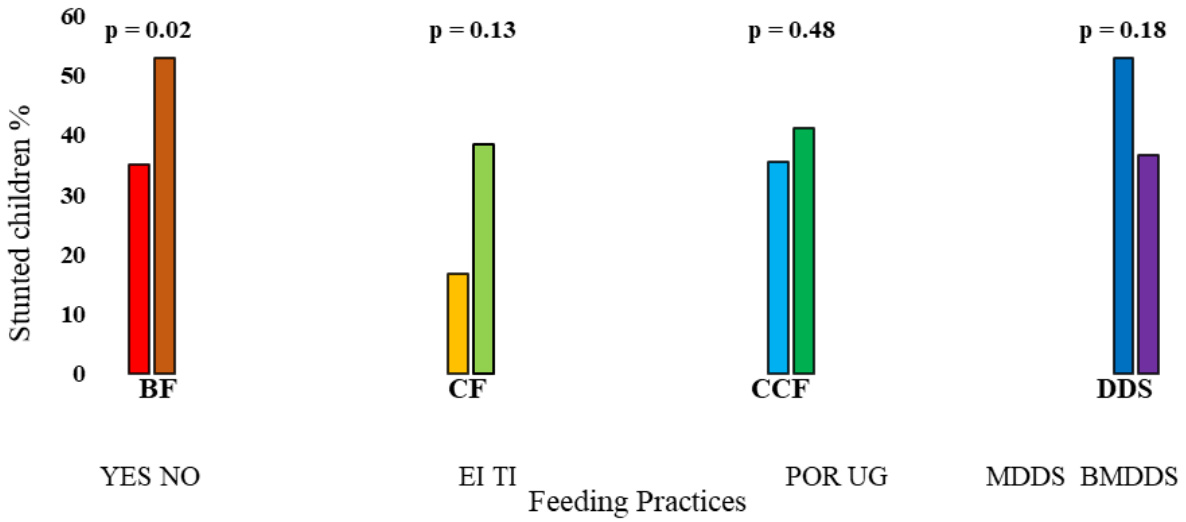

Figure 10: Association between feeding practices and stunting

BF: Breastfeeding; CF: Complementary; feeding; CCF: Commonly Consumed food; DDS: Dietary diversity score; EI: Early introduction; TI: Timely introduction; POR: Porridge; UG: Ugali (stiff porridge); MDDS: Minimum dietary diversity score; BMDDS: Below minimum dietary diversity score

4.1.6 Food handling systems and stunting

Food system and handling has no significance contribution to stunting as in all variables considered in this section has p-value>0.05 in 95% through Chi-square test in descriptive statistic. On preparation of maize/cereals before milling, 95.2% of the mothers/caregivers were just winnowing the produce without washing, and 95.4% were stored the cereals in sacks in a maximum of 1 year (Table 4).

Table 4: Effects of food system and handling to children stunting

Variable	N	%	Stunting	p-value
Food sources				
Home grown	287	99.0	107	**0.888**
Market	3	1.0	1	
Cereal preparation				
Sorting	9	3.1	5	**0.337**
Winnowing	276	95.2	100	
Washing &dried on mats	4	1.4	2	
1,2,3&4	1	0.3	1	
Storage time				
2-5	13	4.5	7	**0.245**
6-9	80	27.7	35	
10-12	180	62.3	60	
>12	16	5.5	6	
Storage facility				
Floor	1	0.3	0	**0.548**
Sacks	288	99.4	108	
Silos	1	0.3	0	

4.1.7 Occurrence of aflatoxin B1 and total aflatoxin in complementary flour

Aflatoxin B_1 contamination was detected in 80 (36.7%) of CF and the level ranged from 0.33 to 23.75 µg/kg where total aflatoxins were detected in 185 (84.9%) foods with the range of 0.47 to 289.28 µg/kg (Table 5). However, in table 6 results show that about 2.7% of the samples exceeded the limit of 5 µg/kg of aflatoxin B1 set by Tanzania Regulatory Authority for food (Tanzania Bureau of Standards [TBS], 2014) and 6.4 % were above the European Union (EU) limits for processed cereals which are 2 µg/kg (EC, 2010). Furthermore, 36.7% of the samples exceed the level of 0.1 µg/kg set by the EU for AFB_1 in baby foods. Thirteen-point three percent of the samples were above the limit of 10 µg/kg set by Tanzania for total aflatoxins.

The highest AFB_1 contamination detected in maize-based flour of up to 289.28 µg/kg and the lowest level of 0.47 µg/kg in another maize-based flour from a different household. The median value of 1.03 µg/kg as the highest was for AFB_1 detected in composite flour compared to the median of AFB1 from other types of foods (Table 5).

Table 5: Occurrence of aflatoxin B1 and total aflatoxin in different foods (n=218)

Type of food	Number of samples	Positive (%) in B1	Positive (%) in total AF	AFB1 (µg/kg		Total aflatoxin (µg/kg)	
				Median values	Range	Median values	Range
Maize	100	40(40.0)	87(87.0)	1.02	0.33-23.75	2.17	0.47-289.28
Millet	16	0(0.0)	10(62.5)	0.00	0.00-0.00	1.66	0.82-3.83
Sorghum	20	5(25.0)	17(85.0)	0.39	0.37-7.99	1.71	1.46-39.52
Composite	62	20(32.25)	51(82.23)	1.03	0.37-4.48	2.73	0.48-50.84
"Magai" flour	9	6(66.7)	9(100)	1.01	0.65-6.16	18.59	1.67-36.54
"Magai" drink	11	9(81.8)	11(100)	0.65	0.60-0.79	3.38	1.11-4.92

Table 6: Number of food samples contaminated with aflatoxin B1 and total aflatoxins above regulatory limits (n=218)

Types of food materials used for complementary feeding	EU limits			Tanzania limits	
	>0.1 µg/kg for AFB1 in baby foods[a]	>2 µg/kg for AFB1[b]	>4 µg/kg for total AF[b]	>5 µg/kg for AFB1	>10 µg/kg for total AF
Maize	40	7	27	4	13
Millet	0	0	0	0	0
Sorghum	5	2	3	0	2
Composite	20	3	16	1	8
"Magai" flour	6	2	6	1	6
"Magai" drink	9	0	4	0	0

[a]Level set for cereal-based baby foods, infants and young children. [b]Level set for all cereal and products derived from cereal

4.1.9 Dietary exposure to children through different complementary foods

Child consumptions of key food products from 180 households ranged from 50 to 456 g per day, with a mean of 117.11±79.8 g. Children's body weight ranged from 6.2 kg to 14.6 kg with a mean of 8.79±1.42 kg. The margin of exposure (MOE) to aflatoxins B1 was below 10 000 (1.0 to 963.0) or above 0.017 ng kg^{-1} BW per day. The median dietary exposure to AFB1 and total aflatoxins were found to be 3.0 ng/kg body weight per day and 34.0 ng/kg body weight per day with the range of 1.0 to 963.0 ng/kg body weight per day and 1.0 to 11,732 ng/kg body weight, respectively.

The mean dietary exposure of AFB1 for children who were using “magai” flour for preparing “magai” drink was higher (157.94 ng/kg body weight per day) compared to their counterparts. The mean dietary exposure due to consumption of susceptible food produce were; *magai* drink (133.36 ng/kg body weight per day), composite flour (106.0 ng/kg body weight per day), maize flour (111.70 ng/kg body weight per day), sorghum flour (94.68 ng/kg body weight per day) and millet flour (92 ng/kg body weight per day). However, there was an insignificant difference (p=0.085) in dietary exposure to AFB1 between children who were using *magai* flour compared to those who were using other types of food materials.

4.1.10 Association between dietary exposure for aflatoxin B1 and nutritional status

The nutritional status was associated with AFB1 exposure and potential confounding factors of growth indicators were adjusted with consideration of 95% CI and p<0.05. The results show that there was no significant association between consumption of the studied cereals based food in complementary feeding and stunted growth, in both crude ratio and adjusted odds ratio with (p=0.972 and p=0.445) respectively (Table 7). On the other hand, the underweight indicator showed significant association with an increase of 3.4 folds in odds due to dietary exposure to AFB1 as compared to those with normal weight (p=0.043 (AOR=3.4; 95% CI:1.04-11.12). However, wasting was protective being caused by dietary exposure to aflatoxin B1 with lower odds of 0.18, p=0.001.

Table 7: Multivariate association between dietary exposures to AFB1 with growth status

	Dietary Exposure for AFB1			
Growth status	**COR (95%CI)**	**ρ value**	**AOR (95%CI)**	**ρ value**
HAZ				
Normal	1			
Stunted	1.00(0.99-0.45-2.15)	.972	0.77(0.39-1.49)	.445
WAZ				
Normal	1			
Underweight	1.04(0.39-2.77)	.942	3.40(1.04-11.12)	.043 *
WHZ				
Normal	1			
Wasting	2.59(0.88-7.59)	.084	0.18(0.07-0.48)	.001 *

COR: Crude odds ratio; AOR: Adjusted odds ratio; CI = Confidence Intervals; *p<0.05
HAZ: Height for age; **WAZ:** Weight for age z-scores; **WHZ:** Weight for height z-score

4.2 Discussions

This section discusses key findings from this study in detail with reference to other studies where necessary.

4.2.1 General overview

Complementary feeding plays a major role in promoting child growth and development especially at the younger age of up to 2 years. There is a direct link between young child feeding practices and poor nutrition status, however, food insecurity (Kulwa *et al.*, 2015) and ignorance have a great contribution to the problem. This study found a significant contribution of age, sex and breastfeeding practices in child stunting in Singida District Council. Furthermore, dietary diversity, food system and handling have no statistical significance to the nutritional status of children though, could show indirect effects as the majority of the children were receiving food varieties below minimum DDS of four food groups per week. Additionally, children introduced to complementary foods were at high risk of being exposed to aflatoxins with statistical significance in contribution to their underweight (p=0.034) and wasting (p=0.001) in multivariate analysis.

4.2.3 Nutritional status of the children

Children with Z-score ≥-2 were grouped as normal and those with <-2 were grouped as stunted, underweight and wasted based on HAZ, WAZ and WHZ respectively (De Onis *et al.*, 2012). When a Z-score falls below -3 it defines severe levels of each of the indices. Stunting indicates low HAZ as a result of long term consequences like food insecurity, infections, illiteracy of the mother, poor

nutritional status of the mother before and during the prenatal period and the like. This is usually used as a sign of chronic malnutrition. Underweight indicates low WAZ and is caused by starvation and it stands as acute and chronic malnutrition. Wasting indicates low WHZ where it shows thinness and usually caused by short term consequences like the occurrence of diseases which may result in less bioavailability and /or intake of foods (Chen, 2016).

However, poor nutritional status of the participants particularly stunting, could be due to low dietary diversity resulted from inadequate harvest of non-cereal-based crops, lack of prioritization by selling out non-cereals crops for income, as well as negligence and slow adoption of nutrition-sensitive agriculture and infant and young child feeding (IYCF) education (Chen, 2016). Furthermore, low income may be supported by the fact that majority of the population are farmers (Kulwa *et al.*, 2015) where the small amount of legumes grown and small animals kept are normally sold for other basic needs. Thus, low dietary diversity plays a major role in the observed nutritional status as very few households (5.8%) could meet the minimum dietary diversity for infants.

4.2.4 Age of the participants

The recruited population was categorized into three groups according to WHO as 6-8, 9-11 and 12-24 months respectively. This as one of the sociodemographic characteristics indicated the significant contribution to stunting with a p-value of 0.000 which is <0.05 CI used. However, a large number (85 out 108) of children from the third group (12-24 months) were stunted. Poor nutritional status of the children particularly stunting, as found out in this study could be attributed to the fact that the majority (62.8%) of the studied children belong to the age group of between 12 to 24 months. This group is at risk of being stunted compared to their counterparts due to the fact that, most of them were no longer or less breastfed. Furthermore, the same group have been on the complementary food at least past six months, and majority relay on family food (WHO, 2003a) which might be nutritionally inadequate to meet child's nutritional requirement (Mittal *et al.*, 2007). On the other hand, exposure to complementary and family foods might have exposed children to environmental contaminants such as aflatoxins, which contribute to stunting (Asfaw *et al.*, 2015). This study also revealed an increase in dietary exposure to AFB1 with an increase in the age of children. This decrease in linear growth seems to be a critical problem from the time of introduction of complementary food, particularly from the first year of life onward (Onyango *et al.*, 2014). Furthermore, Gong *et al.* (2003) found out that majority of children at 12-24 months were no longer breastfed and their complementary feed was susceptible to aflatoxins contamination at levels higher than that of the breast milk. Increased exposure to aflatoxins is

parallel to the high consumption of the susceptible food materials (maize, sorghum and *magai*) as it was observed that, the consumption rate of children at the age group of 12 to 24 months was as high as 456 g per day compared to other groups of 6-8 and 9-11 months which was 350 g and 390 g respectively.

4.2.5 Sex of the children

Sex of the child showed a statistically significant association with stunting where male children seem to be more stunted than their female counterparts with p=0.001. Similar observation was reported in Ethiopia where male children were found to be more stunted than their female counterparts (Asfaw *et al.*, 2015). The fact that boys are introduced to complementary foods earlier than girls (Chirande *et al.*, 2015), poor ability of boy's physiology to suppress environmental stress (Asfaw *et al.*, 2015) and food stress compared to girls. Chronic exposure to natural toxins like mycotoxins was suggested to be among the environmental disasters causing a high risk of aflatoxins exposure in male children than females (Asfaw *et al.*, 2015). Early introduction of complementary foods to male children expose them to aflatoxins which are linked to poor growth outcome (Magoha *et al.*, 2016). This poor growth could be due to their body's inability to digest and absorb useful nutrients and detoxify the levels of toxins found in foods as the digestive system and the liver respectively, are not fully developed (Magoha *et al.*, 2016). In addition, the early introduction of complementary foods may lead to the risk of diarrhea (Marcoux, 2002) contributing to poor nutritional status. A demographic health survey conducted in Tanzania in the year 2015 revealed that male children received supplementary foods earlier than girls even before the age of 6 months with perceptions (Chirande *et al.*, 2015) that, breastmilk is not enough for them.

4.2.6 Breastfeeding

Breastfeeding has shown significant (p=0.02) contribution to stunting as the results indicated that more than fifty percent (52%) of the children who were no longer breastfed were stunted. This could be due to inadequate nutrients from complementary feeding (Mittal *et al.*, 2007). Majority (94.2%) of the studied population were not able to consume ≥4 food groups in a week and cause very low dietary diversity hence inadequate intake of essential nutrients. In this case, unavailability of average amount breastmilk to complement the inadequate diet (Hien & Kam, 2008) could be the main reason for the low height to their age. However, average breastmilk contributes to the energy density, protein (Dewey, 2002) as well as bioavailability of other nutrients essential for growth like minerals and vitamins which strengthen the immunity and could protect the child against frequently

infections and quick recovery on illness (Dewey, 2002). Other studies in Brazil, Bangladesh and Tanzania promoted continuing breastfeeding to 2 years of age as it protects the children against the effects of diarrhea (Briend *et al.*, 1988; Chirande *et al*., 2015; Victora *et al.*, 1992).

4.2.7 Dietary exposure to aflatoxin B1 and its effect on children nutritional status

The nutritional status was associated with AFB1 exposure and potential confounding factors of growth indicators were adjusted with consideration of 95% CI and $p<0.05$. Wasting was protective being caused by dietary exposure to aflatoxin B1 with lower odds of 0.18, p=0.001. The findings were in line with the study conducted in Kenya, where wasting of children who were consuming family flour which was highly contaminated with aflatoxin, was contributed by dietary exposure to aflatoxin with statistical significance of p=0.002 (Okoth & Ohingo, 2004).

However, dietary exposure to aflatoxin could be also a reason for the poor nutritional status which was attributed to the sex of the children where male children were stunted and underweight than the female children (p= 0.001 and p= 0.007) respectively. Asfaw *et al*. (2015) suggested that, environmental stress like chronic exposure to toxins like mycotoxin to male children might be the cause of differences in poor nutritional status to male than female children. Furthermore, male children receive complementary foods as early before the age of six months as it believed that breastmilk couldn't be enough for their body needs (Chirande *et al.*, 2015); thus they are likely to be exposed to these toxins earlier than female children. Because at younger age below six months their immunity is not strong enough to fight against infections and diseases (Marcoux, 2002) which could be attributed by the toxins found in cereal foods, the child is likely to be undernourished than the female who receive complimentary food at the age of six months (Magoha *et al.*, 2016).

4.2.8 Dietary exposure in aflatoxin B1 in different complementary foods

Magai which is a local soft drink made from milled germinated sorghum and common in the study area was used by families as a refreshment and in-between meals. Hand in hand, *magai* was consumed by children as complimentary food (Ezekiel *et al.*, 2018; Nyamete *et al.*, 2016) where there is no other cereal flour for baby's porridge, a traditional complementary food. Of a disadvantage, *magai* was highly exposing children to AFB1 above tolerable levels (Table 6). This study discovered that dietary exposure to AFB1 from *magai* flour consumed as *magai* drink was very high with the mean of 157.94 ng/kg body weight per day as compared to those who were using other complementary flours. *Magai* drink was the first before maize flour (in causing high dietary

exposure to this potent aflatoxin (AF) with the mean of 133.36 ng/kg and 111.7 ng/kg body weight per day respectively. This high level of aflatoxins contamination of *magai* and the resultant dietary exposure could be due to the processes involved during its preparation whereby the dried sorghum is washed and soaked for 2 days and allowed to germinate in 3 days and later on sun-dried on the bare ground/soil or on the mat. Sun-drying of cereals on the bare ground has been widely associated with high levels of mycotoxins contamination (Waliyar *et al*., 2014) as many fungi are harbored in the soil. The washing and soaking processes expose the prior dried sorghum into high moisture content, which favors fungal growth and mycotoxins production if not rapidly dried to the acceptable moisture content. In addition, weather condition and unawareness of mycotoxins contamination of cereals in the study area might delay re-drying of such cereals followed by poor storage of the malt which both favours fungal growth, mycotoxins production and ultimate unacceptable high dietary exposure of these toxins in children and the general population (Ezekiel *et al.*, 2018). Even though there was no statistically significant association between this exposure (p=0.085) and stunting regardless of its high prevalence (37.7%) observed, there could be chronic exposure originating from the mother (Gong *et al.*, 2016; Groopman *et al.*, 2014; Turner *et al.*, 2007) which is later accelerated due to frequent consumption of contamination food. Furthermore, during the preparation of *magai* drink, there could be a carry-over effect resulting to cross-contamination of aflatoxins between batches of *magai* drink as they practiced back-slopping fermentation. This is supported by the fact that, in some cases, the *magai* flour was not or less contaminated compared to the *magai* drink collected from the same household. For instance, a case where *magai* flour was undetected for AFB1 but its product *magai* drink was found to be contaminated with AFB1 at 0.68 µg/kg. On the other hand, high levels of aflatoxin contamination in *magai* flour seems to be reduced after fermentation to *magai* drink. This was revealed in the samples of *magai* flour and *magai* drink collected from the same household where total aflatoxins were reduced from 4.04 µg/kg to 0.46 µg/kg respectively. This is an indication that, when clean culture is used, levels of aflatoxins in the follow-up batch (if contaminated flour used) could be reduced. Reducing of aflatoxin levels in fermented products has been reported to be facilitated by the presence of lactic acid bacteria by fighting the growth of fungus and by detoxifying the toxins (Nyamete *et al.*, 2016).

4.2.9 Food system and handling

Factors that were assessed include sources of food, methods used to clean cereals prior milling, time in which cereals are stored and storage facilities that were used in the storage of cereals. These

factors show no statistical significance to contribution of any of nutritional status of the children Due to levels of aflatoxin contaminations found in the foods that were commonly used as complementary foods and family food as well, these factors could have indirect contribution to stunting through accumulation of small levels of toxins for long time and results into chronic exposure. Chronic exposure could have great effect to nutritional status of the children as these foods consumed as a monotonous diet (Wu *et al.,* 2014) due to economic point of view, and negligence to the little knowledge they have on nutrition throughout the year, since prenatal stage up to the date of survey.

Poor management of crops in the field especially in low fertile land can stress plants and lead to the risk of fungal growth and mycotoxins contamination of the grain. On the other hand, high temperature and humidity are the main factors favoring fungal growth and mycotoxin contamination of crops during storage (Gong *et al.,* 2016). Being the semi-arid region, Singida is highly affected by low and erratic rainfall (435 mm per year) (Lal *et al.,* 2015) subjecting the plants to water stress throughout the production time. This could be the reason for fungal infestation and high aflatoxins contamination of these susceptible food crops. The majority (99.4%) of the households in this study store their produce in sacks for about 12 months (62.3%). Longer storage time of crops, especially in inadequate or poorly managed storage facilities, have been associated with high mycotoxin contamination (Makori *et al.,* 2019; Torres *et al.,* 2014).

On the other hand, poor handling and favourable environment of this region for the growth of fungi and production of the natural toxin could contribute to stunting to the children through their mothers where utero exposure and breastmilk plays a big role. This could be due to chronic exposure from mother to child as most of the cereal foods were consumed as the family food throughout their life cycle. However, (Khlangwiset *et al.,* 2011) reported availability of aflatoxin in blood cord and mother blood, indicating an utero exposure to young children from their mothers. If pregnant women were consuming cereals as the staple food during pregnancy or even after birth via breast milk which might be contaminated as well. Furthermore, in other study a significantly low weight (HAZ) was reported to children whose mothers breast milk was positively contaminated with AFM1 compared to those with negative contamination (Mahdavi *et al.,* 2010). Moreover, all these were the results of the low level of awareness on aflatoxin and its immediate and long term health effects caused by maize and other cereals value chains (Johnson *et al.,* 2018).

4.2.10 Environmental factor

Though environmwntal factors were not assessed in this study, they are acknowledged as confounding factors that might have contributed to the higher prevalence of undernutrition particularly stunting. In an environment where sanitation is poor, stunting may emanate from environmental enteropathy, or suboptimal nutrient absorption due to repeated infections of the gut, soil-transmitted helminths and nematodes, or parasitic worms, diarrheal diseases, acute respiratory infections, and malaria. The high cost of water, including longer time to the water collection point, transportation costs and the costs of medical treatments incurred for recurring infections and diseases (Lestikow *et al.*, 2017) are underlying factors. Studies showed a close association between stunting and poor hygiene and sanitation especially to an environment contaminated with fecal matters from both humans and animals (Dorsey *et al.*, 2018). Being a semi-arid region with limited water supply, characterized by a low level of education and semi pastoral community, Singida is susceptible to poor hygiene and sanitation. Also, in the course of development, infants are actively exposed to the soil and other creatures from the environment which could be contaminated by animal and/or human fecal matters, presenting them at high risk to recurrent infections and diarrhea (Checkley *et al.*, 2008).

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study reported very low dietary diversity among young children with high consumption of cereals-based flour in its wholesome, which are inadequate in essential nutrients and susceptible to aflatoxins contamination. Furthermore, longer time storage of crops, especially in inadequate or poorly managed storage facilities, could be among the reasons of these high levels of mycotoxin contamination. *Magai* drink and maize flour contaminated beyond tolerable limits and the leading complementary food materials responsible for the high aflatoxins exposure in children, posing a public health concern. Therefore, these findings highlighted a need for integrated nutrition interventions to the entire population with the inclusion of strategies for improving dietary diversity and mitigating mycotoxins contamination of staple food crops thereby reducing exposure among infant and the general population.

5.2 Recommendations

Further studies on "*magai*" are recommended to find out a safe method of preparing the local refreshment drink *"magai"* and a positive way of utilizing it to reduce the risk of exposure to aflatoxin in both children and the community at large.

REFERENCES

Alabi, A., Ijadunola, M. Y., Alabi, O., Onayade, A., & Aluko, O. (2016). Assessment of childhood nutritional status: findings from a health and demographic surveillance system. *International Journal.* 4(1), 7-11.

Alberts, J., Lilly, M., Rheeder, J., Burger, H., Shephard, G., & Gelderblom, W. (2017). Technological and community-based methods to reduce mycotoxin exposure. *Food Control*, 73, 101-109.

Arimond, M., & Ruel, M. T. (2002). *Progress in developing an infant and a child feeding index: An example using the Ethiopia demographic and health survey 2000* (No. 583-2016-39595).

Arimond, M., & Ruel, M. T. (2004). Dietary diversity is associated with child nutritional status: evidence from 11 demographic and health surveys. *The Journal of Nutrition*, 134(10), 2579-2585.

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), 1-9.

Briend, A., Wojtyniak, B., & Rowland, M. G. (1988). Breast feeding, nutritional state, and child survival in rural Bangladesh. *British Medical Journal (Clinical research ed.*), 296(6626), 879 -882.

Brown, K. H., Peerson, J. M., Kimmons, J. E., & Hotz, C. (2002). Options for achieving adequate intake from home-prepared complementary foods in low income countries. In *Nestle Nutrition Workshop Series*, 48, 239-256.

Brown, R. L., Chen, Z. Y., Cleveland, T. E., & Russin, J. S. (1999). Advances in the development of host resistance in corn to aflatoxin contamination by Aspergillus flavus. *Phytopathology*, 89(2), 113-117.

Castelino, J. M., Routledge, M. N., Wilson, S., Dunne, D. W., Mwatha, J. K., Gachuhi, K., Wild, C. P., & Gong, Y. Y. (2015). Aflatoxin exposure is inversely associated with IGF1 and IGFBP3 levels in vitro and in Kenyan schoolchildren. *Molecular Nutrition & Food Research*, 59(3), 574-581.

Checkley, W., Buckley, G., Gilman, R. H., Assis, A. M., Guerrant, R. L., Morris, S. S., Mølbak, K., Valentiner-Branth, P., Lanata, C. F., & Black, R. E. (2008). Multi-country analysis of the effects of diarrhoea on childhood stunting. *International Journal of Epidemiology*, 37(4), 816-830.

Chen, J. (2016). Contextualizing Child Stunted Growth and Development within a broader framework of the demographic transition and the epidemiologic transition. *Journal of Social Sciences and Philosophy*, 28 (3), Number 3, 1– 99

Chirande, L., Charwe, D., Mbwana, H., Victor, R., Kimboka, S., Issaka, A. I., Baines, S. K., Dibley, M. J., & Agho, K. E. (2015). Determinants of stunting and severe stunting among under-fives in Tanzania: evidence from the 2010 cross-sectional household survey. *BMC Pediatrics*, 15(1), 1-13.

Demographic, B. (2011). *Health Survey 2014*. https://dhsprogram.com/pubs/pdf/fr321/fr321.pdf

De Onis, M., Dewey, K. G., Borghi, E., Onyango, A. W., Blössner, M., Daelmans, B., Ellen, P., & Branca, F. (2013). The W orld H ealth O rganization's global target for reducing childhood stunting by 2025: Rationale and proposed actions. *Maternal and Child Nutrition*, 9(2), 6 - 26.

De Onis, M., & Branca, F. (2016). Childhood stunting: a global perspective. Maternal & Child Nutrition, 12, 12-26. Dewey, K. (2002). *Guiding principles for complementary feeding of the breastfed child*. https://pesquisa.bvsalud.org/portal/?lang=pt&q=au:"Pan American Health Organization - WHO".

Dewey, K. (2003). *Guiding principles for complementary feeding of the breastfed child*. https://pesquisa.bvsalud.org/portal/?lang=pt&q=au:"Pan American Health Organization - WHO".

Dewey, K., Lutter, C., Martines, J., & Daelmans, B. (2003). *Guiding principles for complementary feeding of the breastfed child.* https://pesquisa.bvsalud.org/portal/?lang=pt&q=au:"Pan American Health Organization - WHO".

Dewey, K. G., & Mayers, D. R. (2011). Early child growth: How do nutrition and infection interact? *Maternal & Child Nutrition*, 7, 129-142.

Dicko, M. H., Gruppen, H., Traoré, A. S., Voragen, A. G., & Van Berkel, W. J. (2006). Sorghum grain as human food in Africa: Relevance of content of starch and amylase activities. *African Journal of Biotechnology*, 5(5), 384-395.

Dorsey, J. L., Manohar, S., Neupane, S., Shrestha, B., Klemm, R. D., & West Jr, K. P. (2018). Individual, household, and community level risk factors of stunting in children younger than 5 years: Findings from a national surveillance system in Nepal. *Maternal & Child Nutrition*, 14(1), 1 -16.

Eroğlu, A. G. (2019). Malnutrition and the heart. *Turkish Archives of Pediatric*, 54(3), 139–140.

Ezekiel, C. N., Ayeni, K. I., Misihairabgwi, J. M., Somorin, Y. M., Chibuzor-Onyema, I. E., Oyedele, O. A., … & Krska, R. (2018). Traditionally processed beverages in Africa: A review of the mycotoxin occurrence patterns and exposure assessment. *Comprehensive Reviews in Food Science and Food Safety*, 17(2), 334-351.

Gong, Y., Egal, S., Hounsa, A., Turner, P., Hall, A., Cardwell, K., & Wild, C. (2003). Determinants of aflatoxin exposure in young children from Benin and Togo, West Africa: The critical role of weaning. *International Journal of Epidemiology*, 32(4), 556-562.

Gong, Y. Y., Turner, P. C., Hall, A. J., & Wild, C. P. (2008). Aflatoxin exposure and impaired child growth in West Africa: An unexplored international public health burden. *Mycotoxins Detection Methods, Management, Public Health and Agricultural Trade*, 53-66.

Gong, Y. Y., Watson, S., & Routledge, M. N. (2016). Aflatoxin exposure and associated human health effects, a review of epidemiological studies. *Food Safety*, 4(1), 14-27.

Groopman, J. D., Egner, P. A., Schulze, K. J., Wu, L. S.-F., Merrill, R., Mehra, S., Shamim, A. A., Ali, H., Shaikh, S., & Gernand, A. (2014). Aflatoxin exposure during the first 1000 days of life in rural South Asia assessed by aflatoxin B1-lysine albumin biomarkers. *Food and Chemical Toxicology*, 74, 184-189.

Hatløy, A., Hallund, J., Diarra, M. M., & Oshaug, A. (2000). Food variety, socioeconomic status and nutritional status in urban and rural areas in Koutiala (Mali). *Public Health Nutrition*, 3(1), 57-65.

Hell, K., Fandohan, P., Bandyopadhyay, R., Kiewnick, S., Sikora, R., & Cotty, P. J. (2008). Pre- and postharvest management of aflatoxin in maize: An African perspective. *Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade*, 219-229.

Hernandez-Vargas, H., Castelino, J., Silver, M. J., Dominguez-Salas, P., Cros, M.-P., Durand, G., Calvez-Kelm, F. L., Prentice, A. M., Wild, C. P., & Moore, S. E. (2015). Exposure to aflatoxin B1 in utero is associated with DNA methylation in white blood cells of infants in The Gambia. *International Journal of Epidemiology*, 44(4), 1238-1248.

Hien, N. N., & Kam, S. (2008). Nutritional status and the characteristics related to malnutrition in children under five years of age in Nghean, Vietnam. *Journal of Preventive Medicine and Public Health*, 41(4), 232-240.

Imdad, A., Yakoob, M. Y., & Bhutta, Z. A. (2011a). Impact of maternal education about complementary feeding and provision of complementary foods on child growth in developing countries. *BMC Public Health*, 11(3), 1-14.

Imdad, A., Yakoob, M. Y., & Bhutta, Z. A. (2011b). Impact of maternal education about complementary feeding and provision of complementary foods on child growth in developing countries. *BMC Public Health*, 11(3), 1 -14.

Johnson, A., Fulton, J., Abdoulaye, T., Ayedun, B., Widmar, N., Akande, A., Bandyopadhyay, R., & Manyong, V. (2018). Aflatoxin awareness and Aflasafe adoption potential of Nigerian smallholder maize farmers. *World Mycotoxin Journal*, 11(3), 437-446.

Kennedy, G., Ballard, T., & Dop, M. C. (2011). *Guidelines for measuring household and individual dietary diversity. Nutrition and Consumer Protection Division, Food and Agriculture Organization of the United Nations. Accessed on 2016/03/06*.

Khandelwal, S., Dayal, R., Bhalla, S., & Paul, T. (2014). A review of Government programmes for women and children in India: Implications for nutrition during the thousand day period. *Indian Journal of Nutrition and Dietetics*, 51, 322-339.

Khlangwiset, P., Shephard, G. S., & Wu, F. (2011). Aflatoxins and growth impairment: A review. *Critical Reviews in Toxicology*, 41(9), 740-755.

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 and Contaminants*, 25(11), 1353-1364.

Kulwa, K. B., 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), 1-11.

Lal, R., Singh, B. R., Mwaseba, D. L., Kraybill, D., Hansen, D. O., & Eik, L. O. (Eds.). (2015). *Sustainable intensification to advance food security and enhance climate resilience in Africa*. *Springer International Publishing*. https://link.springer.com/book/10.1007/978-3-319-09360-4

Lestikow, G., MacArthur, J., Chowdhury, R., Davis, J., & Wei, Y. (2017). *Integrating WASH and nutrition in market-based interventions*: Principles and results from the field. https://repository.lboro.ac.uk/articles/conference_contribution/Integrating_WASH_and_nutrition_in_marketbased_interventions_principles_and_results_from_the_field/9589184/1

Loprieno, N. (1975). International Agency for Research on Cancer (IARC) monographs on the evaluation of carcinogenic risk of chemicals to man:" Relevance of data on mutagenicity". *Mutation Research*, 31(3), 210.

Lukmanji, Z., Hertzmark, E., Mlingi, N., Assey, V., Ndossi, G., & Fawzi, W. (2008). *Tanzania food composition tables*. *MUHAS-TFNC, HSPH, Dar es Salaam Tanzania*. https://www.researchgate.net/profile/GodwinNdossi/publication/265627809_Tanzania_Food_ComposiTion_Tables_Compiled_by/links/5419a5080cf2218008bf990d/Tanzania-Food-ComposiTion-Tables-Compiled-by.pdf

Magoha, H., Kimanya, M., De Meulenaer, B., Roberfroid, D., Lachat, C., & Kolsteren, P. (2016). Risk of dietary exposure to aflatoxins and fumonisins in infants less than 6 months of age in Rombo, Northern Tanzania. *Maternal & Child Nutrition,* 12(3), 516-527.

Mahdavi, R., Nikniaz, L., Arefhosseini, S., & Jabbari, M. V. (2010). Determination of Aflatoxin M 1 in Breast Milk Samples in Tabriz–Iran. *Maternal and Child Health Journal, 14*(1), 141.

Makori, N., Matemu, A., Kimanya, M., & Kassim, N. (2019). Inadequate management of complementary foods contributes to the risk of aflatoxin exposure and low nutrition status among children. *World Mycotoxin Journal,* 12(1), 67-76.

Marcoux, A. (2002). Sex differentials in undernutrition: A look at survey evidence. *Population and Development Review,* 28(2), 275-284.

Marroquín-Cardona, A., Johnson, N., Phillips, T., & Hayes, A. (2014). Mycotoxins in a changing global environment–A review. *Food and Chemical Toxicology,* 69, 220-230.

McKinney, P. (2006). Tanzania: comprehensive food security and vulnerability analysis (CFSVA). *Strengthening Emergency Needs Assessment Capacity (SENAC). Rome, Italy, UN World Food Programme, Vulnerability Analysis and Mapping Branch (ODAV), 95*.

Mittal, A., Singh, J., & Ahluwalia, S. K. (2007). Effect of maternal factors on nutritional status of 1-5-year-old children in urban slum population. *Indian Journal of Community Medicine*, 32(4), 264.

Mulokozi, G., & Svanberg, U. (2003). Effect of traditional open sun-drying and solar cabinet drying on carotene content and vitamin A activity of green leafy vegetables. *Plant Foods for Human Nutrition,* 58(3), 1-15.

Nemati, M., Mehran, M. A., Hamed, P. K., & Masoud, A. (2010). A survey on the occurrence of aflatoxin M1 in milk samples in Ardabil, Iran. *Food Control,* 21(7), 1022-1024.

Nti, C. A. (2014). Dietary diversity is associated with nutrient intakes and nutritional status of children in Ghana. *Asian Journal of Medical Sciences*, 2(2), 105-109.

Nyamete, F., Bennink, M., & Mugula, J. (2016). Potential of lactic acid fermentation in reducing aflatoxin B1 in Tanzania maize-based gruel. *African Journal of Food, Agriculture, Nutrition and Development,* 16(3), 11139-11151.

Okoth, S. A., & Ohingo, M. (2004). Dietary aflatoxin exposure and impaired growth in young children from Kisumu District, Kenya: Cross sectional study. *African Journal of Health Sciences,* 11(1), 43-54.

Onyango, A. W., Borghi, E., de Onis, M., del Carmen Casanovas, M., & Garza, C. (2014). Complementary feeding and attained linear growth among 6–23-month-old children. *Public Health Nutrition,* 17(9), 1975-1983.

Rah, J. H., Akhter, N., Semba, R. D., De Pee, S., Bloem, M. W., Campbell, A., Moench-Pfanner, R., Sun, K., Badham, J., & Kraemer, K. (2010). Low dietary diversity is a predictor of child stunting in rural Bangladesh. *European Journal of Clinical Nutrition,* 64(12), 1393.

Shirima, C. P., Kimanya, M. E., Routledge, M. N., Srey, C., Kinabo, J. L., Humpf, H.-U., Wild, C. P., Tu, Y.-K., & Gong, Y. Y. (2014). A prospective study of growth and biomarkers of exposure to aflatoxin and fumonisin during early childhood in Tanzania. *Environmental Health Perspectives,* 123(2), 173-178.

Smith, L. E., Stoltzfus, R. J., & Prendergast, A. (2012). Food chain mycotoxin exposure, gut health, and impaired growth: A conceptual framework. *Advances in Nutrition,* 3(4), 526-531.

Song, S., Ediage, E. N., Wu, A., & De Saeger, S. (2013). Development and application of salting-out assisted liquid/liquid extraction for multi-mycotoxin biomarkers analysis in pig urine with high performance liquid chromatography/tandem mass spectrometry. *Journal of Chromatography A,* 1292, 111-120.

Stewart, C. P., Iannotti, L., Dewey, K. G., Michaelsen, K. F., & Onyango, A. W. (2013). Contextualising complementary feeding in a broader framework for stunting prevention. *Maternal & Child Nutrition,* 9, 27-45.

Steyn, N. P., Nel, J. H., Nantel, G., Kennedy, G., & Labadarios, D. (2006). Food variety and dietary diversity scores in children: Are they good indicators of dietary adequacy? *Public Health Nutrition,* 9(5), 644-650.

Strosnider, H., Azziz-Baumgartner, E., Banziger, M., Bhat, R. V., Breiman, R., Brune, M.-N., DeCock, K., Dilley, A., Groopman, J., & Hell, K. (2006). Workgroup report: public health strategies for reducing aflatoxin exposure in developing countries. *Environmental health perspectives*, 114(12), 1898-1903.

Sudfeld, C. R., McCoy, D. C., Fink, G., Muhihi, A., Bellinger, D. C., Masanja, H., Smith, E. R., Danaei, G., Ezzati, M., & Fawzi, W. W. (2015). Malnutrition and its determinants are associated with suboptimal cognitive, communication, and motor development in Tanzanian children. *The Journal of Nutrition*, 145(12), 2705-2714.

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.

Thomas, G., Thompson, B., & Traore, M. (2015). *Sustainable Nutrition Security: Restoring the Bridge Between Agriculture and Health*. https://ideas.repec.org/p/ess/wpaper/id7784.html.

Turner, P. C., Collinson, A. C., Cheung, Y. B., Gong, Y., Hall, A. J., Prentice, A. M., & Wild, C. P. (2007). Aflatoxin exposure in utero causes growth faltering in Gambian infants. *International Journal of Epidemiology*, 36(5), 1119-1125.

UNICEF. (2015). Adopting optimal feeding practices is fundamental to a child's survival, growth and development, but too few children benefit. *New York, UNICEF*. https://data.unicef.org/topic/nutrition/infant-and-young-child-feeding/

Victora, C. G., Fuchs, S. C., Kirkwood, B. R., Lombardi, C., & Barros, F. C. d. (1992). Breast-feeding, nutritional status, and other prognostic factors for dehydration among young children with diarrhoea in Brazil. *Bulletin of the World Health Organization*, 70(4), 467.

Waliyar, F., Osiru, M., Ntare, B., Kumar, K. V. K., Sudini, H., Traore, A., & Diarra, B. (2014). Post-harvest management of aflatoxin contamination in groundnut. *World Mycotoxin Journal*, 8(2), 245-252.

World Health Organization. (2021). Levels and trends in child malnutrition: UNICEF/WHO/The World Bank Group joint child malnutrition estimates: key findings of the 2021 edition. In *Levels and trends in child malnutrition: UNICEF/WHO/The World Bank Group joint child malnutrition estimates: key findings of the 2021 edition*. https://data.unicef.org/resources/jme-report-2021.

WHO. (2003a). *Complementary feeding: report of the global consultation, and summary of guiding principles for complementary feeding of the breastfed child*. 37. https://apps.who.int/iris/handle/10665/42739

WHO. (2003b). *Guiding principles for complementary feeding of the breastfed child*. https://www.who.int/nutrition/publications/guiding_principles_compfeeding_breastfed.pdf.

WHO. (2008). *Indicators for assessing infant and young child feeding practices: Part 1: Definitions: Conclusions of a consensus meeting held 6-8 November 2007 in Washington DC, USA, 1 -12*. https://apps.who.int/iris/handle/10665/43895

World Health Organization. (2018). *The state of food security and nutrition in the world 2018: building climate resilience for food security and nutrition*. *Food & Agriculture Org*. https://www.wfp.org/publications/2018-state-food-security-and-nutrition-world-sofi-report

Wild, C. P., & Gong, Y. Y. (2009). Mycotoxins and human disease: A largely ignored global health issue. *Carcinogenesis*, 31(1), 71-82.

Wu, F. (2014). Global impacts of aflatoxin in maize: Trade and human health. *World Mycotoxin Journal*, 8(2), 137-142.

Wu, F., Mitchell, N. J., Male, D., & Kensler, T. W. (2014). Reduced foodborne toxin exposure is a benefit of improving dietary diversity. *Toxicological Sciences*, 141(2), 329-334.

APPENDICES

Appendix 1: Questionnaire

GENERAL INFORMATION			
Village Name **……** VILLAGE CODE……			
Household ID……			
Date of visit…….			
Head of family Name……			
Name of Interviewer……			
A: MOTHERS PARTICULARS			
S/N	**QUESTIONS**	**ANSWERS**	**Correct answer**
1	Name	……	
2	What is your age	Year of birth	years
3	Marital status	1. Single 2. Married 3. Divorced 4. Widow	
4	Level of education	1. No formal education 2. Standard 1-7 3. Form 1-6 4. Others (*specify*)	
5	What is your occupation	1. Farming 2. Livestock keeping 3. Farming and livestock keeping 4. Employed 5. Others (*specify*)	
6	How many children do you have?	1. One 2. Two	

		3. Three 4. Four 5. More than four	
7	What is the size of your family? /how many people live in this family	1. Two-three 2. Four – five 3. Six- seven 4. Eight-nine 5. Others (*specify*)	
8	What type of food do you normally eat in a week?	1. Ugali 2. Dehulled maize 3. Rice 4. Others (*specif)*	
B: CHILD PARTICULARS			
9	Childs name		
10	Date of birth *(from clinic card)*	……..months	……. Months
11	Sex	1. Female 2. Male	
12	Have you ever got any information on child's feeding practices?	1. Yes 2. No	
13	If yes, who gives the education? Mention	1. ……. 2. ……. 3. …… 4. ……. 5. ……	

14	Is the child breast feeding? If yes skip question 14	1. Yes 2. No	
15	If no, at what age did the child stop breastfeeding	…….months	…….months
16	When did you introduce your child with complimentary feeds?	1. At 2 months 2. At 3 months 3. At 4 months 4. >5 months	
17	Which feeds are you normally feed the child	1. Porridge 2. Ugali 3. Banana 4. Rice 5. Others *(specify)*	
18	Is the child having specific foods as the complementary food?	1. Yes 2. No	
19	If yes, what are these?	1. ……. 2. ……. 3. ……. 4. ……	
20	If one of these foods is cereal mixed flour, what is its composition? Mention.....	1. ……. 2. ……. 3. ……. 4. ……. 5. …….	

21	If use the family flour as complementary feed, what type of cereals do normally used in preparation of flour	1. Maize 2. Sorghum 3. Millet 4. Wheat 5. Others *(specify)*	
22	What are the estimations of flour that are normally used in preparing the child's food/porridge? …….. g	……..months	
21	What are other food that are normally feed to child (apart from what you have mentioned in question no. 20)	1. Milk 2. Eggs 3. Fruits 4. Vegetables 5. Beans/legumes 6. others (*Specify)*	
24	How many meals do you give the child per day?	1. 1 2. 2 3. 3 4. Others *(Specify)*	
25	Apart from main dishes, is there any in between meal-snacks that you use to give the child? ……	1. Yes 2. No. (If no skip next question)	
26	If yes, what are those snacks that are normally given to the child?	1. Groundnuts 2. "andazi" 3. Biscuits 4. Fruits 5. Others	

27	Is the child feed in any other house hold?	1. Yes 2. No	
28	If yes, how frequently do the child feed there?	1. ……. per day 2. …….per week 3. ……..per month 4. Others *(Specify)*	
29	What type of food is the child normally feed there?	1. Ugali 2. Porridge 3. Rice 4. Others*(Specify)*	
30	Do you give the child any local drink that is made from cereals? (Specify the type of cereal)…… If yes, how often do you give her/him?	1. .....days in week 2. .....times in a day 3. Others *(Specify)*	
31	What is the source of maize that are normally used in preparation of child/family food?	1. Home grown 2. From the market 3. Others *(Specify)*	
32	If are home grown, how long was the maize stored before consumed?	……..months	.....months
33	How do you store your cereals/grains after harvest?	1. Floor 2. Sacks 3. Silos 4. Air tight containers 5. Others (*specify*)	
34	What do you use to control pest infestation of your storage grains?	1. Pesticides 2. Air tight containers	

		3. Botanicals 4. Nothing	
35	If pesticides in 34 above, what type of pesticide? Mention	1. …… 2. …… 3. …… 4. ……	
36	How do you prepare cereals (maize/sorghum/millet) used in preparation of family/child food?	1. Sorting 2. Winnowing 3. Washing and dried on mats 4. Washing and dried on ground or floor 5. 1,2,3 &4 6. Others *(Specify)*	
37	Whar form of maize do you use for milling of your child/family food?	1. Un-dehulled 2. Dehulled 3. Decorting 4. Others *(Specify)*	
38	In a week, how many times do you feed the child with either of the following foods?	**FOOD**	**FREQUENCY/week**
		Cereal/tubers/roots (potatoes, sweet potatoes, cassava, boiled maize, ugali, porridge "makande"etc	
		Flesh foods (meat, fish, sardines etc.	
		Eggs	
		Dairy (milk, yogurt, cheese, butter etc	

		Legumes (beans, pigeon peas, green peas, etc	
		Vitamin A fruits and vegetables (Carrot, mangoe, pumpkin, tomato etc	
		Other fruits and vegetables (amaranthus, pumpkin leaves, potato leaves, cassava leaves, jute mallow, spider plants and cowpea leaves)	

APPENDIX 3: Ethical clearance certificate

THE UNITED REPUBLIC OF TANZANIA

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NIMR/HQ/R.8a/Vol. IX/2739

30th April 2018

Rufina Fredrick
Nelson Mandela African Institute of Science and Technology
P.O. Box 447
Tengeru-Arusha

RE: ETHICAL CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Assessment of aflatoxin exposure in children through complementary feeding in Singida District Council (Fredrick R. *et al.*) whose supervisor is Dr. Neema Kassim of Nelson Mandela African Institute of Science and Technology has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Site: Singida.

Approval is valid for one year: 30th April 2018 to 29th April 2019.

Name: Prof. Yunus Daud Mgaya

Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Prof. Muhammad Bakari Kambi

Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, COMMUNITY DEVELOPMENT, GENDER, ELDERLY & CHILDREN

CC: RMO of Singida.
DMO/DED of Singida district council

RESEARCH OUTPUTS

Output one: Fredrick, R., Martin, H., Snapp, S., and Kassim, N. (2021). Monoyonous cereal-based complementary feeding contribute to aflatoxin exposure in children. *International Journal of Biosciences*, 18(6), 204- 216.

Output two: Poster presentation

Output two: Poster presentation

SIIL PROJECT

ASSESSMENT OF NUTRITIONAL STATUS AND DIETARY AFLATOXINS EXPOSURE IN CHILDREN IN SINGIDA DISTRICT

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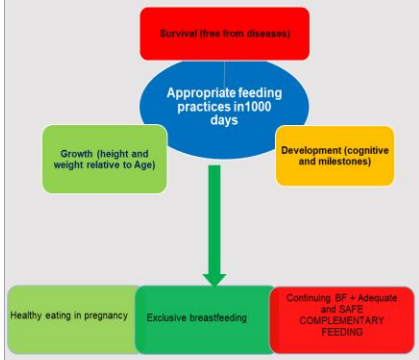
ABSTRACT

Anthropometric measurements of index child were taken, followed by collection of 1 to 3 cereals based food samples used in complementary feeding in 180 households. A total of 218 flour samples made from maize, sorghum, millet, composite and fermented-germinated sorghum locally known as *magai* and *magai* drink; from *magai* flour were collected. Food samples were analyzed for aflatoxins using High-Performance Liquid Chromatography (HPLC). Anthropometric measurements showed that 37.7% were stunted; 20.3% underweight and 8.3% wasted. Furthermore, aflatoxin B1 was detected in 80 (36.7%) out of 218 complementary food samples. Mean dietary exposure were; 133.36, 106.0, 111.70, 94.68 and 92 ng/kg body weight per day for magai drink, composite flour, maize flour, sorghum flour and millet flour respectively.

INTRODUCTION



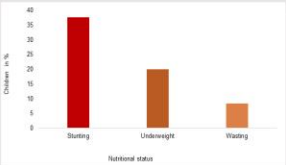
METHODOLOGY

- The study was cross sectional
- The study conducted in ten villages of Singida District Council
- Structured questionnaire was administered to assess dietary habits and consumption frequency of mostly susceptible foods as well as dietary diversification
- A minimum of 200g or 100ml of solids and liquid food respectively, commonly used as complementary food were collected
- Collected food samples were analysed for aflatoxin contamination in HPLC.

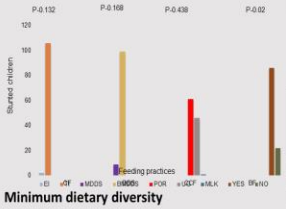
Anthropometric measurement

Analysis of aflatoxin in food samples

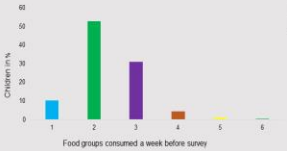
Children nutritional status



Feeding practices



Minimum dietary diversity



RESULTS

Occurrence of aflatoxin B1 and total aflatoxin in complementary foods

Type of food	Number of samples	Positive (%) in B1	Positive (%) in total AF	AFB1(µg/kg)		Total aflatoxin (µg/kg)	
				Median values	Range	Median values	Range
Maize	100	40(40.0)	87(87.0)	1.02	0.33-23.75	2.17	0.47-289.28
Millet	16	0(0.0)	10(62.5)	0.00	0.00-0.00	1.66	0.82-3.83
Sorghum	20	5(25.0)	17(85.0)	0.39	0.37-7.99	1.71	1.46-39.52
Composite	62	20(32.25)	51(82.3)	1.03	0.37-4.48	2.73	0.48-50.84
"Magai" flour	9	6(66.7)	9(100)	1.01	0.65-6.16	18.59	1.67-36.54
"Magai" drink	11	9(81.8)	11(100)	0.65	0.60-0.79	3.38	1.11-4.92

Prevalence of contaminations for aflatoxin B1 and total aflatoxins above regulatory limits

Types of complementary food	EU limits			Tanzania limits	
	>0.1µg/Kg for baby foods	>2µg/Kg for aflatoxin B1[a]	>4µg/Kg for total aflatoxin[b]	>5µg/Kg for Aflatoxin B_1	>10µg/Kg for total aflatoxin
Maize	40	7	27	4	13
Millet	0	0	0	0	0
Sorghum	5	2	3	0	2
Composite	20	3	16	1	8
"Magai" flour	6	2	6	1	6
"Magai" drink	9	0	4	0	0

DISCUSSION

High prevalence of stunting in the study area could be due to low dietary diversity, which resulted in frequent consumption of monotonous diet as 52.8% of the index children were feeding only 2 food groups per week. Majority of the children of 6 to 24 months were less or no longer breastfeeding and were highly relay on family food (WHO, 2003a). These family food might be nutritionally inadequate to meet child's nutritional requirement (Mittal *et al.*, 2007). Furthermore, these complementary food was susceptible to aflatoxins contamination at levels higher than the tolerable limit.

CONCLUSIONS

Complementary food were based on cereals mainly r
and millet which were also susceptible to AF contamina
Nutrition status of the children were 37.7%, 20% and
for stunting, underweight and wasting respectively. Afla
B_1 and total AF contamination were detected in 36.7%
84.8 % of complementary flours and among these, 2.7%
13.3% of the samples contaminated with aflatoxin B1
total AF above the regulatory limit of 5 and 10 µg/kg s
Tanzania respectively. However, there was no signif
contribution to dietary exposure for AFB1 to stunting.

SELECTED REFERENCES

[1] Mittal, A., Singh, J., & Ahluwalia, S. (2007). Effect
maternal f actors on nutritional status of 1-5-year-
children in urban slum population. *Indian Journal
Community Medicine, 32*(4), 264 .

[2] WHO. (2003a). Complementary feeding: report of
global consultation, and summary of guiding princip
for complementary feeding of the breastfed child. 37.

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