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Complementary feeding practices and the risk of exposure to aflatoxins among infants and young children in Kongwa, Tanzania

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COMPLEMENTARY FEEDING PRACTICES AND THE RISK OF EXPOSURE TO AFLATOXINS AMONG INFANTS AND YOUNG CHILDREN IN KONGWA, TANZANIA

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A Thesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

Aflatoxins (AFs) are secondary fungal metabolites that commonly contaminate foods like nuts and cereals and pose major public health concerns like impaired child growth, immune system suppression, liver cancer and death. In sub-Saharan Africa, infants and young children (IYC) feed on complementary foods (CFs) containing ingredients susceptible to contamination with AFs. Although the presence of AFs in foodstuffs has been reported for over 60 years, very few studies have focused on CFs, yet children are more vulnerable due to relatively small bodyweights and underdeveloped immune systems. This study was conducted in Kongwa District, Tanzania in 2017 and 2018, to estimate the contribution of the main CF ingredients to aflatoxin exposure among IYC. The study documented the common ingredients of CFs and the intake of CFs by 35 (6-12-month-old) IYC using multiple-pass 24-h dietary recalls. The levels of AFs contamination in the collected samples were determined using High-Performance Liquid Chromatography while the exposure of IYC to AFs was estimated by a deterministic approach. The study further tested acceptability of Aflatoxin-safe maize-groundnut pre-blended flour (AFSaBF) and groundnut powder (AFSaGP). The key ingredients of CFs were milled maize, sorghum, pearl-millet, rice, and groundnuts (pre- or post-blended with cereals) prepared as thin/stiff porridge. The average per-capita daily intake of CFs flour was 89.45 g. About 82.14% of the samples had AFB_1 levels ranging from 0.27–317 μg/kg, and the exposure levels ranged from 0.33-1168 ng/kg body weight (bw)/day. The Margins of Exposure were < 10 000 for all IYC, signifying a public health concern. The mothers and IYC generally accepted the improved porridge flour and groundnut powder. This is important information for future studies aiming at reducing exposure to AFs in this community. Groundnut and maize flours are the main contributors to the exposure of IYC to AFB_1 in Kongwa district. Community education on mycotoxins-mitigation practices like appropriate pre and post-harvest handling and complementary feeding practices may minimize aflatoxin exposure among IYC in Kongwa and other communities with similar settings. Appropriate feeding practices may entail diet diversification and substitution of groundnut or maize with other food ingredients that are less-prone to AFs contamination.

DECLARATION

I, Clara Justine Mollay do declare to the Senate of Nelson Mandela Africa Institution of Science and Technology that this Thesis is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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CERTIFICATION

The undersigned certify that have read and hereby recommend for acceptance by the Senate of the Nelson Mandela African Institution of Science and Technology the Thesis titled *"Complementary Feeding Practices and The Risk of Exposure to Aflatoxins among Infants and Young Children in Kongwa, Tanzania"* in Fulfillment of the Requirements for the Award of the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology.

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DEDICATION

To my son Jonathan for the love and patience when I was accomplishing this work. Also, to my parents Mr. and Mrs. Justine F. Mollay for their care, love and support in my life.

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LIST OF ABBREVIATIONS AND SYMBOLS

AFs	Aflatoxins
AFSaBF	Aflatoxin-safe maize-groundnut pre-blended porridge flour
AFSaGP	Aflatoxin- safe groundnut powder
BMDL	Benchmark dose lower confidence limit
CAC	Codex Alimentarius Commission
CFs	Complementary Foods
CREATES	Centre for Research, Agricultural Advancement, Teaching Excellence and Sustainability
DAICO	District Agricultural Irrigation and Cooperative Officer
DLLME	Dispersive liquid–liquid microextraction
DMO	District Medical Officer
DNA	De-oxyribo Nucleic Acid
DNuO	District Nutrition Officer
EAC	East African Community
EFSA	European Food Safety Authority
ELISA	Enzyme Linked Immunosorbent Assay
EU	European Union
FAO	Food and Agriculture Organization
FBs	Fumonisins
FDA	US Food and Drug Administration
FGDs	Focus Group Discussions
FLD	Fluorescent Detector
HCC	Hepatocellular Carcinoma
HPLC	High Performance Liquid Chromatography
HPTLC	High-performance Thin-Layer Chromatography
IAC	Immunoaffinity column
IARC	International Agency for Research on Cancer
IYC	Infants and Young Children
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC-MS	Liquid chromatography–mass spectrometry
LODs	Limits of Detection
MBNP	Mwanzo Bora Nutrition Project

ML(s)	Maximum Limit(s)
MMT	Mycotoxin Mitigation Trial
MOE	Margin of Exposure
MoHCDGEC	Ministry of Health, Community Development, Gender, Elderly and Children
NBS	National Bureau of Statistics
NIMR	National Institute for Medical Research
NM-AIST	Nelson Mandela African Institute of Science and Technology
PAHO	Pan American Health Organization
PBS	Phosphate Buffer Saline
RASFF	Rapid Alert System for Food and Feed
RTs	Recipe Trials
SBCC	Social and Behavior Change Communication
SDG	Sustainable Development goals
SSA	sub-Saharan Africa
TBS	Tanzania Bureau of Standards
TDHS	Tanzania Demographic Health Survey
TDI	Tolerable Daily Intake
TFNC	Tanzania Food and Nutrition Centre
TIPs	Trials of Improved Practices
TLC	Thin Layer Chromatography
UNICEF	United Nations International Children's Emergency Fund
USAID	United States Agency for International Development
UV	Ultraviolet
VEOs	Village Executive Officers
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

Complementary foods (CFs) are often introduced as solid and/or liquid components into the diets of infants and young children (IYC) when breast milk is no longer sufficient for their nutritional requirements (WHO/PAHO, 2003). Although the recommended age for the introduction of CFs to infants is 6 months, about 42% of Tanzanian children less than 6 months are introduced to CFs (MoHCDGEC *et al*., 2016). According to Kulwa *et al*. (2015), some children as young as two months old are introduced to CFs in Tanzania. The Tanzania Demographic and Health Survey (TDHS) 2010 showed that 11%, 34%, and 65% of Tanzanian children aged 2 months, 2 - 3 months, and 4 - 5 months respectively are given CFs (NBS & ICF Macro, 2011).

Although the timely introduction of CFs promotes good nutrition and growth in IYC, the period of complementary feeding is also a critical window for growth retardation, nutritional deficiency, and diseases (Mahgoub *et al*., 2006). Feeding practices such as time of introduction and type and/or quality and quantity of CFs are some of the important factors for the child's nutritional status (Kulwa *et al*., 2006). For several years, the choices of CFs have been influenced by cultural and traditional practices without an appropriate focus on their nutritive values or safety. While several ingredients can be used to make CFs, a majority of Tanzanian households depend on cereal-based CFs from aflatoxin prone cereals like rice, maize, sorghum, and wheat (Muhimbula & Issa-Zacharia, 2010; Mamiro *et al*., 2005). Factors that contribute to this are affordability, accessibility, and cultural acceptance (Alamu *et al.*, 2018).

Several reports from Tanzania indicate the contamination of maize with AFs (Aron *et al*., 2017; Kamala *et al*., 2015; Mtega *et al*., 2020; Nyangi *et al*., 2016a). Other studies have reported high AFs exposure in IYC, through maize-based diets (Chen *et al*., 2018; Modest, 2017; Shirima *et al*., 2013). To improve their nutritional value, cereal-based CFs are often mixed with groundnuts (Magamba *et al*., 2017; Makori *et al*., 2019; Muhimbula & Issa-Zacharia, 2010; Shirima *et al*., 2015) which are also susceptible to aflatoxin contamination. Despite their high nutritive value, peanuts are also vulnerable to contamination by AFs as has been established by various studies in many African countries (Anitha *et al*., 2017; Kuhumba *et al*., 2018; Magamba *et al*., 2017; Monyo *et al*., 2012; Mupunga *et al*., 2014). To improve their nutritional value, cereal-based CFs are often mixed with groundnuts (*Arachis hypogea* L.) (Magamba *et al*., 2017; Makori *et al*., 2019;

Muhimbula & Issa-Zacharia, 2010; Shirima *et al*., 2015) which are also susceptible to aflatoxin contamination. The use of such ingredients in CFs may therefore mean that Tanzanian IYC may be exposed to AFs very early in life (Moran & Dewey, 2011) through complementary feeding (Kimanya *et al*., 2014; Magoha *et al*., 2016; Shirima *et al*., 2015).

The exposure of IYC to AFs is a serious public health concern because their daily/routine consumption of CFs contaminated with low levels of AFs can cause chronic exposure (Tola & Kebede, 2016; Wild & Gong, 2010) while the consumption of high concentrations of AFs can cause acute aflatoxicosis and death (Kamala *et al*., 2018b; Liu & Wu, 2020). Aflatoxins are classified as class I human carcinogens which are associated with liver cancer (IARC, 1993), stunting in children (Gong *et al*., 2002, 2003; Turner, 2013), adverse birth defects (Turner *et al.*, 2007), and suppression of the immune system (Jiang *et al.*, 2005; Turner, 2013). This is especially worrying since children are more vulnerable to toxins owing to their lower body weights, less-developed organs, and inability to detoxify (Lombard, 2014), which can subsequently affect their health, and development due to the associated effects of AFs on the immune system, growth, and body organs (Kimanya *et al*., 2014; Magoha *et al*., 2016). As such, there is extensive attention on the risks of dietary exposure of IYC to AFs. Several studies have demonstrated the occurrence of chronic dietary exposure and co-exposure to AFs and other mycotoxins among IYC in Tanzania (Anitha *et al*., 2020; Chen *et al*., 2018; Kamala *et al*., 2018b; Kimanya *et al.*, 2014; Magoha *et al.*, 2016; Makori *et al*., 2019; Shirima *et al*., 2015). Yet, there are limited studies on the contribution of key ingredients of CFs to the exposure of IYC to AFs in Tanzania. The high risk of exposure of IYC to mycotoxins has in the past been associated with poor feeding practices, inadequate knowledge and awareness of AFs, and use of monotonous diets during complementary feeding (Beyene *et al*., 2016; Makori *et al*., 2019), but there is limited data linking these aspects to the risks of exposure of IYC to AFs. The present study was, therefore, designed to assess the complementary feeding practices and estimate the contribution of the main CF ingredients to aflatoxin exposure among the IYC in Kongwa district, Tanzania. Additionally, the study was conducted as part of formative research to inform the design of an IYC feeding intervention as part of a cluster-randomized controlled mycotoxin mitigation trial (MMT) in the district. As such, the study further assessed the acceptability of improved complementary porridge flour with acceptable levels of AFs, which is one of the intervention materials for the trial. In other studies, educational and other interventions on feeding practices have proven useful in improving child nutrition through the reduction of AFs exposure (Aguayo, 2017; Owino, 2006).

1.2 Statement of the Problem

Complementary feeding continues to be a challenge to good nutrition in children of 6–23 months in many parts of the developing world (WHO & United Nations Children's Fund, 1998). Not only are IYC in developing countries introduced to complementary feeding earlier than the recommended age (Gong *et al*., 2016), but also the CFs are often contaminated with AFs (Gong *et al*., 2003; Kuhumba *et al*., 2018; Shirima *et al*., 2013, 2015). Cereals like millet, rice, maize, and wheat, and legumes like groundnuts which are the most common ingredients for CFs in these countries are frequently contaminated with AFs at different stages of the chain such as pre-harvest, harvest, and post-harvest handling (Lombard, 2014; Wagacha & Muthomi, 2008). Several studies in Tanzania have established contamination of CFs ingredients with mycotoxins (Kamala *et al*., 2017; Kimanya *et al*., 2010, 2014; Makori *et al*., 2019). In a study on the exposure of IYC to AFs in the eastern, northern, and southwestern parts of Tanzania, probabilistically estimated exposures were in the ranges of 700 to 716, 13.4 to 14.3, and 9.2 to 9.7 ng/kg/bw/day, respectively (Kamala *et al*., 2017). In another study, the exposure of IYC in Tabora, Iringa, and Kilimanjaro regions of Tanzania to AFs established that the albumins of 84% (geometric mean, 12.9) of the subjects were positive for AFs (Shirima *et al*., 2013).

Although AFs are a universal problem, their economic and health impacts are more established in developing countries owing to their location in tropical/sub-tropical regions (Benkerroum, 2020) where climatic conditions favor the growth of mycotoxin-producing fungi (Bankole *et al*., 2006; Benkerroum, 2020; Gruber-Dorninger *et al*., 2019; James & Zikankuba, 2018; Kamle *et al*., 2019; Lee & Ryu, 2017; Lukwago *et al*., 2019; Medina *et al*., 2014). Tanzania is located between 1°S and 12°S latitudes with an annual average temperature of 25 to 32˚C (Luhunga *et al*., 2018) and a maximum daily temperature of 35 – 38˚C (Kiunsi, 2013; TMA, 2019). This offers a favorable climate for fungal growth and production of AFs in maize whose optimum conditions are temperatures of 35 and 33˚C respectively (Milani, 2013). Many regions in Tanzania also have two short and long rainfall seasons (Luhunga *et al*., 2014; Verheye, 2010). Since areas with short rains are less productive and the country mostly depends on the long rains for crop production, maize is often stored for extended periods to provide for food during the non-productive seasons, making it vulnerable to mycotoxin contamination (Bennett & Klich, 2003; Sasamalo *et al*., 2018).

Several studies have suggested that exposure to mycotoxins through CFs is inversely proportional to child growth (Chen *et al*., 2018; Gong *et al*., 2002, 2003, 2004; Shirima *et al*., 2015; Turner *et al*., 2003; Turner *et al*., 2007; Turner, 2013). The exposure of children to mycotoxins has not only been linked to poor growth and development but also increased vulnerability to diseases (Gong *et al*., 2002, 2003; Kamala *et al*., 2017; Kimanya *et al*., 2010, 2014; Shirima *et al*., 2015; Turner *et al*., 2007; Turner, 2013). The exposure of children to AFs is particularly related to growth impairment or stunting (Chen *et al*., 2018; Kimanya *et al*., 2010, 2014; Shirima *et al*., 2015). According to Akombi *et al*. (2017), Tanzania is ranked first with the burden of stunting approximated at 34.4% compared to neighboring countries like Kenya and Uganda which had 26% and 33.4% respectively. The existing stunting rates in Tanzania are therefore extremely alarming based on the new WHO-UNICEF classification of ≥ 30% which is considered a very high prevalence (UNICEF *et al*., 2018).

1.3 Rationale of the Study

Although there are many different types of mycotoxins, there is massive interest in AFs since they are the most common in staple cereals (Bankole *et al*., 2006; Kimanya *et al*., 2008, 2014) and the most fatal (IARC, 1993; Ostry *et al*., 2017; Paterson & Lima, 2010). Compared to adults IYC are at a higher risk of exposure to mycotoxins owing to their routine consumption of CFs based on cereals and groundnuts that are also prone to contamination by AFs (Gong *et al*., 2002; Kimanya *et al*., 2014; Magoha *et al*., 2014, 2016). Besides, infants are at a higher risk of aflatoxin health effects as they have lower body weights and lower ability to detoxify toxins attributed to incomplete development (Sherif *et al*., 2009), immature metabolic system (Kalantari *et al*., 2011), and lower immunity (Lombard, 2014; Xu *et al*., 2021). It is therefore important to place special emphasis on this group of the population to prevent them from chronic exposure to AFs that can consequently affect their health and development. More attention to the proper handling and formulation of safe CFs is therefore important and will contribute to attaining the 3rd Sustainable Development Goal (SDG) of achieving good health and wellbeing, as well as nutrition-related goals.

Although the consumption of CFs by IYC in sub-Saharan Africa (SSA) continues to increase their exposure to AFs, there are limited studies on the contribution of key ingredients in CFs in Tanzania to the exposure of IYC to AFs. Besides, there is a paucity of information on feeding practices and the perceptions/knowledge of mothers concerning CFs in Tanzania. The present study was conducted to estimate the contribution of the main CF ingredients to AF exposure among IYC in Kongwa district. The study also explored the caregiver's feeding practices to inform the formulation of AF-safe CF flour whose acceptability for infant feeding was also assessed. The study was conducted as part of formative research to inform the design of an IYC feeding intervention as part of a cluster-randomized controlled (MMT in the district. Specifically, the study aimed at informing the MMT of the key ingredients used in CF in Kongwa and the contribution of each to AF exposure among IYC. The study wanted to inform an improved feeding intervention because, improved practices, interventions, and recipe trials (RTs) have been known to improve child nutrition during complementary feeding (Dickin & Griffiths, 1997).

1.4 Research Objectives

1.4.1 General Objective

To assess complementary feeding practices contributing to the risk of exposure to AFs among IYC in Kongwa district of Tanzania.

1.4.2 Specific Objectives

(i) To document caregivers' practices in the feeding of IYC (6-18 months) in Kongwa.

(ii) To assess complementary foods' main ingredients contributing to the risk of dietary exposure to AFs among IYC (6-12 months) in Kongwa district.

(iii) To assess the acceptability of an improved AF-safe flour for complementary feeding in Kongwa district.

1.5 Research Questions

(i) What are the caregivers' practices of complementary feeding for IYC (6 -18 months) in Kongwa district of Tanzania?
(ii) What are the ingredients of CFs in Kongwa? What is the extent of AF contamination in ingredients of CF in Kongwa? What is the risk of exposure of IYC in Kongwa of public health concern? What is the main ingredient contributing to aflatoxin exposure among the IYC in Kongwa?
(iii) What is the acceptability of an improved AF-safe flour for complementary feeding in Kongwa district of Tanzania?

1.6 Significance of the Study

There is a growing concern about the adverse health effects of mycotoxins on humans, with a particular focus on IYC as the most vulnerable population to the risks of exposure to contaminated CFs (de Assunção, 2017). At the moment, data on the risks of dietary exposure of IYC to AFs through ingredients of CFs are scarce. Similarly, the link between dietary exposure to AFs and the knowledge/perceptions of complementary feeding practices among mothers and caregivers in Tanzania has not been well presented. The current study aimed at determining the concentration of AFs in CF ingredients, estimating the dietary exposure of IYC to AFs, and assessing the feeding practices and the acceptability of the provision of an improved formulated complementary porridge flour among mothers in Kongwa District. This is especially important since knowledge/perceptions and complementary feeding practices have been associated with dietary risks of exposure of IYC to mycotoxins (Beyene *et al*., 2016; Makori *et al*., 2019). The information from this study will be important in guiding the trial (MMT) design, and to the policymakers on the risks of exposure to AFs among IYC. Information from this study might be useful in facilitating proper mitigation measures including educating mothers/caregivers on the best feeding practices and preparation of CFs.

1.7 Delineation of the Study

There are several types of mycotoxins (Bennett & Klich, 2003). The present research, however, focused only on AFs which are the most common types of agricultural contaminants (Bankole *et al*., 2006; Kimanya *et al*., 2008, 2014). Similarly, there are several forms of AFs, but the present research was based on AFB_1, AFB_2, AFG_1, and AFG_2 as the most potent forms. Although the risks of exposure to AFs occur in all age groups, the present study only concentrated on IYC (6-12 months) who are the most vulnerable group of the population owing to their low immunity and risks of exposure during complementary feeding (Lombard, 2014). The risk of exposure to mycotoxins can be estimated through urine, blood, and food. The present study evaluated the risk of exposure of IYC to AFs by assessing the quantity of the toxins in some ingredients of CFs alongside the quantities of food they consumed as a direct way of determining the risk of exposure of public health concern in IYC and determining the main ingredient contributing to aflatoxin exposure. The present study also ignored the contribution of AFs from groundnuts that might have been consumed as part of *futari* or added to vegetables relish because these are not common infant foods. Likewise, the current study did not account for the contribution of groundnut powder to the exposure of IYC to AFs since this is not commonly used as a stand-alone ingredient in CFs but rather blended with cereal ingredients like maize. Apart from being added to thin porridge, groundnut powder was also added to vegetables during cooking. The study relied on mothers' memories of food intake by their children in the previous 24 hr which can be subject to recall bias but is a common method in such studies. Besides, there was the use of water to estimate the quantities of thin porridge consumed by the infants which may not be accurate but was employed to approximate the quantities of food consumed since the particular food was not available in its typical form when we visited the studied subjects' homes.

We ignored the contribution of AFs from groundnuts that were consumed in raw form by the IYC, which were added to *futari* and green leafy vegetables relish. Additionally, the moisture content of the AFSaBF and AFSaGP was not controlled during the preparation. Likewise, there was no monitoring of AFs contamination in AFSaBF at the household level. Also, the study considered a small sample size and was only conducted in Kongwa district.

CHAPTER TWO

LITERATURE REVIEW

2.1 History and Origin of Aflatoxins

The term ‘Aflatoxins’ was coined in 1962 when over 100 000 turkeys in England died within a few months from a new infection that was supposedly called the ‘Turkey-X disease’ (Forgacs, 1962). The deaths were linked to acute liver necrosis and hyperplasia of the bile duct (Strosnider *et al*., 2006). It was then discovered that the mortalities were not only limited to turkeys but also ducklings and pheasants and the disease was later on associated with the consumption of peanuts/groundnuts contaminated with secondary metabolites of *A. flavus* and during the analysis of toxic peanut meal, four types of dots that emit blue and green lights were noticed when illuminated with an ultraviolet light (Yitbarek & Tamir, 2013). A thorough analysis of the groundnut meal exposed its toxicity and further evaluations revealed its origin from the fungus, *A. flavus*.

The naming of AFs can be traced back to the species *A. flavus* that produce them since "A" denotes to genus name, and "*fla*", the species name (*A+fla+toxin*) (Ramamurthy & Rajakumar, 2016; Zain, 2011). Generally, research on AFs created a “golden age” of research on mycotoxins or fungal toxins during which several of them were discovered (Bennett, 2010). The AFs are produced as secondary metabolites of *A. vochraceoroseus, A. parasiticus, A. flavus, A. bombycis, A. nomius*, and *A. pseudotamari*. However, *A. flavus* occurs more frequently in food (Mejía-Teniente *et al*., 2011). Other mycotoxins include patulins, fumonisins, and ochratoxins (Bennett, 2010). Among all mycotoxins produced by fungal species, AFs are the most powerful carcinogenic and hepatotoxic compounds and continue to gather more attention (Negash, 2018). Research on these toxins continues to create a growing awareness of the potential dangers linked to their presence in feeds/foods (Al-Fahdawi, 2012).

2.2 Types and Properties of Aflatoxins

There are over 20 known AFs broadly classified as AFB_1, AFB_2, AFG_1, and AFG_2 based on relative chromatographic mobility during thin-layer chromatography and fluorescence under ultraviolet (UV) light (Inan *et al*., 2007). These four classes of AFs occur naturally in foods like sesame, rice, peanut, wheat, and pepper when poorly stored (Al-Fahdawi, 2012). The G and B denote the green and blue fluorescent colors respectively as observed under UV light during thin layer chromatography, while the subscript numbers 1 and 2 show the major and minor compounds respectively (Dhanasekaran *et al*., 2011; Hruska *et al*., 2014). Aflatoxin B_1 is not only the most potent carcinogen but also the most studied (Negash, 2018).

Apart from these major forms of AFs, several other derivatives of AFs such as Q_1, M_1, M_2, and B_{2a}, are biotransformation products of the major metabolites (de Oliveira & Corassin, 2014). For instance, AFM_1 and AFM_2 which are associated with milk and milk products of cows that consume contaminated feeds (van Egmond, 1989), are the hydroxylated metabolites of AFB_1 and AFB_2 respectively (Giray *et al*., 2007; Hussain & Anwar, 2008). These two forms of AFs can also be found in meat, eggs, and urine (Nisa *et al*., 2016). Therefore, the exposure of humans to AFs in both conjugated and unconjugated forms is mostly through the intake of contaminated animal and plant products since these compounds are resistant to heat in conventional treatment temperatures (Negash, 2018). Nevertheless, the mutagenesis and tumorigenesis capability of AFM_1 is less than AFB_1 (Creppy, 2002).

Aspergillus flavus is known to produce only AFB_1 and AFB_2, while *A. paraciticus* produces all the major types of AFs (Midorikawa *et al*., 2008; Pratiwi *et al*., 2015). Apart from *A. flavus*, other *Aspergillus* species have been associated with the production of AFs (Pitt & Hocking, 2006), but with different aflatoxigenic profiles (El Khoury *et al*., 2011) based on DNA (*nor1*) sequence and amplified fragment length polymorphism analyses (Rodríguez *et al*., 2012). Table 1 exhibits the different types of AFs produced by different *Aspergillus* species.

Table 1: Types of aflatoxins produced by some *Aspergillus* species

Aspergillus species	**Type of Aflatoxin(s)**	**References**
A. nomius	B and G	Yan *et al*. (2012)
A. flavus	B	Schmidt-Heydt *et al*. (2010)
A. flavus	G	García-Díaz *et al*. (2019)
A. minisclerotigenes	G	Singh *et al.* (2020)
A. parasiticus	B and G	Levin (2012)
A. ochraceoroseus	B and G	Cary *et al*. (2012)
A. pseudotamarii	B	Baranyi *et al*. (2013)
A. bombycis	B and G	Probst *et al*. (2014)

Table 2 provides the general properties of the four major types of AFs. The toxins are generally odorless, tasteless, and colorless, and thus, not easily detectable. Therefore, laboratory testing is the only assured method of detection (Jallow, 2015). However, certain indicators of mold growth such as severe rotting, discoloration, unusual or offensive smell, or taste on grains or crops can be important in determining contamination (Ramesh *et al*., 2013).

Table 2: Physical and chemical properties of major aflatoxins

Property	**AFB$_1$**	**AFB$_2$**	**AFG$_1$**	**AFG$_2$**
Molecular formula	C17H12O6	C17H14O6	C17H12O7	C17H14O7
Melting point (^oC)	268 - 269	286 – 289	244 – 246	237.- 240
Molecular weight	312	314	328	330
Fluorescence emission (nm)	425	425	450	450
Fluorescence under UV light	Blue	Blue	Green	Green
Crystals	Pale yellow	Pale yellow	Colorless	Colorless
Solubility	Slightly soluble in water and freely soluble in polar organic solvent. Normal solvent Methanol, water: acetonitrile (9:1), trifluoroacetic acid, Methanol: 0.1 N Hydrochloric acid (4:1), Dimethyl sulfoxide and acetone			

Kumar (2018)

2.3 Toxicology of Aflatoxins

Aflatoxins are linked with carcinogenicity and toxicity in both humans and animals (IARC, 1993, 2002). The diseases associated with AFs are generally referred to as aflatoxicoses (Al-Fahdawi, 2012). The consumption of large quantities of AFs in a short period may result in acute aflatoxicosis, resulting in hemorrhages, liver failure, and fatality (Tadesse *et al*., 2020). The common symptoms of acute aflatoxicosis include abdominal pain, vomiting, cerebral edema, convulsions, coma, and death (Strosnider *et al*., 2006). Acute human aflatoxicosis has been reported worldwide but is more common in developing countries (Al-Fahdawi, 2012). The consumption of low or moderate levels of AFs on the other hand results in chronic aflatoxicosis evidenced by cancer and immune system suppression (Hsieh, 1988).

Dietary exposure to AFs is a major Hepatocellular Carcinoma (HCC) risk factor (Shakerardekani *et al*., 2012). The AFB$_1$ is one hepatocarcinogen that presumably causes cancer by inducing DNA adducts leading to genetic changes in liver cells (Wu & Santella, 2012). The adducts are formed when AFB$_1$ is metabolized by cytochrome-P450 enzymes to the reactive intermediate AFB$_1$-8, 9 epoxide which binds to both DNA and proteins to cause acute toxicity/aflatoxicosis (Kew, 2003).

Due to the differences in the susceptibility of test animals to AFs, it is difficult to extrapolate the possible effects of these toxins on humans. Notwithstanding, AFs were put on the Rapid Alert System for Food and Feed (RASFF) of the European Union (EU) in 2008 owing to their severe health effects (European Commission, 2009). The IARC later categorized AFB_1 as a group I carcinogen for humans (IARC, 1993; Negash, 2018). Despite the numerous research and control measures, the presence of AFs in food and agricultural commodities remains a major threat to humans and animals.

Like humans, animals exposed to high levels of AFs in feeds exhibit severe intoxications which can be fatal. Normally, such exposures often lead to liver damage, weight loss, and less productivity which result in huge economic losses. Aflatoxins also affect the growth, reproduction, and immunity of livestock (Anthony *et al*., 2012), and of late, there is a lot of concern about the contamination of milk with AFB_1, especially since milk is often fed to IYC (Fink-Gremmels, 2008).

2.4 Factors that Influence the Production of Aflatoxins in Food

2.4.1 Climatic Conditions and Biotic Stresses

The ability of fungi to grow, survive and contaminate various crops with mycotoxins is greatly influenced by environmental factors like temperature and relative humidity (RH) (Magan *et al*., 2011). According to Milani (2013), fungal development and the contamination of foods with AFs occur due to the interaction among the fungi, the host, and the environment. The environmental factors that favor the development of *A. flavus* and the production of AFs include high soil and/or air temperature, RH and evapotranspiration rates, and water availability (Hell *et al*., 2000). Nevertheless, rainfall and humidity are more important than altitude in predicting mycotoxins (Nyangi *et al*., 2016a). This is because the regulatory genes (*aflR; aflS*), and structural genes (*aflD*) for the production of AFs are influenced by the interaction of temperature and water activity (a_w) (Mahato *et al*., 2019). The general growth conditions for aflatoxigenic fungal species are moisture contents of 80 - 85% or more and temperatures of 13 - 42^{o}C (Coppock *et al*., 2018).

The contamination of foods by AFs is more common in the tropics and sub-tropical regions of SSA where temperature and rainfall are strongly suitable for the growth of *A. flavus* (Pratiwi *et al*., 2015). Therefore, countries that are located between 40ºN and 40ºS latitude such as Tanzania favor the growth of molds and the risk of exposure of populations to AFs (Anthony *et al*., 2012). According to Lahouar *et al*. (2016), high temperatures favor the production of *A. flavus* conidia, their dispersal, and kernel infection rate, thereby contributing to the accumulation of AFs. This particularly occurs in lower altitude areas which are usually warmer, and have high temperatures and humidity as opposed to higher altitude areas which are normally cooler and have lower humidity and temperatures (Nyangi *et al*., 2016a). The prevailing conducive weather in SSA is characterized by high humidity and temperatures coupled with dryness; which promotes fungal growth and production of AFs (Abbas *et al*., 2009). According to Patel *et al*. (2015), under favorable temperature and humidity, these fungi grow on foodstuffs like maize, groundnuts, rice, figs, and other dried foods from contamination before, during, or after harvest. Aflatoxigenic fungi can also grow under long-term storage from heat and high humidity (Hell *et al*., 2010).

Apart from temperature and humidity, other pre-disposing conditions leading to fungal growth and the production of toxins may include poor soil fertility, monsoons, and unseasonal rains during harvest (Kamala *et al*., 2015). Besides, other factors like nitrogen stress that affects plant growth during pollination can also elevate the quantities of AFs produced by the *Aspergillus* (Wagacha & Muthomi, 2008).

2.4.2 Insect Pests and Biotic Stresses

Among the factors which promote the production of AFs is insect damage to plants (Kamala *et al*., 2015). Insects are the primary biotic stressors that influence fungal colonization and the production of toxins in maize. As reported by Widstrom *et al*. (2003), several studies have shown a positive correlation between ear-feeding insects and the presence of mycotoxins in kernels. This is probably because damaged grains are more prone to fungal contamination, and, therefore, toxin formation (Ostry *et al*., 2015). According to Kebede *et al*. (2014), insects feeding on developing kernels, and other biotic and abiotic stress facilitate fungal infection and production of AFs. The damage of kernels or ears by insects or birds creates portals for entry of aflatoxigenic fungi thus, insect pests and other biotic plant stresses like birds increase the vulnerability of crops to *Aspergillus* colonization and contamination with AFs in the field or during storage (Benkerroum, 2020). In this regard, controlling insect damage can reduce the risk of fungal infection and mycotoxin production (Coppock *et al*., 2018).

2.4.3 Harvesting and Post-Harvesting Practices

Aflatoxin contamination is an intricate process that starts in the field when fungi originating from plant debris and soil contaminate crops and continues as the crop grows to maturity and during storage, especially if subjected to a conducive environment for fungal growth (Probst *et al*., 2014)

This is especially because the *Aspergillus* grows over a broad temperature range (10 – 50ºC) (Dagenais & Keller, 2009). The contamination of crops with AFs before harvest has previously been linked to drought stress (Kebede *et al*., 2012; Klich, 1987). For instance, dry soil conditions that are associated with higher temperatures favor infestation by *Aspergillus* and the development of AFs in groundnuts before harvest (Negash, 2018). Normally, pre-harvest contamination of foodstuff with AFs provides the inoculum and subsequent contamination during storage (Craufurd *et al*., 2006). Poor harvesting practices, and substandard conditions during transportation, storage, processing, and marketing can also promote fungal growth and enhance mycotoxin production (Mahmoudi *et al*., 2013). Although cereals are usually harvested at elevated moisture content and dried to reduce the moisture content before storage, this delayed drying increases the risks of mold growth and the production of AFs. Besides, many smallholder farmers often store maize harvests under sub-optimal conditions for several months before use and/or sale, creating conducive conditions for fungal growth and contamination with AFs (Hell & Mutegi, 2011).

Post-harvest contamination of food commodities with AFs can occur when the prevailing conditions are conducive to the growth of aflatoxigenic fungi at harvest, or during transport, storage, and manufacturing (Coppock *et al*., 2018). *Aspergillus flavus* has comparatively high moisture requirements than other fungi, thus, grain contamination is elevated by high seed moisture contents. For instance, the occurrence of AFs in maize after harvesting is influenced by high temperatures and moisture contents in the ranges of 12 - 40℃ and 3-18% respectively (Negash, 2018). Contamination after harvest can occur due to delayed drying and during storage if water exceeds the critical values that support fungal growth (Waliyar *et al*., 2015). According to Coppock *et al*. (2018), delayed harvest due to wet conditions with adequate heat to support the growth of aflatoxigenic fungi can cause high contamination. Damage to kernels or nuts during harvesting, cleaning, and handling can also weaken seeds and promote fungal invasion and contamination (Coppock *et al*., 2018).

Post-harvest contamination can also occur during storage under conditions that favor mold growth, especially if moisture can exceed critical values (Chulze, 2010). In a recent study, the storage of grains after heavy rains increased spoilage and contamination (Pratiwi *et al*., 2015). Apart from the moisture content of grains, RH is another important variable during storage concerning fungal contamination (Wilson & Payne, 1994). In developing countries, AFs occur naturally in agricultural products particularly because the harvesting and storage methods are sub-standard (Negash, 2018). The duration of storage also counts. For instance, a significant correlation between the levels of AFs in maize after prolonged storage in agroecological zones with humidity in dry regions (Hell *et al*., 2000).

During storage, the production of AFs may increase when the equilibrium RH of agricultural commodities is between 90 and 99% (Benkerroum, 2020). This may occur if crops are not dried properly or kept in poorly ventilated areas with high RH (Benkerroum, 2020; Kaaya *et al*., 2006). According to Villers (2014), aflatoxigenic fungi thrive when RH surpasses 65% during storage. This is because temperature and a_w have a significant effect on *Aspergillus* growth and the expression of genes for biosynthesis of AFs (Abdel-Hadi *et al*., 2012; Bernáldez *et al*., 2017). Nevertheless, the minimum a_w for growth differs depending on nutrient availability and temperature. For instance, the minimum a_w for *A. flavus* growth was established to be 0.91 in sorghum (Lahouar *et al*., 2016), and 0.83 and 0.85 in rice (Mousa *et al*., 2011). A comparable range was observed in shelled peanuts where a lower growth rate was observed for *A. flavus* at a_w < 0.85 or temperature < 20°C, while higher growth occurred at an elevated a_w and around 28 – 40°C (Liu *et al*., 2017). Interestingly, *A. flavus* growth can occur over broader a_w and temperature ranges compared to the production of AFs (Abdel-Hadi *et al*., 2012; Liu *et al*., 2017). According to Abdel-Hadi *et al*. (2012), the optimum a_w and temperature levels for *A. flavus* were 0.99 a_w and 30 – 35°C while the optimum conditions for AFB_1 production were 30 – 35°C at 0.95 a_w, and 25 – 30°C at 0.99 a_w. In a previous study, optimum *A. parasiticus* growth occurred at 35°C. However, the production of AFB_1 and AFG_1 was optimum at > 37°C and 20 – 30°C, respectively (Schmidt-Heydt *et al*., 2010).

2.5 Measures to Prevent and Reduce Aflatoxin Contamination and Exposure

Since most aflatoxin contaminations occur before and after harvest, appropriate storage and handling of food crops at and after harvesting can be handy in preventing and reducing contamination with AFs and exposure. According to Udomkun *et al*. (2017), measures that can reduce the contamination of foods with AFs before and after harvesting can equally reduce exposure to these toxins. Several studies, for instance, in the Democratic Republic of Congo (Kavugho, 2019; Udomkun *et al*., 2018) and Tanzania (Kamala *et al*., 2018a) have confirmed that farmers in SSA employ various before and after harvest practices to control the contamination of foodstuffs with AFs. Some of these techniques are described in subsections 2.5.1 to 2.5.6 below.

2.5.1 Pre-Harvest Practices

Regarding pre-harvest practices for reducing aflatoxin contamination and dietary exposure, crop rotation is a common practice. According to a study by Mutegi *et al*. (2009), the continuous production of groundnuts on the same farm may lead to heavy fungal infestations and aflatoxin contamination. Subsequently, crop rotation with non-host crops can reduce the survival of fungal strains between seasons (Mutegi *et al*., 2012). In a study by Marete *et al*. (2020) in Kenya on the effects of various agricultural practices on levels of AFs in maize, crop rotation was found to be a significant factor. This confirms that crop rotation reduces mycotoxin contamination of crops by disrupting the cycles and accumulation of aflatoxigenic fungi (Marete *et al*., 2020). However, the intercropping of grains, especially wheat and maize should not be done since both are prone to contamination with aflatoxigenic fungi (Achaglinkame *et al*., 2017). Since insects also contribute to the contamination of crops with AFs by creating avenues of entry for fungi, spraying with insecticides in the field may reduce contamination incidences with, and thus exposure to AFs. In a study by Udoh *et al*. (2000) in Nigeria, farmers who reported insect problems in their storage facilities were established to be more likely to have AFs in their foodstuffs.

2.5.2 Sorting, Winnowing and Washing of the Grains

Some of the most common pre-storage post-harvest interventions involve decontamination, removal, and degradation of AFs by a variety of physical methods. According to Ayieni (2021), physical interventions like hand-sorting, cleaning/washing, and winnowing can effectively reduce mycotoxin exposures and contamination levels. For instance, sorting, which can be done by hand or floatation and density segregation, can remove existing aflatoxin contamination in contaminated kernels. The sorting process seeks to remove grains with substandard quality based on physical properties like size, colour, density, shape, and the noticeable identification of fungal growth. By removing discolored or damaged grains, sorting reduces the occurrence of AFs and contaminants in foods and feeds (Fandohan *et al*., 2005). According to Shakerardekani *et al*. (2012), AFs contamination in pistachio nuts can be reduced by more than 95% by color-based sorting. Xu *et al*. (2017) also established a > 90% reduction of aflatoxin contamination in peanuts in Rural Gambia by sorting. Zivoli *et al*. (2016) also established similar results in sorted apricot kernels. Nevertheless, sorting and other physical methods are labor-intensive and generally impractical on large scale, limiting their use. In yet another study in Kenya, Marete *et al*. (2020) established that farmers sorted shelled maize. Generally, sorting helps to reduce aflatoxin contamination since damaged seeds are more prone to infestation by aflatoxigenic fungi compared to good ones.

Another mechanism of sorting involves floatation whereby grains are immersed in water followed by the removal of bad ones. Due to the density differences, if contaminated and non-contaminated grains are immersed in water, the former float and are easily discarded alongside the AFs they contain (Fandohan *et al*., 2005).

2.5.3 Drying and Dehulling

Drying and dehulling can significantly prevent or reduce the contamination of foods/feeds with AFs. A previous community-based intervention in Guinea entailed the proper drying and storage of groundnuts in farming villages (Turner *et al*., 2005). The trial attained > 50% reduction in mean serum aflatoxin levels in the people. This demonstrates that simple and cheap postharvest techniques can significantly reduce exposure to AFs. These have also been confirmed to reduce the dietary exposure of infants to AFs in maize (Kamala *et al*., 2018a). Other traditional methods of reducing AF contamination of foods include milling, fermentation, and roasting to reduce water activity, fungal growth, and contamination as has been established by Olagunju *et al*. (2018) in Bambara groundnuts.

2.5.4 Use of Radiations and Ozone

Radiations have been shown to reduce the contamination of food with AFs in various studies. According to Jalili *et al*. (2010), gamma radiation can effectively prevent the contamination of food products with AFs. Unlike other physical prevention mechanisms, radiations reduce contamination by destroying the aflatoxigenic fungi. Several studies have reported the use of gamma radiations on decreasing AF contamination as outlined in Udomkun *et al*. (2017). Markov *et al*. (2015) also investigated the destruction of aflatoxin-producing fungi and the subsequent reduction of AFB_1 through gamma radiations and established that the growth, germination, and sporulation of the aflatoxigenic fungi could be prevented by gamma radiation. However, the efficiency of gamma-irradiation is dependent on several factors, like the type and number of fungal strains, dosage, food composition, and the prevailing humidity (Jalili *et al*., 2010; Kanapitsas *et al*., 2015). Furthermore, there are conflicting reports about the potential of gamma radiation for the mitigation of contamination of foods with AFs are rather inconsistent Udomkun *et al*. (2017). Reports are also present regarding the use of ultraviolet (UV) radiation as a non-thermal, affordable technology for the destruction of AFs in various foods. For instance, Atalla *et al*. (2004) established that AFB_1 and AFG_1 were removed in wheat grains after UV radiations were applied for 30 min, while AFB_2 was decreased by 50 - 74% following exposure to UV radiations.

The detoxification of foodstuffs using ozone has also been shown by some studies to reduce AFs in food commodities. Ozone is among the strongest oxidants and disinfecting agents (Udomkun *et al*., 2017). Ozone inhibits aflatoxigenic fungi by oxidizing important cellular components like amino acids and polyunsaturated fatty acids to smaller fragments. Besides, ozonation can degrade the cell envelope of unsaturated lipids to cause leakage (Daş *et al*., 2006). A study by de Alencar *et al*. (2012) on groundnuts established the detoxifying effects of ozonation regarding the reduction of contamination levels of AFB_1 and total AFs. Another study by Diao *et al*. (2013) also established that the levels of AFB_1 in groundnuts significantly reduced after ozonation.

2.5.5 Good Storage Practices

Since poor storage facilities can fuel the infestation of foodstuffs by insects and pests that damage grains and promote contamination by toxin-producing fungi, several post-harvest interventions also involve improving storage practices. According to Peraica *et al*. (2002), proper storage can deter mould growth, and prevent the contamination of foods with aflatoxigenic fungi. As such, optimal storage humidity, temperature, and moisture, levels can also decrease mould growth and prevent the production of AFs. For instance, reducing the moisture content of harvested foodstuffs can create unfavorable conditions for fungal growth and metabolism (Lanyasunya *et al*., 2005). Although the existing storage and processing practices in industrialized countries can prevent post-harvest contamination with AFs, the practices in developing countries are largely inadequate (Khlangwiset & Wu, 2010). Controlling contamination at storage is especially important because inappropriate storage conditions are likely to curtail other intervention strategies due to the increased accumulation of toxins during storage (Ayeni *et al*., 2021).

2.5.6 Dietary Interventions

Several dietary interventions can reduce the exposure of consumers to AFs and the associated health risks. Where feasible, the consumption of less groundnut and maize meals can significantly lower the exposure levels (Bandyopadhyay *et al*., 2007). Where it is difficult to make such dietary shifts, other dietary interventions may be required.

2.6 Aflatoxin Regulations

Aflatoxins are becoming a major barrier to the global trade of plant and animal products. Due to their various health effects, there are several international and national regulations on the Maximum Limits (MLs) in food products to protect human and animal health. According to Van-Egmond *et al*. (2007), most countries have not only established various regulations for levels of

AFs in foods/feeds but also limit the import of contaminated products to limit exposure to mycotoxins in general. The limits differ among countries and the regulatory values are mainly derived from available knowledge on toxicity and the potential existence of animal products (Negash, 2018). For instance, animal feed grains in the United States are allowed up to 300 µg/kg AFs (Wolde *et al*., 2018). The regulatory limits of AFs in some countries are shown in Table 3.

The regulation of AFs began back in the 1960s and now exists in about 100 countries worldwide covering approximately 90% of the global population (van Egmond & Jonker, 2004). Many countries now have specific regulations for AFB_1 or total AFs in food and agricultural products (Wu & Guclu, 2012). Of the four AFs, AFB_1 is not only the most toxic but also the most frequent (Negash, 2018). The European Union has set an ML of 0.10 µg/kg for AFB_1 in baby foods (European Commission, 2010). The common limits for AFB_1 and total AFs in foods are 5 and 15 µg /kg respectively, but more stringent regulations were established for peanuts, dried fruits, nuts, and cereals by the EU (European Commission, 2006).

The number of countries regulating AFs has significantly increased over the years. Internationally, the EU regulation, US Food and Drug Administration (FDA), and Codex Alimentarius Commission (CAC) have widely been accepted as the guidelines for establishing the MLs for AFs in foods and feeds. Due to the toxicity of AFs, their presence in food supplies is strictly regulated in developed countries (Masomo, 2020), but their regulation is still a challenge in developing nations like Tanzania where food supplies are already limited and legal measures may inflate food prices.

Table 3: Aflatoxin regulatory limits in different countries

Country/Organization	Type of Aflatoxin	Type of food	Maximum µg/kg
East African Community (EAC) countries including Tanzania, Kenya, Uganda, Burundi, Rwanda and South Sudan)	Total	Maize and peanut	10
	AFB1	Maize and peanut	5
European Union (EU)	AFB$_1$	Peanuts	8
	Total	Peanuts	15
	AFB$_1$	Peanut products	2
	Total	Peanut products	4
	AFB$_1$	Rice	2
	Total	Rice	4
FDA	Total	Peanuts	20
Codex	Total	Peanuts	15
China	AFB$_1$	Peanut/corn	20
Hong Kong	Total	Peanuts and peanut products	20
India	AFB$_1$	All food	30
Indonesia	Total	All food	35
	AFB$_1$	Peanut and corn	15
	Total	Peanut and corn	20
Japan	Total	All foods	10
South Korea	AFB$_1$	Grains, cereal products	10
Malaysia	Total	Raw peanuts	15
	Total	Peanut products	10
Morocco	AFM$_1$	Pasteurized milk	5
Nigeria	Total	Maize	4
Philippines	Total	All food	20
Singapore	Total	All food	5
Sri Lanka	Total	All food	30
Taiwan	Total	Peanut and corn	15
Thailand	Total	All food	20
Vietnam	Total	All food	10

Norlia *et al*. (2019), EAC (2011), and TBS (2014a)

By 2003, regulations on AFs existed only for five African countries (van Egmond & Jonker, 2004). According to Hell and Mutegi (2011), research on AFs should be intensified in SSA to inform policymakers on the need for increased implementation of interventions for food safety and security. This is because the accessibility of the toxicological data and information on analysis and sampling are the key aspects of the decision-making process of setting up the regulation limit (van Egmond & Jonker, 2004). The levels of AFs in food and food products still exceed international MLs in many African countries. For example, research by Assefa *et al*. (2012) from Northern Ethiopia on groundnut revealed that from the total samples analyzed, 83.9% were unsafe for direct human consumption as per the EU MLs and 46.6% were unfit for export to EU counties (as per the EU safe limit for import of groundnut); and based on the Food and Agriculture Organization of the United Nations (FAO) ML, 16.6% of the samples surpassed the 30 µg/kg limit. The average concentration for the total samples had 10 times greater than the recommended maximum level of AFs. Similarly, Fuffa & Urga (2001) also reported that in many parts of Africa human food staples exist which contain 10 to 30 times the recommended maximum limit.

The risks of human aflatoxicosis from the consumption of maize contaminated with AFs are still high in EAC despite the existing regulatory standards (Masomo, 2020). In Tanzania, this is partly because of the failure of regulatory authorities to deal with the widely scattered small-scale maize farmers who grow, consume, and sell their maize to the rest of the population (Abt, 2013). The regulations on AFs have massive effects on international trade, especially for developing countries like Tanzania. For instance, (FAO, 2002) reported that developing countries account for approximately 95% of the world's groundnut production, but cannot sell the groundnuts to the international market because of contamination with AFs (Akeberegn *et al*., 2018).

Peanut contamination with AFs is regulated in most countries since peanuts are naturally vulnerable to contamination with *Aspergillus* spp. Most countries have an ML of 4 to 20 µg/kg for total AFs in peanuts (Wu, 2013). The EU has the stringiest regulations which allow only 2 µg/kg and 8 µg/kg of AFB_1 in peanut products for direct human consumption and those intended for further processing, respectively (European Commission, 2010). A maximum level of 20 µg/kg of total AFs in peanuts has also been imposed by the FDA (2005) Most other countries regulate the total AFs in peanuts and peanut based-products to a maximum limit of 10 – 35 µg/kg except for Singapore where the limit is 5 µg/kg and Malaysia where the limit is 10 µg/kg and 15 µg/kg for total AFs in ready-to-eat peanuts and raw peanuts intended for further processing, respectively (Food Act 1983, 2014). The MLs set for AFs in peanuts by Codex is 15 g/kg (Codex, 1995) while the Tanzania Bureau of Standards (TBS) has set the maximum acceptable limit for peanuts at 10 µg/kg for total AFs and 5 µg/kg AFB_1 (EAC, 2011; Gheysens, 2015; TBS, 2014b).

The MLs of AFs in foods and products for human consumption range from 0.5 µg/kg in milk to 20 µg/kg for processed foods (USAID, 2012). To protect public health, the EU has set a maximum level of AFB_1 and total AFs (2 µg/kg and 4 µg/kg, respectively) in rice (European Commission, 2006), while the maximum levels of AFB_1 and total AFs were set at 5 µg/kg and 10 µg/kg, respectively in rice (European Commission, 2010). A comparable regulatory limit for total AFs has been reported in Brazil (30 μg/kg), India (30 μg/kg), Mexico (20 μg/kg), Canada (15 μg/kg), USA (20 μg/kg), Taiwan (10 μg/kg), and 10 μg/kg for AFB_1 in Japan, Korea and China (FAO, 2004). The least regulatory limit for AFB_1 of 1 μg/kg (in cereals and beans) has been reported in Bosnia and Herzegovina (Jager *et al*. 2011), and (in all foods) in Switzerland (Creppy, 2002).

Due to the carcinogenic nature of AFs, tolerable daily intake (TDI) cannot be considered a safety factor, and human exposure should be reduced as much as possible (Aydin *et al*., 2011). For a toxin where adverse effects show a threshold, a TDI is established. However, for AFs, where carcinogenicity is the basis of concern, TDIs are not applicable (EFSA, 2020).

2.7 Occurrence of Aflatoxins in Common Food Commodities around the World

Many food substrates have been shown to support the growth of aflatoxigenic molds and the subsequent formation of AFs all over the world. According to Coppock *et al*. (2018), almost all food or feedstuffs can support the growth of aflatoxigenic fungi and the production of AFs. Previous estimates show that 25 – 50% of the world's crops are contaminated with AFs (Muthomi *et al*., 2012). A most recent study indicated that more than 60−80% of the world's cereal grains are contaminated with mycotoxins (Eskola *et al*., 2020). Cereals like maize, millet, sorghum, wheat, and rice, and cereal-based products which are the major human foods worldwide form the bulk of this estimate (Temba *et al*., 2017). Apart from cereals, oilseeds like sunflower, groundnut, soybean, black pepper, chilies, turmeric, and tree nuts such as walnuts, pistachio, almonds, and coconuts are also affected by AFs (Weidenbörner, 2013).

A summary of the foods which have shown contamination with AFs worldwide is provided in Table 4.

Table 4: **Occurrence of aflatoxins in foods and feeds around the world from 2005 to 2021**

Country	Food matrix	Aflatoxin	Range or mean (µg/kg/l)	Detection technique	References
Turkey	Maize Flour	AFB_1	0.041-1.12	HPLC	Kara *et al*. (2015)
	Almond	AFB_1	1-13	TLC	Gürses (2006)
	Butter	AFM_1	< 0.001-0.100	ELISA	Aycicek *et al*. (2005)
	Infant formula	AFM_1	< 0.03-0.02	HPLC	Torović (2015)
	Lentil	AFB_1	0.57-7.14	HPLC	Baydan *et al*. (2016)
	Cheese	AFM_1	0.1-0.70	ELISA	Yaroglu *et al*. (2005)
	Breast milk	AFM_1	0.00366	ELISA	Yalçin *et al*. (2020)
	Figs	Total	0.1-28.20	HPLC	Kabak (2016)
	Red-chili powder	AFB_1	0.025-40.90	ELISA	Giray *et al*. (2007)
Brazil	Cashew nuts	Total	0.60-31.50	ELISA	Milhome *et al*. (2014)
	Milk (cow)	AFM_1	0.17-2.59	HPLC	Bahrami *et al*. (2016)
United States	Chilies	AFB_1	2-94.9	ELISA and TLC	Singh and Cotty (2017)
Costa Rica	Corn	Total	0.48–- 500	ELISA and HPLC	Granados-Chinchilla *et al*. (2017)
Zimbabwe	Corn	AFB_1	0.75-26.6	HPLC	Murashiki *et al*. (2017)
India	Corn	AFB_1	48-383	HPLC	Mudili *et al*. (2014)
Serbia	Corn	Total	1.01-86.10	ELISA	Kos *et al*. (2013)
Vietnam	Corn	AFB_1	1.0-38.40	ELISA	Chu *et al*. (2017)
Uganda	Peanuts	Total	7.3 – 12.4	Fluorimeter	Kaaya *et al*. (2006)
	Poultry feeds	Total	7.5 – 393.5	Fluorimeter	Nakavuma *et al*. (2020)
	Maize and Peanuts	AFB_1	9.1-20.2	ELISA	Wacoo *et al*. (2020)
Kenya	Peanuts	Total	4-7525	ELISA	Mutegi *et al*. (2009)
	Milk	AFM_1	0.00 – 0.69	ELISA	Langat *et al*. (2016)
	Cereals and groundnuts	Total	0.3-740	HPLC	Awuor *et al*. (2021)
Nigeria	Ginger	Total	0.11-9.52	HPLC	Lippolis *et al*. (2017)
	Nuts	Total	0.3-20	ELISA	Tor *et al*. (2020)
	Cereals and Cassavas	Total	1.75 – 173.3	ELISA	Ekpakpale *et al*. (2021)
Ethiopia	Groundnuts	Total	15-11.900	HPLC	Chala *et al*. (2013)
Côte d'Ivoire	Maize	AFB_1 Total	0.79 – 130.31 2.63 – 169.13	HPLC	Bamba *et al*. (2021)
Serbia	Hazel nut	AFB_1	0.07-43.60	HPLC	Baltaci *et al*. (2012)
Greece	Milk Products	Total	0.47-2.10	ELISA	Abd-Elghany and Sallam (2015)
Saudi Arabia	Nuts	Total	0.2-513.40	HPLC	Kumagai *et al*. (2008)
South Korea	Soybean paste	Total	0.01–281.92	HPLC	Lee *et al*. (2022)
Burkina Faso	Peanut oils	Total	0.17-35.33	HPLC	Zio *et al*. (2020)
Togo	Maize	AFB_1	1-1-75.9	HPLC	Hanvi *et al*. (2021)
Jordan	Wheat and corn	Total	1.14–- 4.12	HPLC and ELISA	Omar *et al*. (2020)
Iran	Milk	AFM_1	< 0.005-0.07	HPLC	Tsakiris *et al*. (2013)
	Yogurt	AFM_1	0.006-0.021	HPLC	Bahrami *et al*. (2016)
	Bean	AFB_1	0.113-0.351	DLLME and HPLC	Asadi (2020)
	Milk (Cow)	AFM_1	0.061 ± 0.008	HPLC	Hajmohammadi *et al*. (2020)

Country	Food matrix	Aflatoxin	Range or mean (µg/kg/l)	Detection technique	References
	Spices	Total	0.6–21.3	HPLC	Zareshahrabadi *et al*. (2021)
Italy	Milk (cow)	AFM$_1$	1.0-110	HPLC	Picinin *et al*. (2013)
	Spices	AFB$_1$	0.59-5.38	HPLC	Prelle *et al*. (2014)
Portugal	Milk (cow/buffalo)	AFM$_1$	0.1-40.60	ELISA	De Roma *et al*. (2017)
Japan	Milk (cow)	AFM$_1$	0.015-46.60	HPLC/HPTLC	Duarte *et al*. (2013)
Malawi	Nut-based foods	AFB$_1$	0.1-40.60	HPLC	Matumba *et al*. (2014)
	Groundnut products	Total	13-670	ELISA	Magamba *et al*. (2017)
	Maize	B$_1$, B$_2$, G$_1$, G$_2$	5.7–- 44.7	LC-MS/MS and HPLC	Matumba *et al*. (2015)
	Groundnuts	AFB$_1$	4-20	ELISA	Monyo *et al*. (2012)
	Maize	Total	2.13-33.37	HPLC	Jere *et al*. (2020)
Zambia	Peanuts	AFB$_1$	0.015-46.60	HPLC	Bumbangi *et al*. (2016)
Taiwan	Peanut products	Total	0.2-513.40	HPLC	Chen *et al*. (2013)
China	Rice	AFB$_1$	0.03-20	HPLC	Lai *et al*. (2015)
	Peanuts	Total	3.93-37.63	HPLC	Qin *et al*. (2021)
	Rice	AFB$_1$	0.1-136.80	HPLC	Sun *et al*. (2011)
India	Rice	AFB$_1$	0.1-308	ELISA	Reddy *et al*. (2009)
	Corn	Total	13.49-53.2	LC-MS	Sailaja *et al*. (2021)
Sudan	Edible oils	AFB$_1$, AFB$_2$, AFG$_1$, AFG$_2$	0.2 – 0.8	HPLC	Idris *et al*. (2010)
Pakistan	Rice	AFB$_1$	0.04-21.30	HPLC	Iram *et al*. (2016)
	Milk	AFM$_1$	0.252	ELISA	Sadia *et al*. (2012)
	Dried fruits	AFB$_1$	0.04-9.80	HPLC	Masood *et al*. (2015)
	Peanut oils	Total	0.12–- 55	TLC	Hussain *et al*. (2021)
Malaysia	Spices	AFB$_1$	0.58-4.64	ELISA	Reddy *et al*. (2011)
	Wheat	AFB$_1$	0.55-5.07	ELISA	Reddy *et al*. (2011)
Tunisia	Sorghum	AFB$_1$	0.4-25.1	HPLC	Ghali *et al*. (2010)
	Wheat	AFB$_1$	0.12-18	HPLC	Ghali *et al*. (2010)
Ghana	Maize	Total	0.78 - 339.3	HPLC	Kortei *et al*. (2021a)
	Groundnuts	Total	0.38–- 230.21	HPLC	Kortei *et al*. (2021b)

TLC = Thin Layer Chromatography, ELISA = Enzyme Linked Immunosorbent Assay, HPLC = High Performance Liquid Chromatography, LC-MS = Liquid chromatography–mass spectrometry, DLLME = Dispersive liquid–liquid microextraction, HPTLC = High-performance thin-layer chromatography

Maize is one of the most vulnerable cereals to AFs. According to Kpodo *et al*. (2000), maize is a suitable substrate for mold growth and the production of mycotoxins either pre-harvest or post-harvest (Chulze, 2010). Apart from maize, *Aspergillus* can also colonize other grains and cereals during pre-harvesting, or harvesting/transport/storage (Kader & Hussein, 2009). For instance, crops like wheat and barley are commonly contaminated with AFs due to inappropriate storage. Nevertheless, the extent of fungal growth and production of AFs in cereals depends on soil type, temperature, moisture, and storage conditions (Achaglinkame *et al*., 2017). Aflatoxins can also be present in milk and/or milk products like cheese, yogurt, and butter (Ketney *et al*., 2017; Lindahl *et al.*, 2018; Tadesse *et al.*, 2020; Xiong *et al.*, 2020) as AFM_1 which is the hydroxylated metabolite of AFB_1 (Creppy, 2002).

AFM1 is secreted in milk after the consumption of AFB_1-contaminated feeds (Fallah *et al.*, 2009; Škrbić *et al.*, 2014). The exposure of humans to AFM_1, therefore, occurs from the consumption of contaminated milk and milk products (Langat *et al.*, 2016). In this regard, infants are the most vulnerable to these toxins due to the high consumption of milk and milk products (Sadia *et al.*, 2012). Apart from milk and milk products, AFs have also been associated with other animal products like eggs, meat, and meat products (Awuchi *et al.*, 2020; Peles *et al.*, 2019; Tarus *et al.*, 2019; Wang *et al.*, 2018). This way, the toxins infect humans through the consumption of contaminated animal products (Bennett & Klich, 2003). Many other agro-food products have also been documented to be potentially contaminated with different types of AFs (Hammami *et al.*, 2014; Leong *et al.*, 2010; Rubert *et al.*, 2011).

Aflatoxins can contaminate crops in the field or even post-harvesting (Kumar *et al.*, 2008). Exposure to these toxins poses serious health hazards to humans (Umoh *et al.*, 2011). However, the susceptibility of food commodities to infection by aflatoxigenic fungi is extremely variable. For instance, food commodities such as corn, peanuts, and coconut are highly susceptible to contamination by AFs (Idris *et al.*, 2010), while oats, wheat, rice, millet, soybeans, barley, cassava, beans, sorghum, pulses, and other agricultural products are seldom contaminated (Bankole *et al.*, 2010).

2.8 Dietary Exposure of Infants and Young Children to Aflatoxins through Complementary Feeding

Complementary feeding is the introduction of solid and/or liquid foods into the diets of IYC in addition to breast milk when breast milk can no longer meet their nutritional needs (Burdette *et al.*, 2006; WHO/PAHO, 2003). Although breastfeeding may continue beyond the second year of life, the target age for complementary feeding is normally 6 - 24 months (Dewey & Adu-Afarwuah, 2008). Several reports around the world show that IYC are continually exposed to AFs through complementary feeding.

Table 5: Studies portraying exposure of infants and young children to aflatoxins worldwide

Country	Food ingredient	Exposure (ng/kg. bw/day)	References
Togo	Maize	700.8–701.2	Hanvi *et al*. (2021)
Nigeria	Cereal and Peanut butter	40.5–54 892	Ojuri *et al*. (2019)
Nigeria	Cereal and nut-based	25.7–54 892	Ojuri *et al*. (2018)
Kenya	Maize	0.011-0.49	Kang'ethe *et al*. (2017)
Nigeria	Maize	763.6- 1909.1	Adetunji *et al*. (2017)
Serbia	Several	0.79–1.10 and 1.20–1.66	Udovicki *et al*. (2021)
Kenya	Dairy foods	0.8-46	Ahlberg *et al*. (2018)
Colombia	Rice and Bread	>0.017	Martinez-Miranda *et al*. (2019)
Iran	Commercial cereal-based	5.81 -8.55	Bashiry *et al*. (2021)
Vietnam	Rice, Wheat, Legumes	104.9- 124.2	Huong *et al*. (2019)
Ghana	Maize	50 – 1150	Kortei *et al*. (2021)
Chile	Dairy consumption	0.07	Foerster *et al*. (2020)
Ghana	Cereals and cereal-based foods	3.9×10^{-3} -0.899	Kortei *et al*. (2019)
Ghana	Groundnut	0.014 – 0.55	Omari & Anyebuno (2020)

The exposure of IYC to AFs can occur in the uterus via the transfer of AFs from the mother to the fetus through the placenta (Watson *et al*., 2017) or breast feeding (Magoha *et al*., 2014), or direct from foods consumed (Gong *et al.*, 2003, 2016). Complementary foods still contribute the bulk of exposure of IYC to AFs worldwide. Although some studies, for instance, Ortz *et al*. (2018) in Ecuadorian highlands, Gummadidala *et al*. (2019) in India, and Foerster *et al*. (2020) in Chile have shown the absence of common AFs in CFs and/or modest exposure of IYC to AFs through complementary feeding, a lot of other studies have shown different results. A very recent survey and meta-analysis by Bashiry *et al*. (2021) show that about 75% of babies aged 2 years in African and European countries consume foods that contain high levels of AFs which put them at high risk of exposure.

In France, Vin *et al*. (2020) established that French children are also exposed to several mycotoxins, among them, AFs, and concluded that appropriate efforts should be put in place to decrease the exposure of infants to the toxic molecules. Similar results have also been obtained in Portuguese by Assunção *et al*. (2018) where 95% of cereal-based foods were established to contain mycotoxins and the levels of exposure to AFs suggested potential adverse health effects. A recent study in Spain has also established the presence of AFs in 20% of cereal-based foods meant for infant consumption, some of which surpassed the EU ML for AFB_1 of 0.10 µg/kg.

People living in tropical and sub-tropical countries are supposedly exposed to high levels of AFs (Kiarie *et al*., 2016). A fairly recent review by Achaglinkame *et al*. (2017) on the contamination of cereals and legumes used as ingredients of CFs for Ghanaian infants with AFs demonstrated that feeding on contaminated food ingredients exposed the IYC to poor growth and development. An earlier study in Ghana by Blankson & Mill-Robertson (2016) also established aflatoxin contamination and exposure of IYC through processed cereal-based CFs. The study demonstrated that about 70% of the processed foods meant for infant consumption had AFB_1 (0.18 - 36.1 µg/kg) and estimated the aflatoxin intake for the infants to be 0.005 to 0.838 µg/kg bw/d. Other studies that have evidenced the dietary exposure of IYC to AFs through complementary feeding are summarized in Table 5.

Analysis of the aflatoxin levels of traditional CFs fed to 6-24 month-old children in southern Zambia recently established that maize-based porridge samples were the most contaminated with mean aflatoxin levels of 5.8 ± 15.93 mg/100 g (Alamu *et al*., 2018). In Ethiopia, over 90% of food samples used for the preparation of CFs were shown to contain 2.3-88 µg/kg AFs. In another study conducted in Ethiopia, the exposure of children to different types of AFs was also shown to be high (Tessema *et al*., 2021). Most of these studies have established extremely high levels of AFs in CFs that put the IYC at a high risk of exposure. For instance, the study by Ojuti *et al*. in Nigeria showed high exposures to several mycotoxins, among them, AFs that surpassed the established reference standards multi-fold, indicating an important source of health concern. The study by Kortei *et al*. (2021) on the exposure and risk assessment of AFs through the consumption of maize in different parts of Ghana also established that 0ver 50% of the studied samples had AFs that exceeded the limits of EFSA and Ghana Standards Authority. A number of these studies have also established that the high levels of exposure of IYC to AFs result from the over-reliance on maize and nut-based CFs which are extremely susceptible to contamination with aflatoxigenic fungi.

Rather than quantifying exposure in terms of dietary intake and levels of contamination in CFs, some studies have also quantified exposure of IYC to AFs in terms of urinary biomarkers (Abebe, 2017; Ayelign *et al*., 2017; Jolly *et al*., 2021) and blood samples (Gong *et al*., 2003). According to Schwartzbord *et al*. (2016), urinary biomarkers are used to detect the existence of AFM_1 in urine samples which indicates the contamination of diets meant for IYC with AFB_1.

2.9 Occurrence of Aflatoxins in Common Food Commodities in Tanzania

Like other parts of SSA, AFs are common in Tanzania and several studies have documented their presence in major food crops in the country (Table 6). The incidences are reported in almost all parts and agroecological zones of the country.

For instance, Kimanya *et al*. (2008) established that 52% of maize samples in four agro-climatic regions of Tanzania had up to 6125 µg/kg (median, 206 µg/kg) contamination with AFB_1 while the levels of contamination ranged from 5 – 90 µg/kg (median, 38 µg/kg) in 12 % of the samples. Similarly, Nyangi *et al*. (2016a) reported that some maize products meant for consumption by humans and animals in Babati in Northern Tanzania contained AFs at levels above the East Africa Community (EAC) MLs.

Several factors have been associated with the contamination of crops with AFs in Tanzania. In a study by Mohamed (2017) on the contamination of maize with AFs, the factors associated with higher post-harvest contamination were the duration and type of storage, sorting, store treatment, and crop treatment. The occurrence of AFs may also differ according to agroecological regions. Similarly, unpublished studies in Tanzania show that the contamination of foods with AFs differs significantly among agroecological regions (Kamala *et al*., 2015; Mtega *et al*., 2020).

It is hypothesized that the expression of *aflR*, *aflS,* and *aflD* genes which are involved in the biosynthesis of AFs is significantly subject to temperature and water availability (Benkerroum, 2020; Dooso Oloo *et al*., 2019).

Table 6: Occurrence of aflatoxins in common food commodities in Tanzania

Type	**Food/crop**	**Range/Mean (µg/kg)**	**Stage**	**Place**	**Reference**
AFB_1	Bambara nut, Groundnut, Maize, Sorghum, Sunflower	28.6-116	Pre-harvest and post-harvest	Njoro (Kitelo district) Chitego, Mlali, Moleti, and Laikala (Kongwa district)	Anitha *et al*. (2017)
Total	Maize	2.3-70.5	Harvest and storage	Dodoma (Kongwa district)	Sasamalo *et al*. (2018)
AFB_1	Peanut-enriched composite flour	1.24 to 60.64	Processing	Dodoma, Arusha, Iringa, Dar es Salaam, Morogoro and Kilimanjaro	Kuhumba *et al*. (2018)
Total	Maize and maize products	9.99 ± 1.43	Field, storage	Kongwa and Njombe districts	Mtega *et al*. (2020)
AFB_1, AFG_1, AFB_2 and AFG_2	maize, sorghum, groundnuts, millet, composite flour (millet and maize) and rice	10-51,100	Stored	Dodoma and Manyaraa	Kamala *et al*. (2018b)
B_1, B_2, G_1, G_2 and total AFs	Maize-based complementary flour	0.24-1.39	Post-harvest	Sokoni, Matitu, Miembeni, Nyerere, Lugongo, and Mbuyuni Villages in Bahi	Aron *et al*. (2017)
Total	Maize	13.12 - 19.39	Harvest and storage	Kongwa district	Mohamed (2017)
Total	Maize	2.94-26.2	Pre-harvest	Babati district	Nyangi *et al*. (2016a)
Total	Maize	2.1–16.2	Marketing and Processing	Babati district	Nyangi *et al*. (2016b)
AFM_1 and AFB_1	Milk and feeds	0.026 – 0.364	Processing	Singida	Mohammed *et al*. (2016)

Type	Food/crop	Range/Mean (µg/kg)	Stage	Place	Reference
Total	Maize	1-158	Stored	Iringa, Ruvuma, Kilimanjaro and Tabora	Kimanya *et al*. (2008)
Total	Maize, groundnuts	123 - 136	Storage	Kilosa District	Magembe *et al*. (2016)

Thus, in regions where highly toxigenic *Aspergillus* strains exist, ecological stresses may intensify the production of AFs. Central Tanzania receives low rainfall and is generally warm and dry. Therefore, crops there are more vulnerable to drought stress and more susceptible to fungal attacks (Gheysens, 2015). Some studies in Tanzania have established that the occurrence of AFs in major food supplies is within the EAC standard of 10 μg/kg for total AFs. For instance, the study on pre-harvest maize contamination with AFs in Babar district in Northern Tanzania recently established a mean of 2.94 μg/kg which is within the EAC standard of 10 μg/kg (Nyangi *et al*., 2016a).

2.10 Complementary Foods in Tanzania

The traditional CFs in Tanzania are based on starchy staples and cereals like maize, rice, sorghum, finger millet, or non-cereals like cassava, sweet potato, yams, round potato, and green bananas (Mosha, 2004). In this thesis, the emphasis is placed on the major cereals like maize, sorghum, millet, and finger millet together with groundnuts. These are discussed in more detail in the subsections below:

2.10.1 Maize

Maize is an important cereal crop that is cultivated widely in various agroecological regions and consumed by many people in SSA (Macauley & Ramadjita, 2015). Maize is grown for food in all regions of Tanzania (Ismail *et al*., 2015; Nyaruhucha *et al*., 2006) where the national consumption is approximated to be > 3 million metric tons annually, and the daily per-capita consumption at 450 g (Smith & Subandoro, 2007). The crop contains high concentrations of energy and carbohydrates (Mamiro *et al*., 2005), and is used as an ingredient for CFs (Kuhumba *et al*., 2018). In numerous parts of Tanzania, maize forms the main proportion of cereals CFs alongside other cereals like rice, sorghum, and finger millet (Gheysens, 2015).

Like many parts of developing countries, maize in Tanzania is most often contaminated with AFs (Kamala *et al*., 2015; Mtega *et al*., 2020; Nyangi *et al*., 2016a). The toxins can contaminate maize in the fields or during storage, thus making the grains unsafe for consumption. Outbreaks of aflatoxicosis and associated mortality have been reported in Eastern Africa including Tanzania in 2016 (Kamala *et al*., 2018b), and studies and the presence of AFs in maize-based CFs in Tanzania (Aron *et al*., 2017). Studies in Tanzania have similarly reported high exposure in IYC to AFs through maize-based diets (Chen *et al*., 2018; Modest, 2017; Shirima *et al*., 2013). This is a source of public concern because the daily consumption of foods contaminated with low levels of AFs can cause chronic exposure associated with impaired growth, immune suppression, reduced life expectancy, and cancer (Tola & Kebede, 2016; Wild & Gong, 2010).

2.10.2 Groundnuts/Peanuts

Peanuts or groundnuts are the main crops in Tanzania with various nutritional and economic benefits (Kuhumba *et al*., 2018). The various nutritional aspects of groundnuts are provided in Table 7. They are nutritious sources of carbohydrates, lipids, proteins, minerals, vitamins, and fibre which are important to human health and nutritional needs (Arya *et al*., 2016; Settaluri *et al*., 2012).

Table 7: Nutritional aspects of groundnuts per 100 g

Nutrient	Nutrient value	Percentage of RDA
Protein	25.80 g	46
Carbohydrates	16.13 g	12
Energy	567 Kcal	29
Dietary Fiber	8.5 g	22
Total fat	49.24 g	165
Vitamins		
Thiamin	0.640 mg	53
Pyridoxine	0.348 mg	27
Niacin	12.066 mg	75
Riboflavin	0.135 mg	10
Folates	240 µg	60
Pantothenic acid	1.767 mg	35
Vitamin E	8.33 mg	55.5
Electrolytes		
Sodium	18 mg	1
Potassium	705 mg	15
Minerals		
Calcium	92 mg	9
Selenium	7.2 µg	13
Magnesium	168 mg	42
Copper	1.144 mg	127
Manganese	1.934 mg	84
Iron	4.58 mg	57
Phosphorus	76 mg	54
Zinc	3.27 mg	30

Arya *et al*. (2016)

The nuts also have all the essential amino acids like lysine, tryptophan, leucine, threonine, isoleucine, phenylalanine, methionine, valine, histidine, and tyrosine which makes them a significant component of human diets, especially in communities where animal proteins are not affordable or readily available (Arya *et al*., 2016; Settaluri *et al*., 2012). Being plant-based, most of the fat in peanut protein is unsaturated and the fibre is a complex carbohydrate which makes them the best form of human nutrition (Arya *et al*., 2016). Since peanuts are legumes, their protein contents are higher protein relative to other nuts (Arya *et al*., 2016).

Peanut-enriched flour is a common infant weaning food in Tanzania owing to its high protein content (Kuhumba *et al*., 2018). The use of groundnuts as an ingredient of CFs is especially advocated for because of their availability and culturally accepted as a source of fat and protein and it is also tasty (Lymo & Muzanila, 2014). Despite these nutritional benefits, there have been conflicting opinions about their use in infant feeding especially because of its vulnerability to contamination by AFs as has been established by various studies in many African countries (Anitha *et al*., 2017; Kuhumba *et al*., 2018; Magamba *et al*., 2017; Monyo *et al*., 2012; Mupunga *et al*., 2014).

The high nutritive value of peanuts makes them a conducive substrate for fungal growth and production of AFs (Mupunga *et al*., 2017). The humid and hot climates which are common in tropical countries like Tanzania also promote fungal growth which contaminates the crop in the field and/or during storage (IARC, 2002). Peanuts can also get contaminated with AFs if not dried immediately to reduce post-harvest moisture levels. Inadequately-dried peanuts can favor the growth of aflatoxigenic fungi during storage (Dorner, 2008), which is very challenging because peanuts are naturally hygroscopic and can absorb moisture during storage (Waliyar *et al*., 2015). Besides the risk of contamination with AFs, the high-fat content in peanuts also causes rancidity and shortens the shelf life of groundnut flour (Cämmerer & Kroh, 2009).

2.10.3 Rice

Rice is an essential dietary staple that is mostly consumed as a significant part of the human diet worldwide (Sales & Yoshizawa, 2005). In developing countries, rice forms 20% of dietary protein intake (Ok *et al*., 2014). Rice is the main food source for much of the world's population. Its protein content is 5 - 7%, which is less than that of most other cereals (Gheysens, 2015). The crop is generally cultivated in sub-tropical environments with humid and hot climates that encourage fungal growth and production of AFs (Ali, 2019; Aydin *et al*., 2011; Gheysens, 2015). Rice can be contaminated by aflatoxigenic fungi under favorable climatic conditions in the field, or during harvesting, and storage (Park *et al*., 2005). The contamination of rice with AFs has been reported in several parts of Tanzania (Chen *et al*., 2018; Routledge *et al*., 2014). Rice is generally cultivated in paddy fields with high moisture levels that favor the growth of molds and subsequent contamination with mycotoxins such as AFs as has been reported in other countries (Majeed *et al*., 2018; Sales & Yoshizawa, 2005). Aflatoxigenic fungi can grow on rice during floods and heavy rainfalls during harvest and storage. Inadequate drying and inappropriate storage can also make rice prone to fungal contamination (Majeed *et al*., 2018).

Although the contamination levels in rice in Tanzania are low and rarely reported, there are indeed a few reports on the same. For example, Kimanya *et al*. (2016) state that the aflatoxin contamination levels in the 101 rice samples from the three main rice-producing districts of Tanzania (Kilosa, Morogoro, Mbarari, Mbeya, and Misungwi, Shinyanga), were ranged from 0.01-3.83 µg/kg. Kuhumba *et al*. (2018) also report that Rice used to make complementary flours in urban markets in Tanzania had a median level of 1.82 µg/kg of total AFs. Nevertheless, the contamination of rice is normally lower than in maize or wheat

2.10.4 Sorghum

Sorghum is among the most important staple foods in Africa (Elbashir & Ali, 2014). In Tanzania, sorghum ranks fourth after maize, rice, and wheat with average yielding of < 1 ton/ha. It is the basic food in the central zone (Msongaleli *et al*., 2017), and is seldom contaminated with AFs (Bankole *et al*., 2010). However, sorghum is low in essential amino acids like lysine and threonine (Gheysens, 2015), and is not generally recommended for consumption by small children majorly because of insufficient energy and digestibility (Friedman, 1996). Sorghum also contains some antinutritional constituents such as phytic acid (101 g/kg protein) and tannins (up to 79 g/kg) which can inhibit iron absorption in infants (Gilani *et al*., 2012).

2.10.5 Millet and Finger Millet

Millets are considered superior cereals owing to their high concentration of proteins, minerals, and fats. According to Bankole *et al*. (2010), millets are also seldom contaminated with AFs, however, the presence of various anti-nutrients like tannins (up to 72 g/kg) (Gilani *et al*., 2012), poor digestibility, and low palatability largely affect its utilization (Kaur *et al*., 2014). According to Sirma *et al*. (2016), millet can also be contaminated with AFs.

Although previously unfashionable, finger-millet is slowly gaining popularity. The nutritional characteristics of finger-millet-based CFs have extensively been studied by Mwikya *et al*. (2002). According to Rao and Deosthale (1983), the micronutrient density of finger millet is higher than that of rice and wheat. Finger millet is exceptionally rich in calcium (344 %) relative to all other cereals and has 283% phosphorus, 3.9% iron, and many other trace elements and vitamins (Shobana *et al*., 2013). Besides, finger millet is an essential source of several phenolic compounds which have certain health benefits (Dykes & Rooney, 2007).

Finger millet is the cereal of choice for the preparation of children's porridge in many parts of Africa. It is more palatable and has a greater mineral content than other cereals like rice and wheat (Mwikya *et al*., 2002; Saleh *et al*., 2013). Although the 8 - 11% total protein content of finger millet is comparable to that of other cereals, it is limiting in lysine but has sulfur-containing amino acids at levels equal to that of milk protein (Mwikya *et al*., 2000). According to Tatala *et al*. (2007), there occurs marked improvement in the level of hemoglobin in children fed on finger millet. Besides, studies on the contamination of finger millets with AFs are limited, indicating that they might be less susceptible to fungal infection.

2.10.6 Common Beans

Common beans are the world's most vital source of food (Namugwanya *et al*., 2014). They are important for nutrition security and are considered a cheap option for improving the diets of resource-poor consumers in developing countries. Tanzania has been one of the major producers of common beans in East Africa where over 75% of rural households depend on the crop for subsistence (Kalyebara & Buruchara, 2008). Although there are several reports concerning the contamination of beans with aflatoxigenic fungi (Costa & Scussel, 2002; Marcenaro, 2018; Marcenaro & Valkonen, 2016), it is important to note that the detection of toxigenic fungi in food does not necessarily translate to contamination with mycotoxins, especially if the fungi are not exposed to conditions that promote the production of secondary metabolites. However, the presence of toxigenic fungi indicates potential risks of mycotoxin contamination. Besides, some studies have confirmed the presence of AFs in beans (Buruchara *et al*., 2011).

2.10.7 Soybeans

Soybean is an annual crop that is popular in infant foods because of its abundance and nutritional qualities. According to Martin *et al*. (2010), soybean is an abundant and economical source of protein that is cheaper than animal proteins and contains all essential amino acids. Despite its nutritional benefits, soybean requires careful home processing to reduce anti-nutritional factors, undesirable flavor, bitterness, toxic proteins, haemagglutinins (Martin *et al*., 2010). Although it is being used as a complementary food in Tanzania, one of the challenges of using soybean is the presence of phytates and trypsin inhibitors which need to be removed to improve its nutritional contribution to the diet (Martin *et al*., 2010). This anti-nutritional content inhibits the bioavailability of nutrients like iron, zinc, calcium, and proteins which are critical for infant development (Gibson *et al*., 2010). Besides, low levels of AFs have been reported in soybeans (Sobolev & Dorner, 2002).

2.10.8 Composite Flour

Composite flours are often a blend of several cereals and/or legume seeds in the ratio of 70:30 for use in making thin porridge as is common practice in several communities in Tanzania (Dewey & Adu-Afarwuah, 2008). Peanut-enriched flour is one of the most common CFs for IYC in Tanzania because of its high protein content (Kuhumba *et al*., 2018). Peanuts are often incorporated to increase the proportion of proteins in the flours. According to the United States Department of Agriculture (2015), peanuts are a cheap and rich source of protein (25.8 g of protein/100 g) and energy (567 kcal/100 g). However, peanuts are also associated with food safety risks (Kuhumba *et al*., 2018) due to their susceptibility to contamination with AFs in developing countries (Chang *et al*., 2013). In Tanzania, previous surveillance indicated that the contamination of peanuts with AFs ranged from 10.3 to 40.3 g/kg (Abt, 2013), which is more than acceptable levels (Kuhumba *et al*., 2018).

Kuhumba *et al.* (2018) also established the presence of AFs B_1, B_2, G_1, and G_2 in all samples of peanut-enriched complementary flour (n = 65) from six regions of Tanzania (Arusha, Dar es Salaam, Dodoma, Iringa, Kilimanjaro, and Morogoro). The study also reported that 71% of the studied samples had total aflatoxins above the acceptable levels of 10 μg/kg. Apart from peanuts, the studied complementary flours also contained other cereals and food products; 94% contained both finger millet and maize, 88% contained soybeans, 82% contained rice, 53%296 contained wheat, and 29% contained both sorghum and dried vegetables.

2.11 Complementary Feeding of Infants and Young Children in Tanzania

About 42% of Tanzanian children < 6 months are fed on CFs, contrary to WHO recommendations. According to Shirima *et al*. (2001), the introduction of solid foods to infants in Tanzania starts when they are 1 – 8 months old in rural and 2 – 6 months in urban areas. However, other studies have established that children as young as two months old are introduced to maize-porridge mixed with milk (Kulwa *et al*., 2015; NBS & ICF Macro, 2011; Nyaruhucha *et al*., 2006). In a study by Kulwa *et al*. (2006), the mean age for the introduction of cereal-based CFs in urban areas reported was 3 months even in urban areas like Dar es Salaam. Recent information regarding exclusive breastfeeding and the introduction of CFs in Tanzania as compiled during the 2010 Tanzania Demographic Health Survey (NBS & ICF Macro, 2011) indicates that 11% of children below 2 months of age, 34% of children aged 2 - 3 months, and 65% of children aged 4 - 5 moths are given CFs. Generally, many infants are introduced to CFs before they reach 6 months, and the main food given to them is composed of maize in the form of thin porridge (*uji*) (Mamiro *et al*., 2005; Nyaruhucha *et al*., 2006).

During the transition period from breastfeeding to eating CFs, IYC are fed small amounts of solid and semi-solid foods all through the day. Although the timely introduction of CFs promotes good nutrition and growth in IYC, the complementary feeding period is a critical window for growth retardation, nutritional deficiency, and disease infections (Mahgoub *et al*., 2006). Nutritional deficiencies are especially very prevalent when children are predominantly fed on high levels of cereal-based CFs (Bankole & Adebanjo, 2003). Sub-standard feeding practices, poor quality of foods, repeated infections, and micronutrient deficiencies underwrite the high malnutrition prevalences and mortalities among IYC (Black *et al*., 2008). Feeding practices such as time of introduction and type and/or quality and quantity of CFs given are some of the most important factors for children's nutritional statuses (Kulwa *et al*., 2006).

Adequate nutrition during complementary feeding remains a big challenge (Dewey & Vitta, 2013). For several years, the choices of CFs have been dictated by cultural and traditional practices without an appropriate focus on their nutritive values. Food safety has neither been taken care of alongside the choices and preparation of CFs. A majority of Tanzanian households depend on formulated cereal-based CFs (Muhimbula & Issa-Zacharia, 2010). Much of the composite complementary flours are homemade and may include several types of cereals like sorghum, millet, finger millet, maize, and rice as dictated by accessibility and cost (Mamiro *et al*., 2005). In addition to cereals, most of the mixtures contain groundnuts which are very susceptible to contamination with AFs (Muhimbula & Issa-Zacharia, 2010; Shirima *et al*., 2015). The proportions and combination of each ingredient are solely dependent on the mothers or caregivers. These feeding practices may explain the high exposure levels of Tanzanian children to AFs through complementary feeding (Kimanya *et al*., 2014; Magoha *et al*., 2016; Shirima *et al*., 2015).

The main CFs consumed in Tanzania are thin porridges made from maize flour whose composition depends on the age of the children. For instance, 3-5 month-old children consume thin porridge while older ones (6 - 11 months) consume thicker or composite-flour porridge. Although the main ingredient in the product is maize flour, other ingredients can be added and differ between households, such ingredients include sugar or salt, and other cereal grains like peanut, finger millet, and rice composite flour porridge (Muhimbula & Issa-Zacharia, 2010). Apart from cereals, vegetables and fruits, legumes and nuts, fish, meat, poultry and eggs, roots and tubers, and milk and milk products are also used as CFs in Tanzania (NBS & ICF Macro, 2011). Most foods and ingredients for CFs are highly prone to contamination with AFs which can result in acute and chronic dietary exposure. Besides the risk of exposure to AFs, infant diets in resource-constrained areas are often cereal-based CFs which are generally low in protein and important amino acids like tryptophan and lysine (Osundahunsi & Aworh, 2003), and do not provide adequate nutrition (Aron *et al*., 2017). The various types of foods used for complementary feeding in Tanzania are further discussed in the first section of 2.10.

2.12 Dietary Exposure of Infants and Young Children in Tanzania to Aflatoxins through Complementary Feeding

Literature suggests that Tanzanian IYC are exposed to unacceptable levels of AFs at a very young age (< 12 months) (Lombard, 2014; Shirima *et al*., 2013), indicating that the CFs are introduced before the age of 6 months contrary to the recommendations of WHO on complementary feeding. According to the 2015-2016 TDHS, 16, 41, and 73% of infants less than 2 months, between 2 -3 months, and between 4 -5 months respectively, were introduced to CFs (NBS, 2016). This suggests that the infants may be exposed to diets containing AFs very early in life (Moran & Dewey, 2011). This is especially worrying since children are more vulnerable to toxins owing to their lower body weights, less developed organs, and inability to detoxify (Lombard, 2014), which can subsequently affect their health, and development due to the associated effects of AFs on the immune system, body organs, and growth (Kimanya *et al*., 2014; Magoha *et al*., 2016).

A majority of CFs in Tanzania are cereal-based (Lombard, 2014), which can be contaminated with aflatoxigenic fungi during growth, pre-and post-harvesting, drying, transportation, or storage (Shabani *et al*., 2015), which increases their exposure to AFs. According to Lopriore and Muehlhoff (2005), most CFs in SSA are made of maize, groundnuts, sorghum, and millet. Like Kenya, maize-based gruels are common CFs in Tanzania (Mamiro *et al*., 2005). Sorghum, rice, millet, cassava, yams, and potatoes are also common CFs in Tanzania (Mamiro *et al*., 2005). Several studies in the country have established the high exposure in IYC to AFs through maize-based diets (Kimanya *et al*., 2008, 2014; Shirima *et al*., 2013; Suleiman *et al*., 2017), and breast milk from mothers whose primary diets consist of maize (Magoha *et al*., 2014). For instance, 32% of children in Rombo district were established to consume flours with detectable levels of AFs (range, 0.11–386 µg/kg) and 30% of the homemade CFs in the district contained AFs above the maximum tolerable limit of 10 µg/kg (Kimanya *et al*., 2014). Since the exposure of children to AFs increases markedly through complementary feeding reducing the levels of AFs in CFs is crucial (Gong *et al*., 2004).

The high exposure can be possibly explained by the early introduction of infants to cereal foods, and increased consumption of cereal-based foods as their age increases, which is further associated with dietary exposure as reported by Shirima *et al*. (2013). The consumption of food composed of maize and other grains plus groundnuts compared to other food compositions has with high exposure of children to AFs. Thus, the exposure of IYC to AFs is common in several SSA countries where maize & groundnuts are dietary staples and included in CFs (Khlangwiset *et al*., 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was carried out in the Kongwa District of Dodoma Region in central Tanzania (Fig. 1). Dodoma Region was selected purposively as a study area due to the high stunting rates of 56 and 37% reported in 2010 and 2016, respectively. Kongwa was selected because of its higher population size compared to other districts in the region (MoHCDGEC *et al*., 2016; NBS & OCGS, 2013). Also, Kongwa district was purposively selected due to its high production and reliance on crops like maize (Johnson *et al*., 2020) and groundnuts (Mwatawala & Kyaruzi, 2019; Okori, 2014) which are highly susceptible to contamination by AFs as staple foods, and the weather in Kongwa that may favour mycotoxin production in food. Moreover, the district is geographically adjacent to Kibaigwa international groundnut and maize market, thus increasing the likelihood of access to foods for the preparation of CFs.

Kongwa district lies between latitude 5^{o} 30′ to 6^{o} 0′ South and longitude 36^{o}15′ to 36^{o}East, with an area of approximately 4041 km^{2}. Its elevation ranges from 900 to 1000 meters above sea level towards the leeward side of Ukaguru Mountains (Mkonda & He, 2017). The vegetation in Central Tanzania is thicket or bush. The mean annual precipitation of the area is 400–600 mm with a mean annual temperature is 26˚C. The current population of the district is approximately 318,995. With an annual growth rate of 2.4%. The number of households is 60 301, out of which 90% are involved in farming (Mkonda & He, 2017).

3.2 Study Design

This study was performed as part of formative research to inform the design of an IYC feeding intervention as part of a cluster-randomized controlled MMT, that used the Trials of Improved Practices (TIPs) methodology (Dickin & Griffiths, 1997). The TIPs methodology involves a series of visits to selected homes to test new behaviors to improve feeding practices with mothers and children (Dickin & Griffiths, 1997). The study first conducted formative research using focus group discussions (FGDs) and RTs to explore and document the household’s typical feeding practices.

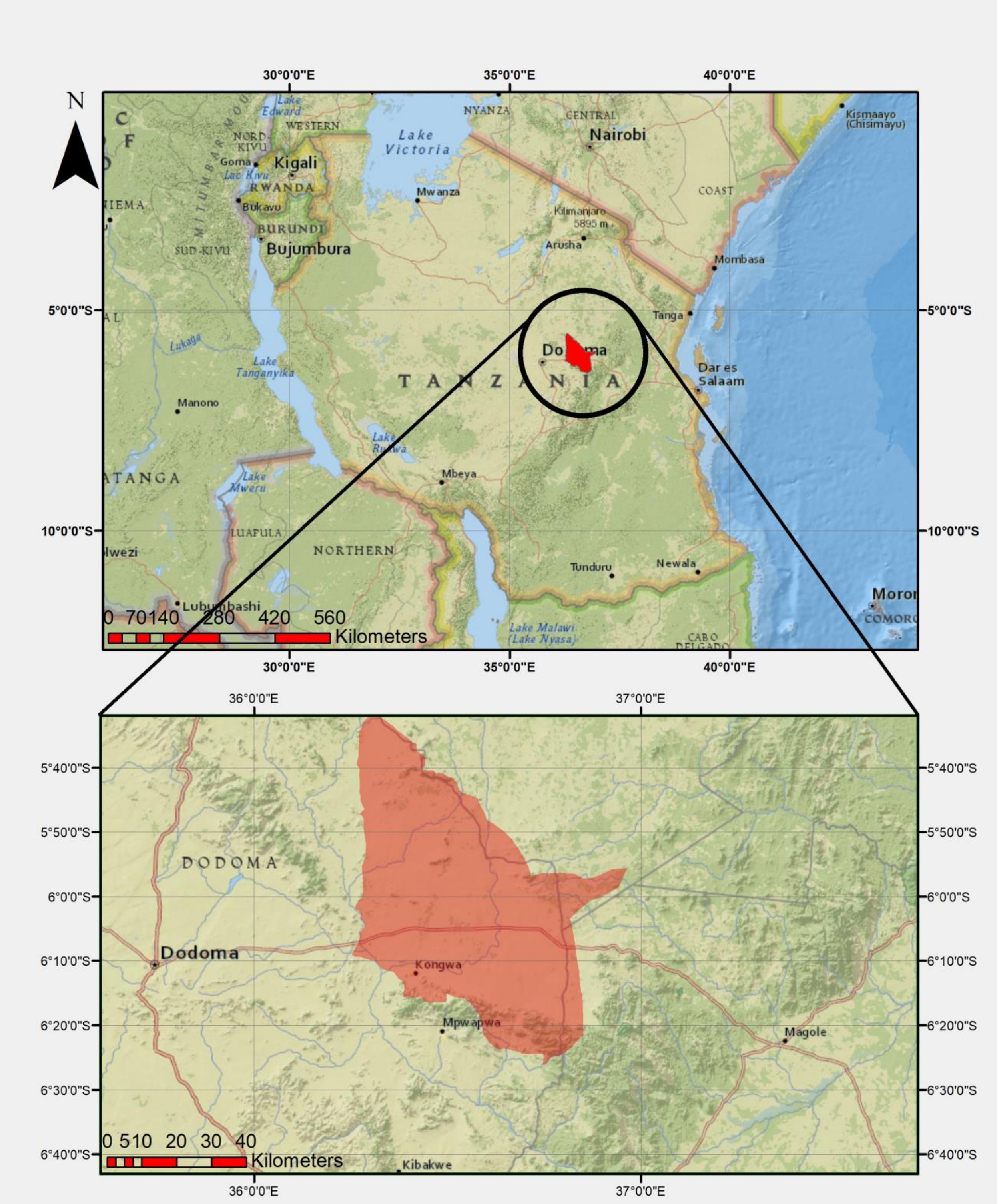

Figure 1: **Kongwa District in Dodoma region, the central part of Tanzania**

Data from the FGDs and RTs were followed by second formative research composed of two short trials. Specifically, the FGDs and RTs were conducted to inform the messages and interaction with mothers (during short trials) while assessing the acceptability of the AF-safe maize-groundnuts pre-blended flour (AFSaBF) for the preparation of thin porridge and AF-safe groundnuts powder (AFSaGP). The AFSaBF and AFSaGP were improved in terms of safety hence the name AF-safe.

It was observed that mothers in Kongwa were normally formulating CF flours from cereal (largely maize) and groundnuts in different proportions. The preparation procedures for AFSaBF and AFSaGP were therefore informed by feeding practices previously identified from FGDs and RTs (Mollay *et al*., 2021).

For the FGDs and RTs, a purposive sample of 60 respondents about 12 from each village) was selected from five villages (Ibwaga, Nghumbi, Songambele A, Pandambili B, and Mkoka) out of the 87 villages in Kongwa district. The villages were selected to represent remote and less remote settings. The respondents were mothers of children aged 6 - 18 months, identified with the help of community health workers in the villages.

In the first visit of short trial 1, thirty-six mother-child (6 - 12 months of age) pairs were randomly enrolled from a list of eligible children from the Reproductive and Child Health clinic registers at the health facilities in each of the four villages (Mtanana A, Pandambili A, Sagara, and Machenje) that were selected purposively because of high production of maize and groundnuts. The information on maize production and consumption in Kongwa was provided by the District Agricultural Irrigation and Cooperative Officer (DAICO) and the District Nutrition Officer (DNuO). The enrolment and data collection were performed between mid-February and early March 2018, approximately 6 - 9 and 10 - 11 months after the harvesting of maize and groundnuts, respectively. This timing was scheduled to account for contamination of food ingredients with AFs during storage.

The food samples were collected at two-point visits during the first short trial, before the assessment of the trial acceptability. The provision of the AFSaBF and AFSaGP was done during the second short trial, a time at which the acceptability data discussed in this research were collected. Therefore, the second trial was used to test the acceptability of an improved formulated AFSaBF and AFSaGP with a reduced quantity of AFs in the same villages and mother-child pairs in Kongwa district. A total of 35 children were characterized for the data collection on acceptability (Dickin *et al*., 1997).

3.3 Study Methods

3.3.1 Focus Group Discussions

These discussions were designed to explore the types of ingredients used in CFs and how the mothers fed their children as well as what informed their choices/practices. Predesigned open-ended questions guided the discussion in each FGD, and responses were noted. In each session, there was three research staff with; one moderator, and two note-takers. The moderator facilitated mothers to discuss feeding practices and foods for their IYC, the proceedings were well captured. Before the study day, mothers in each village were requested to carry to the study point at the VEO's office, all household items, and ingredients, together with the typical; kitchen utensils used in the preparation of CFs. Discussion points were taken alongside the age at which the mothers introduced CFs to their IYC, foods used for complementary feeding, porridge ingredients and preparation, cooking, porridge consistency, and feeding.

3.3.2 Preparation of Trial Complementary Flours

The AFSaBF and AFSaGP were prepared as trial CF ingredients by using maize and groundnuts. The safety of AFSaBF and AFSaGP were improved unlike what the mothers commonly practiced during IYC feeding in Kongwa. The maize and groundnut ingredients we used to formulate the pre-blended flour and the groundnut powder were processed to minimize AF contamination levels of the final product, pre-blended flour, and groundnut powder. These ingredients were sourced in the same locality, winnowed, and then carefully sorted. The maize and groundnut ingredients were further tested for AF contamination levels before formulation. Only lots whose samples tested < 5 μg/kg of AFB_1 (acceptable maximum limit) (EAC, 2011; TBS, 2014a; TBS, 2014b) were taken for the blend formulation, and the groundnut powder. The improved AFSaBF was blended at a ratio of 4:1 (maize: groundnuts). The proportion of maize and groundnuts (4:1 ratio) in the pre-blended flour was estimated following the findings from previous exploratory research to study what and how mothers fed the IYC in Kongwa, using FGDs and RTs. The feeding practices identified showed that CFs were mainly thin porridge containing cereal (largely, maize) mixed with groundnuts, and the ratio of groundnut to cereal ranged from 1:3 to 1:4 (Mollay *et al*., 2021). For the AFSaBF formulation, a 4:1 ratio of maize: to groundnuts was chosen due to its practicality in milling the pre-blend which is a common practice.

Table 8: Nutritional values of aflatoxin-safe blended flour (AFSaBF) per 100 g

Nutrient	Nutrient value
Energy	403 KCal
Protein	11.64 g
Fat	12.72 g
Carbohydrate	64.74 g
Dietary fiber	7.54 g
Zinc	2.1 mg
Iron	3.72 mg
Vitamin B6	0.3 mg

Additionally, the formulations of these products were further informed by the other formative research that preceded the FGD s and RTs that uses a 24-hour recall and tested AF contaminations in CF ingredients consumed that indicated both blended flour and groundnut powder formulated by mothers to be used as CF ingredients were contaminated with AFs to a level that contributed to the risk of AFB_1 exposure to IYC and signified a public health concern (Mollay *et al.*, 2022). As such the safety of the ingredients was improved during the formulation of AFSaBF and AFSaGP by hand sorting and winnowing the ingredients and testing them for AFB_1 concentration before use in the preparation of AFSaBF and AFSaGP. For the maize and groundnuts ingredients used in the preparation of AFSaBF and AFSaGP, as the AFB_1 concentration levels tested below 5 μg/kg, the assumption is other control measures to minimize AFs in food such as good agricultural practices (GAP) and good storage practices were also adhered to in the entire process. This is because there was no control for practices associated with AFs contamination from these ingredients during as from production to storage.

3.3.3 Recipe Trials

The recipe trials (RTs) comprising one group per village a day were used to get extensive observations and views for use as a foundation for designing better and more effective complementary feeding practices. During the RTs, mothers were randomly separated into sub-groups of 3 - 6 mothers. They were then instructed to make complementary feeding porridges and feed their children in the same manner they normally would at home. Requested ingredients were provided to mothers who were unable to bring ingredients from home. Porridge from pre-blended maize-groundnut (3:1) flour was made by one group of each village during the RTs to evaluate the adequacy of this formulation for future trials. This formulation was prepared following the DNuO's recommendations. All through the RTs, researchers asked questions and observed the practices, while noting down the mothers' explanations, comments, and reactions to each other's statements or actions whilst cooking. They also observed and noted the children's responses to complementary feeding, the amounts served and consumed, and the mode of feeding or reassurances used. Questions regarding the ingredients incorporated in the complementary feeding porridge were also asked by the researchers. The questions touched on reasons for using a particular ingredient, the age of the child for whom a particular ingredient was used, and if the choices/uses of ingredients differed with seasons. The consistency of the prepared foods was also observed. The researchers sought to know whether or not the consistencies varied with the age of the child. They also noted mothers' feelings towards the smell, appearance, feeding frequencies and portions, and porridge consistency of babies at a given age. Open-ended questions were administered (Appendix 1) and opinions regarding the use of groundnuts in CFs were raised and debated. The variation of ingredients of the prepared CFs were also noted by the researcher during RTs.

3.3.4 Assessment of Complementary Food Intake

A multiple-pass 24-hr dietary recall, as described by Gibson & Ferguson (2008) was used to collect information on the intake of CFs and estimate the food intake of the IYC with minor modifications (Appendix 2). Passes 1 and 2 of the 24-hr dietary recall interview were used to generate a list of the drinks and foods taken by the targeted child in the past 24 hr. Pass 3 described the foods consumed, amounts, and recipes used. The household kitchen utensils such as cups, bowls, and spoons used by the mothers/caregivers were used to measure actual foods or ingredients,or in some cases, water was used to estimate the weights or volumes of ingredients used in the recipes of CFs. The researchers converted the amounts into weight and volume equivalents by using calibrated measurements. Pass 4 narrated the portion/size of food consumed by the children. Direct weighing was used to measure the portion/size of stiff porridge (actual food) consumed, in grams. The respondents used their cups to estimate the volume of thin porridge consumed using water. The volume was then converted to volume equivalents (mL) using standard measuring cups. To ensure the correct measure of the consumed portions/sizes, the respondents were asked to eliminate the leftovers, thus the final portions consumed by IYC were the ones measured by the researchers. The 24-hr dietary recall questionnaire was administered repeatedly at two-point visits at an interval of 10 days.

3.3.5 Sampling Complementary Foods Ingredients

From each of the respondent families, samples of the susceptible foods consumed by an infant in the past 24 hr were collected. Thus, food samples were collected at the time of each 24-hr recall based on what the child ate as discovered from the recall, for the analysis of AFs. About 250-500 g of each sample was collected depending on the stock and amount each household was willing to offer. If a household had less than 1 kg of a required foodstuff, a request was made for a sample of about 250 g only. In cases where valuable food items like groundnut powder or *pre-blended* flour were used in the CFs and only small quantities of it were available in the households, a sample of about 100 g was collected. A food collection form (Appendix 3) was used to guide the enumerators on the procedures for sampling cereal grains and groundnut-based CFs. Each sample was thoroughly mixed by inverting the storage bag up and down vigorously about five times and samples were drawn from different parts of the bag and mixed thoroughly before drawing the amount required as a representative sample. Information about the source (market or home farm) of the cereal grains and groundnuts that were used in CFs was also collected. For each of the samples collected, families were compensated based on the market price per unit. Each sample was then separately coded and kept in a clean Ziplock bag. During packing, all air inside the Ziplock bag was squeezed out, and the bag was tightly zipped and stored in a refrigerator at 4°C at the Kongwa District Hospital before being transported to NM-AIST laboratory in Arusha for analysis.

3.3.6 Extraction, Clean-Up, and Aflatoxin Detection

The content of each Ziplock bag was mixed thoroughly for 3 min using a laboratory mixer. The determination of AFs in the sample was performed using the method described by Stroka and Anklam (2000). About 25 g of the evenly-mixed sample was homogenized in a blender jar at a high speed for 3 min with 100 mL of water/methanol in the ratio of 4:6 (v/v) for cereals or 2:8 (v/v) for groundnut samples. Using filter papers (Whatman No. 1), the extracts were filtered and 4 mL of each filtrate was diluted with 8 mL of phosphate buffer saline (PBS) at pH 7.3 (Sigma Aldrich). The extracts were loaded into the immunoaffinity column (IAC) after dilution [VICAM, USA], topped with a syringe barrel, and fixed onto a vacuum manifold. The extract was allowed to pass through at a flow rate of 3 mL per min. The IAC was then rinsed twice with 10 mL of distilled water and a vacuum pump was applied to ensure that the solvent and water content were drained to completion.

Table 9: Recoveries for total aflatoxin in different ingredients used as complementary foods in Kongwa District

Aflatoxin	Spiking concentration levels (µg/kg)	Percent recovery (%)					
		Maize	Groundnuts	Rice	Pearl millet	Sorghum	Pre-blended flour
AFG2	0.5	122.00	100.00	123.40	106.12	116.67	87.49
	1	102.26	107.20	118.90	119.99	80.24	115.95
	2	84.90	80.05	89.45	78.05	115.72	120.32
AFG1	0.5	121.00	80.83	122.00	107.40	114.94	85.77
	1	103.40	68.22	119.10	101.80	84.07	104.09
	2	93.95	85.31	82.55	80.05	87.63	119.94
AFB_2	0.5	114.59	96.55	116.85	118.35	80.10	103.51
	1	103.59	95.78	124.63	107.08	106.55	106.38
	2	101.43	103.69	82.66	79.64	109.02	120.08
AFB_1	0.5	122.00	95.42	115.55	108.12	119.29	89.46
	1	101.00	100.21	117.48	92.47	113.95	116.73
	2	80.54	104.76	89.59	79.38	111.27	123.27
Total AFs	0.5	119.90	93.20	119.45	110.00	107.75	91.56
	1	102.56	92.85	120.03	105.33	96.20	110.78
	2	90.20	93.45	86.06	79.28	105.91	120.90

Spiking concentration levels were used as previously described by Kimanya *et al*. (2014) and Magoha *et al*. (2016). The bounded AFs were eluted from the IAC using 1 mL of acetonitrile (Sigma Aldrich) to an amber vial. During this process, acetonitrile was left on the column for a few seconds before elution to provide for intensive contact with the adsorbent pad to detach the bound toxins. About 400 µL of the eluted extracts were mixed thoroughly with 600 µL of a derivatizing reagent made from acetic acid, trifluoroacetic acid, and water in the ratio of 1: 2: 7 (v/v/v). The mixture was then conditioned at 65°C for 15 min on a water bath and cooled at room temperature (25 ± 2˚C) before injecting 10 µL of each sample into the High-Performance Liquid Chromatography (HPLC) system (Shmadzu, Japan) connected to a fluorescence detector (FLD) (RF-20A Shimadzu, Japan) for the detection of AFs B1, B2, G1, and G2. The mobile phase was composed of acetonitrile, methanol, and water in the ratio of 1: 4: 5 (v/v/v) at a flow rate of 1 mL per min. The FLD was fixed at 450 nm for the emission wavelength and 365 nm for the excitation wavelength. The reverse-phase C18 column (250 x 4.6 mm) [Phenomenex, USA] was used at a temperature of 40˚C.

Table 10: Test of intra-day precision through comparison between the resulted concentration levels detected and the run reference material (5 µg/kg)

Aflatoxin	Day 1					Day 2		
	Batch 1	**Batch 2**	**Batch 3**	**Batch 4**	**Batch 5**	**Batch 1**	**Batch 2**	**Batch 3**
G2	4.94	4.88	4.94	5.15	4.89	5.05	4.83	4.91
G1	4.94	4.88	4.94	5.00	5.13	4.94	5.26	4.95
B2	5.07	4.99	5.07	4.95	4.98	4.82	4.92	4.95
B1	5.04	5.00	5.04	5.01	4.88	4.94	5.18	4.82
Total	**19.99**	**19.75**	**19.99**	**20.11**	**19.87**	**19.74**	**20.18**	**19.63**

The suitability of the AF determination method was tested by spiking maize, *pre-blended*, groundnut, pearl millet, rice, and sorghum flours with a standard mix/solution of AFs (B_1, B_2, G_1, and G_2) at 3 different concentrations of 0.5, 1 and 2 µg/kg. The results of the individual average recoveries of AFs spiked at three different concentrations indicated in Table 9. Respectively, the average percentage recoveries of maize, groundnuts, rice, pearly millet, sorghum, and pre-blended flour were 104.22, 93.17, 108.51, 98.20, 103.29, and 107.75%. The Limit of detection LOD was calculated as described by Sengul (2016), using the formula that LOD = 3 * SD+ Bave where SD = Standard deviation of the measurement; Bave = Average concentration of the spiked sample. Therefore, the LOD for AFB_2, AFB_1, AFG_2, and AFG_1 were 0.27, 0.25, 0.28, and 0.39 µg/kg respectively. Intra-day precision was assessed by running a standard of 5 µg/kg after every 10 samples for 50 samples analyzed in a day (Table 10) whereas inter-day precision was measured by running standards at 5 levels (2, 5, 10, 15, and 20 µg/kg) for 2 consecutive days. These were done as checkpoints and the results showed no deviations.

3.3.7 Estimation of Daily Flour Intake

The estimates of dietary exposure of IYC to AFs in the present study are based on flours consumed in stiff/thick (*ugali*) or thin (*uji*) porridge only because they are not only susceptible to contamination with AFs but also, the most commonly and frequently consumed foods in the study area. The proportion of flour consumed through a thin or stiff porridge was computed based on Kimanya *et al*. (2014) who estimated that the flour content of thin and thick porridge consumed in Tanzania is 17 and 36% (w/w), respectively. The weight in grams of the thin or stiff porridge prepared from either cereal only or cereal-groundnut-based flour as estimated from each of the two 24-hr recalls was used to estimate the average flour intake per day per child. Before calculating the amount of consumed flour, cooking and adjusting volumes and weights of the thin porridge enabled us to estimate its specific gravity to be 1.1 g/mL. Therefore, the volumes (mL) of the thin porridge were multiplied by the specific gravity (g/mL) to obtain their consumed weight in grams.

3.3.8 Estimation of Dietary Exposure to Aflatoxins

The estimation of dietary exposure to AFs in the current study was based on AFB_1 only because it is the most toxic and carcinogenic aflatoxin for which a reference Margin of Exposure (MOE, 10 000) for assessment of health risk is available. The dietary exposure of individual children to AFB_1 was estimated by the deterministic approach (Equation 1) (JECFA, 2010). The estimated exposure of a child to AFB_1 (ng/kg bw/day) was calculated as a sum of individual AFB_1 exposures through different CF ingredients consumed by the child. Exposure through an ingredient was estimated by multiplying the daily intake of contaminated ingredients and the contamination of AFB_1 level in the consumed food ingredient and dividing by the child's body weight.

$$Z = \sum_i^n \frac{X_i Y_i}{W} \qquad \text{(Equation 1)}$$

Where; Z stands for the child's estimated daily intake (ng/kg bw/day), Xi, the contamination level of AFB_1 in food ingredient i (i = 1…, n) measured in (ng/g), Yi is the flour in grams consumed per day, and W is the child's body weight in (kg).

There was a case where two different samples of similar ingredients (maize) were taken from a single household during the same visit. This was because the child consumed CF prepared from similarly purchased maize, but one maize ingredient was milled as undehulled flour whereas the other maize ingredient was dehulled ⇒ soaked ⇒ dried ⇒ milled before use in the preparation of CFs. The estimated contamination levels of AFB_1 (µg/kg) in maize on a particular day were calculated through adjustment whereby:

$$X_i = \frac{\sum_{k=1}^{s} a_k p_k}{\sum_{k=1}^{s} p_k} \qquad \text{(Equation 2)}$$

Xi is the contamination level of AFB_1 in food ingredient i (i = 1…, n), a_k is the contamination level (µg/kg) of ingredient i present in k^{th} sample (k=1…s), p_k is the amount consumed by a child (flour in kg) in k^{th} sample.

It's also worth noting that, since we had the proportions of groundnuts and cereal in blended flour, the adjustment for AFB_1 contamination level presented in post-blended flour was done by the following formula;

$$X_i = \sum_{r=1}^{n} \omega_r a_r \qquad \text{(Equation 3)}$$

Xi, the adjusted contamination level of AFB_1 in post-blended flour sample, a_r is the contamination level (µg/kg) of ingredient r present in post-blended flour, ω_r is the proportion of food ingredients used to prepare the post-blended flour.

Descriptively, if a child consumed one kind of food ingredient during the two visits, the average consumption in g/day was multiplied by the average contamination level of AFB_1 in the food ingredients in (ng/g) divided by the average child's bodyweight taken during the two visits. The summation of estimated daily intake was calculated when a child consumed (on the same day or different days) more than one kind of food ingredient, for example, maize-based thin porridge and pre-blended flour-based thin porridge, maize-based thin porridge, and sorghum-based stiff porridge or post-blended flour-based thin porridge and maize-based stiff porridge. Where AFB_1 was not detected, samples were assigned half the value of LOD (EFSA, 2010).

Each child's weight was recorded from the Reproduction and Child Health clinic cards, during the data collection period for the computation of their dietary exposure to AFs.

3.3.9 Estimation of the Risk of Exposure of a Public Health Concern

The MOE was used to determine the risk levels of exposure to AFB_1 per child. The MOE is considered the best approach to characterize risks for genotoxic or carcinogenic substances (Barlow *et al.*, 2006; Benford *et al.*, 2010; EFSA, 2020). The benchmark dose lower confidence limit, ($BMDL_{10}$) (for a benchmark dose-response of 10% for the incidence of hepatocellular carcinoma in male rats following AFB_1 exposure) of 400 ng/kg bw/day was used to calculate MOE of each child to AFB_1 (EFSA, 2020). EFSA used the animal experiments-based BMDL because the calculation of a BMDL from the human data proved inappropriate (EFSA, 2020). A MOE of 10 000 which is equivalent to an exposure of 0.04 µg /kg bw/day (400 ng/kg bw/day divided by 10 000) marked the cut-off point of low public health concern. This also implies that exposure above 0.04 µg /kg bw/day or MOE < 10 000 is a public health concern (EFSA, 2020). Therefore, a child whose MOE was < 10 000 was considered at risk and represented a public health concern.

3.3.10 Proportion of Groundnuts in Pre-and Post-Blended Flour

We estimated the fraction of groundnut ingredients present in pre-blended flour and post-blended flour by taking the groundnut amount in grams divided by the total amount of flour in grams in the total blended flour. The amount of groundnuts used in pre-blended and post-blended flour was estimated on a digital weighing scale using the weight equivalents provided by the mothers/caregivers during the multiple pass 24-hr dietary recall (pass 3), using the actual household kitchen utensils. In the case where water (mL) was used by caregivers to estimate the amount of the missing ingredient, the amounts were converted to weight equivalents by researchers using measurements of actual food by a digital weighing scale, following 3 consecutive measurements, in which the average was taken as the amount of actual ingredient in grams.

3.4 Test of Acceptability of Improved Complementary Porridge Flour and Groundnut Powder

Apart from the exclusion and inclusion criteria described in Section 3.6, the participants enrolled in the study to test the acceptability of AFSaBF and AFSaGP were mothers who were previously involved in the short trial 1 as they were using both maize and groundnuts in CFs. Thus, mothers with IYC 7–15 months (n=35 mother-child pair) (Dickin & Griffiths, 1997) participated. Mothers were provided with 1 kg of AFSaBF and a half kg of AFSaGP in zip bags at the baseline. They were given recommendations on the use of AFSaBF and AFSaGP ingredients in preparations of CFs that requires those products. Mothers were instructed to use AFSaBF when preparing thin porridge (as a post- blended flour) and AFSaGP in cases when they want to add groundnut powder into vegetable relish, *mamungunya,* or any other CFs they would prefer to use those kinds of ingredients. Mothers were also recommended to continue feeding IYC with other foods including breastfeeding in a typical way they were doing at home. Mothers were also instructed on how to air-tight the zip bag after each use of the flour, store it away from direct moisture contact and keep them in a safe place in the house. The provided amount of pre-blended flour and groundnut powder per household was enough for about 7 days. This proportion was estimated following the experience observed during the first mini-trial visit of this study when we conducted the 24-hr multiple pass dietary recall for the IYC food intake. After about a week, a follow-up study was done to interview mothers concerning the recommended new feeding practices. The interviews were conducted by using a semi-structured questionnaire with open-ended questions.

The test of acceptability of those improved complementary porridge flour and groundnut powder was conducted during the second mini/short trial as part of formative research that used the TIPs methodology (Dickin & Griffiths, 1997). Specifically, the test sought to find out the mothers' acceptability (willingness to use and continue use) of an improved AFSaBF and AFSaGP to inform the future MMT.

3.5 Data Analysis

The transcripts of the responses from the FGDs and RTs were fed into Excel sheets and translated from Swahili to English. Codes were generated by allocating short expressions to main data sets that represent crucial (recurring) themes along with memo-writing as explained by Corbin and Strauss (2008). Further management and final data analysis were conducted in ATLAS.ti Version 7 (ATLAS.ti. Scientific Software Development GmbH) (Friese, 2016) in which the results were structured using the determined final quotations and themes. IBM SPSS Statistics (Version 26) was used for normality testing and descriptive data analysis. The Shapiro-Wilk test showed that the datasets were skewed even after different transformations, thus all further statistical analyses involved non-parametric tests. The variables were analyzed with Kruskal-Wallis and Mann-Whitney rank tests for differences among different subgroups. Microsoft Excel (2016) was used for the computation of groundnuts proportions in blended flour and dietary exposure to AFB_1

3.6 Participant's Inclusion and Exclusion

3.6.1 Inclusion Criteria

(i) If the baby previously consumed groundnuts.

(ii) The baby has never shown any signs of potential groundnut allergy, including a runny nose, a rash or swelling of his/her skin, etc.

(iii) If the baby was generally healthy, with no report of fever, acute diarrhea, vomiting, or breathing problems, and if the baby appears alert. If the baby is sleeping, rely on the maternal report.

(iv) If the baby was able to eat and drink normally with no reports of poor appetite or chewing or swallowing problems, acute or chronic.

(v) If the woman and baby were available for the time during which the study visits were conducted.

3.6.2 Exclusion Criteria

(i) If the baby has not previously consumed groundnuts

(ii) If the baby has shown any sign of a potential groundnut allergy, including a runny nose, a rash or swelling of his/her skin, etc.

(iii) A woman below 16 years of age

3.7 Ethical Considerations

Approval of the present study was obtained from the National Medical Research Institute of Tanzania (NIMR) (Permit numbers NIMR/HQ/R.8c/Vol.I/951 and NIMR/HQ/R8a/Vol.IX/2874). The approval to carry out the study was also given by the Kongwa District authorities. Before data collection, mothers/caregivers of eligible IYC signed written informed consent to take part in the study. For mothers who could not read and write, researchers read the informed consent statement to them and only those who verbally consented were provided with a stamp pad to embed their fingerprint signature.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Demographic Information of Participants at Enrollment

A total of 36 mother-child pairs were enrolled to take part in the short trials 1, 1 pair dropped out for an unknown reason. The demographic characteristics of the studied IYC is shown in Table 11. In total, there were 31 infants and 4 young children aged 6 – 11 and 12 months respectively. The average body weight of IYC was 8.9 kg.

Table 11: Demographic information of the subjects enrolled in the study

Sex	**n**	**%**
Male	19	54.3
Female	16	45.7
Age in months		
6-11	31	88.6
12 and above	4	11.4
Weight in Kgs		
7-7.9	8	22.9
8-8.9	8	22.9
9-9.9	12	34.3
10-10-9	6	17.1
11-11.9	1	2.9

4.1.2 Complementary Foods and Feeding Practices

(i) Common Complementary Foods

During the FGDs, most mothers stated having introduced CFs to their infants at 6 months of age. A few mothers, however, practiced earlier (at 5, 4, and 3 months) and late (at 8 months) initiation of infants to CFs. Table 12 displays the common CFs per study village in Kongwa district while Table 13 displays the types of CFs in the district. The types of cereal and groundnut-based flours used in the preparation of CFs included maize, groundnuts, *lishe* (composite flour), sorghum, rice, and millet. Several CFs in the study area were maize-based and administered in form of usual family food or porridge (thick or thin). Legumes like beans were given as meal portions or sometimes added to complementary porridge. Other CFs contained groundnuts, cow's milk, Irish potatoes, fish, eggs, sardines, vegetables, and fruits. Eggs were mentioned only in Ibwaga village while meat, roots, poultry, cheese, and yogurt did not appear at all. Except for groundnut flour, rice, and *lishe* which were solely used to prepare thin porridge, ingredients like maize, pearl millet, and sorghum flour were used to prepare both stiff and thin porridges. Unlike *lishe* in which groundnuts were mixed with maize or other cereal(s) before milling, a proportion of groundnut flour as preferred by the mother/caregiver was separately added into the maize-based thin porridge while cooking.

Table 12: Common complementary foods in Kongwa District

Village	Main food	Other protein-based foods	Other foods (fruits and Vegetables)	Snacks/others
Nghumbi	Mashed irish potatoes, thin porridge, stiff porridge.	Sardine sauce, milk.	Mango, Ripe banana	Artificial juice (commercial) Tea.
Ibwaga	Stiff porridge, thin porridge, Irish potatoes.	Milk, groundnuts, eggs.	Oranges, fruits	Biscuits, Doughnut,
Songambele A	Mashed Irish potatoes, stiff/thin porridge, and banana (puree) with potatoes.	Boiled cow's milk.	Natural (fresh) orange juice, mangoes, natural mangoes juice.	Artificial (commercial) mango juice, water, and soda.
Pandembili B	Thin porridge, stiff porridge, potatoes (puree), thin rice, spaghetti.	Cow's milk.	Fruits like mangoes and bananas.	Tea, juice, doughnut.
Mkoka	Thin porridge, banana, potatoes (puree) mashed rice.	Cow's milk, boiled fish, beans soup, sardines.	Mangoes, amaranth soup, dried green vegetables, mangoes, watermelon and banana.	
Overall	Thin/stiff porridge, thin rice porridge, Irish potatoes, spaghetti, and bananas.	Milk, groundnuts, eggs, boiled fish, sardine sauce, beans soup	Mango, Oranges, dried green vegetables, ripe banana, amaranth soup, and watermelon.	Biscuits, Doughnut, artificial tea, soda, water

Table 13: Types and consumption of complementary foods

Household	Ingredients	Source	Ingredient in blended flour	Total amount of flour consumed (g/day)
1	Maize	Market		46.04
2	Maize	Market		32.94
3	Maize	Market		88.35
4	Maize	Market		67.83
5	Maize	Market		67.00
6	Post-blended flour	Market	Maize +groundnuts	14.03
7	Maize	Market		25.50
	Post-blended flour	Market	Maize +groundnuts	18.33
8	Maize	Market		46.75
	Post-blended flour	Market	Maize +groundnuts	49.74
9	Maize	Market		19.26
10	Maize	Market		22.95
	Post-blended flour	Market	Maize +groundnuts	24.31
11	Maize	Market		73.52
	Sorghum	Market		36.36
12	Sorghum	Market		106.18
13	Pre-blended flour	Home grown	Maize +groundnuts	63.92
	Maize	Home grown		60.29
14	Maize	Market		149.60
	Pre-blended flour	Market	Maize +groundnuts	48.62
15	Pre-blended flour	Home grown	Maize +groundnuts +sorghum	19.55
	Sorghum	Home grown		19.55
	Maize	Home grown		18.72
	Post-blended flour	Home grown	Maize +groundnuts	57.04
16	Sorghum	Home grown		73.44
	Maize	Market		43.56
	Post-blended flour	Home grown	Sorghum +groundnuts	38.34
	Post-blended flour	Market	Maize+ groundnuts	15.90
17	Pre-blended flour	Home grown	Maize +groundnuts	50.15

Household	Ingredients	Source	Ingredient in blended flour	Total amount of flour consumed (g/day)
	Maize	Market		23.4
	Rice	Market	*	32.64
18	Maize	Home grown		62.99
19	Maize	Home grown + market[a]		89.16
20	Pear millet	Market		28.05
	Maize	Home grown		20.52
21	Post-blended flour	Market	Maize +groundnuts	54.23
22	Maize	Market		42.73
	Post-blended flour	Market	Maize +groundnuts	54.23
23	Maize	Market		28.05
	Post-blended flour	Home grown + market[b]	Maize + groundnuts	38.34
24	Pre-blended flour	Market	Maize +groundnuts	107.10
	Maize	Home grown		73.80
25	Maize	Market		65.25
	Post-blended flour	Market	Maize +groundnuts	87.89
26	Maize	Market		19.44
	Post-blended flour	Market	Maize +groundnuts	110.33
27	Maize	Market		29.47
28	Pre-blended flour	Market	Maize +groundnuts +rice	35.38
	Maize	Home grown		31.68
29	Pre-blended flour	Market	PRUP (KIBOKO)	37.4
	Maize	Market		12.24
30	Maize	Market		22.32
	Post-blended flour	Market	Maize + groundnuts	36.47
31	Maize	Market		62.28
	Pre-blended flour	Market	Maize +groundnuts +rice +millet	73.95
32	Maize	Home grown + market[c]		40.78
	Post-blended flour	Home grown	Maize + groundnuts	46.75

Household	Ingredients	Source	Ingredient in blended flour	Total amount of flour consumed (g/day)
33	Maize	Market		184.89
34	Maize	Home grown + market[d]		48.59
	Post-blended flour	Home grown + market[e]	Maize + groundnuts	27.11
35	Maize	Market		105.50

a. Maize was sourced from purchased and home-grown during visit one and visit two respectively.
b. Groundnuts were purchased while maize was home-grown.
c. Maize was purchased while groundnuts were home-grown.
d. Maize was from home-grown during visit one and purchased during visit two.
e. Maize was sourced from home-grown during visit one and purchased during visit two.

PRUP: Purchased Ready to Use Pre-blended flour (namely KIBOKO). The PRUP was the only purchased blended flour, the rest were homemade by mothers/caregivers.

The rice, pearl millet, pre, and post-blended flours were used to prepare thin porridge only while maize and sorghum were used for preparing thin or stiff porridge.

Other CFs given to IYC in the present study were identified as roasted maize, vegetables in form of green leaf vegetable relish, *mandazi, mamung'unya*, tea, *futari*, sweets, raw groundnuts, potatoes, bread, beans relish, fruits (watermelon, ripe banana, mangoes). *Mandazi* is normally made by deep-frying small pieces of wheat flour dough. *Mamung'unya* is a squash-like fruit of a flowering plant grown in the area of study during the rainy season (normally during the maize production season) and is prepared by chopping the fruit into small pieces and boiling the pieces in water with the addition of some salt for taste. *Futari* in this context means a boiled and mashed mixture of peeled *mamung'umya* with groundnut powder with some oil, onion, and salt. The quantities of these foods consumed during the survey time were too small to contribute considerably to the energy intake of the children. Although, the consumed quantities of these foods are smaller compared to maize flour, or blended groundnut-based foods, the risk of exposure to AFs from them cannot be ignored. This suggests that interventions to minimize aflatoxin exposure among IYC in Tanzania should also address minor ingredients of maize and groundnut origin.

(ii) Ingredients and Preparation of *Lishe*

Ingredients used in preparing complementary porridge flour were maize, sesame, sorghum, rice, wheat, finger millet, and groundnut. The ingredients were prepared and mixed at home and then milled at nearby hammer mills as was discovered during FGDs. Blended flour is commonly referred to as *lishe.* The mothers stated over 10 different *lishe* formulations as indicated in Table 14. The most frequently used ingredients were groundnuts and maize. Other less commonly used ingredients included rice, sorghum, millet, sardines, soya-bean, beans, baobab powder, wheat, finger millet, and tamarind. About 75% of the *lishe* blends contained 66.7 to 80.0% (w/w) cereals. All (100%) of *lishe* were constituted of 7.7 to 33.3% (w/w) groundnuts. In very infrequent incidents, other ingredients like sugar, Baobab juice, and *Blue-Band* margarine were added to the porridge during preparation.

(iii) Sources of Ingredients

During both RTs and FDGs, the mothers reported purchasing maize and groundnuts from January to May and July to December, respectively, after they ran out of self-produced food. Other ingredients like sugar, salt, *Blue-Band* margarine, wheat, rice, and soya-bean were bought and utilized depending on the availability of funds.

> *In most cases, we use these as ingredients but not all. What we use depends on what we can afford at the moment* [said one of the respondents].

Table 14: Lishe ingredients in Kongwa district

Village	Porridge ingredient	Ingredients weight in Kg	Cereal composition in composite flour %[1]	Cereals: groundnuts ratio	Groundnut composition in %[2]	Others (e.g., Baobab, Legumes, sardines) composition in %[3]
Ibwaga	Finger millet	1	80.0	12:1	7.7	12.3
	Groundnuts	0.25				
	Rice	0.5				
	Soybeans	0.5				
	Maize (whole)	0.528				
	Sorghum	1				
	Finger millet	0.5	80.0	4:1	20.0	-
	Maize	1				
	Groundnuts	0.5				
	Rice	0.5				
Nghumbi	Maize (whole)	1	80.0	4:1	20.0	-
	Groundnuts	0.25				
	Rice	0.25	75.0	3:1	25.0	-
	Groundnuts	0.25				
	Maize	0.25				
	Finger millet	0.25				
Songambele A	Groundnuts	0.216	80.0	4:1	20.0	-
	Rice	0.314				
	Whole maize	0.264				
	Millet	0.276				
	Maize	0.528	80.0	4:1	20.0	-
	Finger millet	0.56				
	Groundnuts	0.25				
Mkoka	Maize	1	44.4	2:1	33.3	42.9
	Groundnuts	0.5				

Village	Porridge ingredient	Ingredients weight in Kg	Cereal composition in composite flour %[1]	Cereals: groundnuts ratio	Groundnut composition in %[2]	Others (e.g., Baobab, Legumes, sardines) composition in %[3]
	Sardines	0.75				
	Rice	0.25	50.0	3:1	25.0	40.0
	Beans	0.5				
	Maize	0.5				
	Groundnuts	0.25				
	Wheat	1	50	4:1	20	42.9
	Soy beans	1				
	Groundnuts	0.5				
	Sesame	0.5				
	Rice	0.5				
	Baobab[†]	0.5				
Pandembili B	Maize	1	75.0	3:1	25.0	-
	Rice	0.5				
	Groundnuts	0.5				
	Maize	1	66.7	2:1	33.3	-
	Groundnuts	0.75				
	Rice	0.5				
	Finger millet	0.56	75.0	3:1	25.0	-
	Rice	1				
	Groundnuts	0.5				

1. Computed from cereals and all other ingredients including groundnuts.
2. From groundnuts and cereals only.
3. From cereals and others (e.g., Sardines, Legumes, or baobab) excluding groundnuts.

[†] Baobab powder was mixed with water t produce a juice in which other porridge ingredients were mixed during the preparation of porridge

(iv) *Lishe* Preparation Knowledge and Practices by Mothers

A majority of mothers were cleaning *lishe* ingredients, roasting groundnuts, and decoating as well as drying soon after milling to decrease the moisture content. Individual ingredients were cleaned through sorting, removing sand, and winnowing. Additionally, soya beans were boiled, cleaned/washed, and sun-dried. Sometimes, groundnuts were roasted before mixing with the other ingredients. The observed activities were good practices to promote in the study area as they have been reported to reduce AF contamination in CFs (Kamala *et al.,* 2016). It was however observed during the FDGs that some mothers failed to sort, wash or winnow groundnuts and cereals (mostly maize). Most of the mothers reported sorting rice as a normal practice to remove husked rice but they did not de-hull cereals like wheat or maize. Some mothers reported storing the milled *lishe* for up to two weeks.

(v) Porridge Consumption and Feeding Frequency

During the FGDs, a majority of the mothers stated preparing complementary feeding porridge for their children once to thrice a day 7 days/week. The observation during RTs revealed that a child consumed approximately 1 tablespoon to a whole cup (about 250 ml) of a thin porridge in each feeding.

(vi) Preferences, Beliefs, and Perceptions about *Lishe*

A majority of the mothers added groundnuts to *lish*e flours from the belief that it is nutritious and can improve child health. When mothers were asked about the thickness of the porridges they prepared, some of them favoured thick to thin porridge claiming it satisfied the babies, particularly the hungry ones. One mother, when asked why she preferred thick porridge for her child, said that *"Thick porridge stays in a child's stomach longer and provides him energy."* Some of the mothers, however, expressed concern that thick porridge does not cook well and, in some cases, makes babies vomit. The younger infants (6-12 months) mostly refused to drink thick porridge. Others preferred thin porridge because it is easier to feed and swallow. When a mother was asked about her preference for thin porridge, she said, *"Thin porridge is good, the child prefers it and it is easy to swallow."*

(vii) Feeding Styles

The mothers were asked to feed their IYC during the RTs as they usually do at home to assess their affection and responsiveness. Mothers put the complementary porridges in cups and fed their infants with spoons. During feeding, most mothers put their infants on their laps and encouraged them to eat in various ways, for example, by shaking their hands, petting them, or saying “Take, this is sweet!” while looking at them. Moreover, some mothers fed their children while allowing them to play around making them to generally accept and enjoy the porridge offered to them. From the observations, mothers’ styles and practices encouraged their children to feed.

(viii) Undesirable Experiences of Feeding on Groundnuts

Some mothers believed that groundnuts may comprise diarrhea-causing fungi and enquired instructions and guidelines for proper preparation, cooking, and storage of groundnut-containing *lishe* flour to avoid fungal infestation. The mothers knew about the negative effects of feeding children on groundnut-based porridge, although this was not directly proven during the observations. When probed if they knew the health effects of groundnuts, some mothers said, *“When stored for a long time, groundnuts develop fungus and can affect the child.”*

“Yes, if you add groundnut into your child’s food, they will suffer from allergies like skin rashes.”

Despite such beliefs about groundnuts, mothers still preferred feeding their children groundnuts. When asked if they would be willing to feed their children groundnut-based porridge if someone suggested so, they all agreed agreed.

4.1.3 Risk of Dietary Exposure to Aflatoxins

(i) Aflatoxin Contamination in Complementary Food Flours

In total, 84 samples of ingredients meant for the preparation of CFs were collected from Kongwa district, and AFB_1 occurred in 82.14% of the samples in the range of 0.27 to 317 µg/kg with a median of 3.96 µg/kg. Out of the contaminated samples, 53.62% had AFB_1 proportions more than 50% of the total contamination by AFs. The distribution of AFB_1 and total AFs in the main ingredients of CFs (maize, *blended flour*, groundnuts, pearl millet, and sorghum) are summarized in Table 15. All groundnut-based blended flours were contaminated with AFs (Tables 15 and 16).

Maize samples had AFB_1 contamination levels of 0.28 -7.75 µg/kg (Table 15). The highest AFB_1 contamination occurred in the maize samples (median = 4.17 µg/kg), followed by pearl millet (median = 1.45 µg/kg) and then sorghum (median = 1.35 µg/kg). Only one rice sample was tested and established to be contaminated with AFB_1 (0.27 µg/kg) and total AFs (2.34 µg/kg) (Table 15).

Table 15: The occurrence of aflatoxins in main complementary flour ingredients

Aflatoxin	Ingredients	Households (n=35)	No. of samples	≥LOD n (%)	Range ≥LOD (µg/kg)	Median ≥LOD (µg/kg)	>ML n (%)
AFB1	Maize	35	52	41 (78.85)	0.28-7.75	4.17	20 (48,78)
	Groundnuts	14	15	14 (93.33)	0.55-317	6.87	11 (78.57)
	Pre-blended	8	9	100	0.67-6.48	1.45	2 (22.22)
	Sorghum	4	5	2 (40)	1.33-1.36	1.35	0
	Pearl millet	1	2	100	1.44-1.47	1.45	0
	Rice	1	1	100	0.27	0.27	0
	Post-blended	15	NA	NA	0.69-103.04	4.73	6 (40)
	Overall	35	84	69 (82.14)	0.27-317	3.69	33 (47.83)
Total AFs	Maize	35	52	51 (98.08)	0.28-14.91	7.51	17 (33.33)
	Groundnuts	14	15	100	1.1-428.55	13.25	11 (73.33)
	Pre-blended	8	9	100	2.29-18.97	5.3	2 (22.22)
	Sorghum	4	5	4 (80)	2.33-24.74	3.92	1(20)
	Pearl millet	1	2	100	2.69-7.26	4.98	0
	Rice	1	1	100	2.34	2.34	0
	Post-blended	15	NA	NA	1-133.04	5.43	7 (46,67)
	Overall	35	84	82 (97.62)	0.28-428.55	7.12	31 (37.80)

- *Total AFs is the sum of AFG_1 + AFG_2 + AFB_1+ AFB_2. The limit of detection (LOD) for aflatoxin B1 in the current study is 0.25µg/kg. The LODs for AFB_2, AFG_1, and AFG_2 were 0.27, 0.39, and 0.28 respectively.*
- *The maximum limit (ML) for aflatoxin B1 and total AFs in groundnuts and cereal flours intended for human consumption are 5µg/kg and 10µg/kg respectively* (EAC, 2011; TBS, 2014a; TBS, 2014b)
- *NA stands for not applicable since the post-blended flours were not directly tested in the laboratory, only the materials (groundnuts + maize or sorghum) formulated it was tested, and the AFs were adjusted as per equation 3.*

In reference to the maximum limit (ML) of 5 µg/kg for AFB_1 set by the EAC, 78. 57 and 48.78% of the groundnut and maize samples had higher levels of AFB_1. Besides, the contamination levels established in the present study are far above the ML of 0.10 µg/kg established by the EU for AFB_1 in baby foods and processed cereal-based foods intended for IYC.

(ii) Proportion of Groundnuts in Pre-And Post-Blended Flours and Contribution to Contamination

The blended flours contained one or more types of these cereals and groundnuts. In *pre-blended flour* groundnuts were mixed with maize or other cereal(s) before milling, and in post-blended flours, groundnut powder was mixed with any of the cereal flours after milling (and just before cooking). These flours were solely used to prepare thin porridges.

Table 16: Proportion of groundnuts and AFB_1 in cereal-groundnuts mix flour

Flour ingredients type	Groundnuts proportion	AFB_1 (µg/Kg)
Post-blended flour	0.066	0.176
Post-blended flour	0.273	2.084
Post-blended flour	0.220	2.646
Post-blended flour	0.645	6.442
Pre-blended flour	0.250	3.156
Pre-blended flour	0.250	6.469
Pre-blended flour	0.250	6.485
Post-blended flour	0.200	0.131
Post-blended flour	0.303	2.386
Post-blended flour	0.449	5.962
Pre-blended flour	0.250	1.395
Post-blended flour	0.348	0.728
Post-blended flour	0.127	0.688
Post-blended flour	0.455	7.474
Pre-blended flour	0.250	1.453
Post-blended flour	$<0.001^m$	7.753
Post-blended flour	0.297	89.661
Pre-blended flour	0.200	0.996
Pre-blended flour	PRUP	1.616
Post-blended flour	0.322	103.041
Pre-blended flour	0.056	0.737
Post-blended flour	0.211	3.506
Post-blended flour	0.103	0.169

Groundnuts proportional range (m-0.645)
m= 0.00028098
PRUP: Purchased Ready to Use Pre-blended flour (namely KIBOKO).

The proportion of groundnuts in pre-blended and post-blended flour ranged from 0.000281 to 0.65 with an average of 0.25 (Table 16). There was a strong and significant correlation between groundnut proportions in the post and pre-blended flour and the AFB_1 contamination level ($r = 0.5$, $p = 0.02$). This implies that as caregivers increased groundnuts in the formulation of pre- or post-blended flour, there was an increase in the overall flour AFB_1 contamination levels. The total AFs were computed as the sum of AFG_1, AFG_2, AFB_1, and AFB_2. Groundnuts were contaminated with AFB_1 in the range of 0.55-317.00 µg/kg and contained the highest contamination levels with a median of 6.87 µg/kg than cereals like maize (median = 4.17 µg/kg) and sorghum (median = 1.35 µg/kg). All cereal groundnut-based samples were contaminated with AFB_1. The results also revealed that cereal groundnut-based flour formulations were the major contributors to the exposure of IYC to AFB_1 (Table 16).

(iii) The Risk of Dietary Exposure to Aflatoxins

Table 17 shows the ingredients of CFs and estimated daily intake as used in the AFB_1 exposure assessment and the MOE results. The dietary exposure to AFB_1 estimated in the current study ranged from 0.33 to 1168 ng/kg bw/day with a median of 23.08 ng/kg bw/day. Consequently, the estimated MOE for IYC in the present study ranged from 0.34 to 1212 with a median of 17. The estimated dietary exposure to AFB$_1$ per ingredient of CFs for IYC is also indicated in Table 17.

Table 17: Ingredients of complementary foods and estimated daily intake as used in AFB$_1$ exposure assessment and MOE

S/N	Food ingredients	AFB$_1$ Dietary intake (ŋg/kg bw/day)	MOE	Group
1	Maize	3.91	102.	Young Children
2	Maize	15.90	25	Infants
3	Maize	41.97	10	Young Children
4	Maize	8.49	47	Infants
5	Maize	59.76	7	Young Children
6	Maize	8.14	49	Infants
7	Maize	23.39	17	Young Children
8	Maize	26.83	15	Infants
9	Maize	11.53	35	Young Children
10	Maize	122.64	3	Young Children
11	Maize	1.47	273	Young Children
12	Maize, Pearl millet	23.08	17	Infants
13	Maize, post-blended	5.52	73	Infants
14	Maize, post-blended	58.91	7	Infants
*15	Maize, post-blended	29.92	13	Infants
16	Maize, post-blended	30.89	13	Young Children
17	Maize, post-blended	58.62	7	Infants
18	Maize, post-blended	117.83	3	Infants
19	Maize, post-blended	1167.55	0.3	Young Children
20	Maize, post-blended	418.45	0.96	Infants
21	Maize, post-blended	23.06	17	Young Children
22	Maize, post-blended	18.42	22	Infants
23	Maize, pre-blended flour	27.90	14	Infants
24	Maize, pre-blended flour	139.49	3	Infants
25	Maize, pre-blended flour	15.76	25	Infants
26	Maize, pre-blended flour	6.18	65	Young Children
27	Maize, pre-blended flour	9.11	44	Infants
28	Maize, pre-blended flour	47.66	8	Infants
29	Maize, rice, pre-blended flour	14.36	28	Infants
30	Maize, sorghum	66.86	6	Infants
31	Maize, Sorghum, post-blended flour	27.26	15	Infants
32	Maize, Sorghum, post-blended flour, pre-blended flour	17.50	23	Infants
33	Post-blended	0.33	1212	Infants
34	Post-blended	4.70	85	Infants
35	Sorghum	8.94	45	Infants

MOE = Margin of Exposure

The estimated dietary exposure to AFB$_1$ for IYC was mainly contributed to by a combination of maize and post-blended flour ingredients for which the highest exposure of 1168 ng/kg bw/day (Table 17) was estimated in this study. The maximum dietary exposure of AFB$_1$ was slightly higher for young children (1168 ng/kg bw/day) than for infants (418 ng/kg bw/day) but the difference was not statistically significant ($p = 0.86$). On the other hand, a higher risk of exposure to AFB_1 was determined for cereal-groundnut-based CFs consumed by 60% of children compared to maize only-CFs consumed by 31.40% of the children, and the difference was not statistically significant ($p = 0.33$).

4.1.4 Acceptability of the Improved Complementary Porridge Flour

Following up of the use of the trial CFs flours recommended was made at each household. The results indicated that several mothers, (57.1 %) prepared AFSaBF at least once per day in a week (Table 18). That is an indicator that both mothers and children liked the products.

Table 18: Mothers' frequency of preparation of AFSaBF porridge per week (n = 35)

Frequency/week	No of mothers (n)	Percent (%)
1-5	15	42.9
6-10	16	45.7
11-14	2	5.7
15-19	2	5.7

The mothers appreciated the porridge made from AFSaBF whereby 37.1% said that it had a good taste, 34.3% said that babies ate well, 11.4 % mentioned that it was delicious and fills a baby, 5.7% said that the porridge was thick and satisfied the baby because it has a lot of nutrients and improves health and only one mother said that the porridge flour provided reduces the cost of buying cereals (Table 19).

Table 19: Reasons for appreciating AFSaBF porridge flour (n=35)

Statement	Frequency (n)	Percent (%)
Good taste	13	37.1
A baby eats well	12	34.3
Delicious and fills a baby	4	11.4
It has a lot of nutrients and improves health	2	5.7
It becomes thick and fills a baby	2	5.7
It reduces the cost of buying cereals	1	2.9
Its recipe is good	1	2.9

Table 20 and 21 also show the reasons for mothers who intended to proceed with the implementation of AFSaBF porridge cooking and consumption and the reasons for appreciating AFSaGP respectively.

Table 20: Reasons for continuing implementation of AFSaBF porridge cooking and consumption (n=35)

Reasons	Frequency (n)	Percent (%)
I like its thickness	11	31.4
It fills a baby	7	20
It has a good ratio	4	11.4
I want to see good growth progress for my baby	4	11.4
It is easy to prepare	4	11.4
A baby liked it	2	5.7
The flour is good	1	2.9

Table 21: Reasons for appreciation of AFSaGP (n=35)

Reason	Frequency (n)	Percent (%)
It have good taste on vegetable	10	33.3
Reduction of the cost of buying and time of grinding groundnuts	10	28.6
It is good	4	11.4
They contain a lot of fat fat	3	8.6
It is smooth and is prepared in a good way	2	6.7
The vegetable soup becomes thick when has groundnuts	2	5.7
It was well packed	1	2.9
It makes vegetable taste good and contains nutrients	1	2.9

Table 22: Other people who tasted the porridge in the household (n=35)

Number of Tasters	Households	Percent (%)
6	1	2.9
5	3	8.6
4	5	14.3
3	9	25.7
2	12	34.3
1	5	14.3

The present study further showed that other household members tasted the porridge in addition to the index child and they revealed that it was delicious and had good smell (Table 22). During the tasting, 88.6% of the household had tasters who consumed a little amount of porridge while 11.4% of the household had tasters who consumed more than the indexed child.

Table 23: Foods consumed by IYC during the AFSaBP and AFSaGP recommendation period (n=35)

Food	Frequency (n=35)	Percent (%)
Breast milk	34	97.1
Groundnuts in any thin porridge	33	94.3
Mamung’unya	9	25.71
Vegetables with groundnuts	9	25.7
Thin porridge (maize)	7	20
Roasted maize (*Mahindi ya kuchoma*)	5	14.29
Watermelon	5	14.29
Boiled eggs	4	11.43
Mamung’unya with groundnuts	3	8.57
Burns (Maandazi)	3	8.57
Orange	3	8.6
Banana	3	8.6
Tea	3	8.6
Biscuit	2	5.7
Beans soup	2	5.7
Sweets (pipi)	2	5.7
Meat soup	2	5.7
Okra soup	1	2.9
Potato with groundnuts	1	2.9

During the study period, at baseline, each household was given about 1 kg of the AFSaBF, refilled during the follow-up visit (after (about 7 days). At follow-up, 48.57% of the participants had consumed the AFSaBF at more than half the amount of AFSaBF provided per household in the previous visit, 20% consumed more than three-quarters of the amount provided and 14.29% consumed less than a quarter of the provided amount. On the other hand, each household was given 0.5 kg of the AFSaGP which during the follow-up visit 100% reported mixing with either porridge or vegetable relish. It was revealed that 14.29% of the household had finished the amount provided, 77.14% consumed more than half of the provided amount, and only 8.57% used AFSaGP less than a quarter of the amount provided. All mothers reported enjoying feeding IYC with the AFSaGP.

Several foods were consumed by the IYC during the AFSaBP and AFSaGP recommendation period as illustrated in Table 23. A majority of the households (97.1%) also fed on breast milk and 94.3% of households gave groundnuts in thin porridge. Fruits like watermelon, oranges, and bananas were consumed by IYC in little proportions. Other foods such as *Mamung’unya,* thin maize porridge, and vegetables with groundnuts were also used in moderate proportions.

4.2 Discussion

4.2.1 Caregivers Practices on Complementary Foods

This study provides an empirical description of mothers' practices and perceptions about porridge ingredients, feeding and cooking techniques, as well as perceptions about maize, groundnuts, and other common ingredients for CFs in Kongwa. The research findings revealed that porridge flour formulations were homemade with various cereals, with groundnuts and maize being the most common. It was also noted that certain formulations had more than one cereal (rice, maize, and finger millet), one legume (groundnuts), two types of cereal (rice and maize), or 2 legumes (groundnuts and beans).

The RTs showed that the mothers' practiced the authoritative responsive child feeding style (Birch & Fisher, 1995; Hodges *et al*., 2008). Comparable results were obtained in a cross-sectional study in Southern Ethiopia (Wondafrash *et al*., 2012). These results, nevertheless, differ from others studies by Kinabo *et al*. (2017) who showed that caregivers in Unguja Islands, Tanzania, rarely practiced responsive feeding and Dharmasoma *et al*. (2020) where no caregivers in Sri Lanka practiced responsive feeding fully. Some studies suggest that responsive feeding may contribute to the healthy growth, development, and well-being of children (Black & Aboud, 2011; Daniels, 2019). On the other hand, non-responsive feeding (authoritarian) is linked to the increased risk of overweight and/or obese children (Hurley *et al*., 2011; Vollmer & Mobley, 2013) due to a lack of self-regulated feeding.

The WHO recommends the gradual increase of food consistency from 6 months onwards (WHO, 2005). The mothers agreed that the right consistency of complementary porridge was dependent on the age of the child. However, some mothers reported that thick porridge can choke children. The mothers also appeared to adhere to similar methods of preparing complementary porridge and the style of feeding their children. The uniformity of methods was notable in terms of the ingredients of complementary porridge with little diversity of food groups.

These findings suggest that the mothers might have learned some good feeding practices like the consistency of IYC food given depending on age from the Mwanzo Bora Nutrition Project (MBNP) which among other priorities promotes exclusive breastfeeding for infants 0-6 months and continued breastfeeding for those aged 6 to 24 months (District nutrition officer, unpublished communication). Among other objectives, the MBNP project aimed at reducing child stunting in Dodoma region where the present study was carried out. The interventions of

MBNP included training the mothers on nutrition education packages using its Social and Behaviour Change Communication (SBCC) Kit, the 1,000 Days SBCC Kit (*Mkoba wa siku 1000).* The kit was developed by Tanzania Food and Nutrition Centre (TFNC) in collaboration with MBNP to influence positive nutrition behaviors and improve maternally and child nutrition.

The results of this study also showed that most mothers initiated their infants to CFs at 6 months of age. These findings are contrary to those of Tanzania demographic and health Surveys (MoHCDGEC *et al*., 2016) and numerous studies in Tanzania that have observed poor timely initiation of CFs (de Bruyn *et al*., 2018; Kinabo *et al*., 2017; Kulwa *et al*., 2006; Maonga *et al*., 2016; Mgongo *et al*., 2013; Nkala & Msuya, 2011; Shirima *et al*., 2001). We probed for true behaviors since mothers were well-educated on the recommended time for the introduction of complementary feeding which is at 6 months of age. In the current study, some mothers practiced both early (before 6 months) and late (over 8 months) introduction of CFs to infants. These practices are known to be major contributors to infant malnutrition since they are associated with inadequate nutrient intake and high infection rates (Sellen, 1998).

The IYC in Pandambili B and Nghumbi villages fed on few varieties of food compared to the other villages. The low diet diversification was related to limited nutrition knowledge in the study area. These findings relate to those of Christian *et al*. (2016) in Ghana and Kuchenbecker *et al*. (2017) in Malawi where caregivers' nutrition knowledge and attitude may influence children's dietary quality. According to Ochieng *et al*. (2017), training in nutrition and food preparation can significantly influence dietary diversity in Tanzania. This is important because diversified diets are negatively associated with stunting and underweight among children, and can eventually decrease under-nutrition in the country (Khamis *et al*., 2019).

Mothers in the present study were not aware of mycotoxin contamination of ingredients used in *lishe*. Some of them did not sort groundnuts, maize, or other cereals, they used nor de-hulled maize; practices that may reduce mycotoxin contamination in complementary feeding porridge (Anitha *et al*., 2020; Kamala *et al*., 2018a; Mutegi *et al*., 2018). However, some mothers seemed to know about the effect of moisture on stored *lishe* and reported drying the flour before storage. This is in line with findings from a qualitative study in Dodoma and Singida regions of Tanzania by Ngoma *et al*. (2020) where participants were aware of the effects of mold contamination of improperly dried CFs with AFs. The knowledge of AFs in this context might be from the efforts of the Tanzanian government and its international partners in mitigating the contamination of foods with AFs. For example, from 2017 – 2019, FAO in partnership with the government of Tanzania executed a project on the mitigation of AFs through the dissemination of appropriate postharvest management technologies and awareness-raising in Dodoma and Manyara regions (FAO, 2019).

It is established that CFs in Tanzania are cereal-based (Kimanya *et al*., 2010; Kinabo *et al*., 2017; Kulwa *et al*., 2015; Muhimbula *et al*., 2011; Vitta *et al*., 2016). Such diets have low nutrient content (Moursi *et al*., 2008; Vitta *et al*., 2016) and are also susceptible to contamination with mycotoxins. Maize and groundnuts are prone to contamination with AFs (Agbetiameh *et al*., 2017; Kimanya *et al*., 2008). The central part of Tanzania where the present study was carried out is the site of a recent outbreak of acute aflatoxicosis that claimed 20 lives, including young children. The outbreak was associated with the consumption of groundnuts and cereal-based foods contaminated with high levels of AFs (10 - 51,100 µg/kg) alongside fumonisins (Kamala *et al*., 2018b). Therefore, the IYC in Kongwa Tanzania may be at risk of exposure to AFs and stunted growth (Chen *et al*., 2018; Kamala *et al*., 2017; Kimanya *et al*., 2014). Depending on cereal-based CFs might also limit the attainment of nutrient adequacy and bioavailability of micronutrients which may altogether negatively impact the nutritional status of children (Makori *et al*., 2017).

Following the procedures described by Kimanya *et al*. (2014) regarding the conversion of stiff and thin porridge weights into amounts of flour consumed, the average sum of complementary flour consumption in the current study ranged from 14.02 to 198.22 g/child/day with a grand average of 89.45 g/child/day The range reported in this study is higher than the maize-based flour intake reported by Kamala *et al*. (2017). (0.13 to 185 g/child/day), and lower than the maize-based flour intake reported by Kimanya *et al*. (2014) (16 to 254 g/child/day) indicating that flour consumption by IYYC in Tanzania differs from one place of the country to another. Thus depending on the AFs contamination levels in the flour, risk of exposure to those toxins may also vary in one region to another.

4.2.2 Risk of Dietary Exposure to Aflatoxins

About 82.14% of the 84 samples of the ingredients used to prepare the CFs in Kongwa district had AFB_1 ranging from 0.27 to 317 µg/kg with a median of 3.96 µg/kg. Similarly, about 53.62% of the food samples had AFB_1 proportions at > 50% of the total contamination by AFs. Past studies elsewhere have established that AFB_1 is the most toxic and frequently detected in aflatoxin-contaminated samples, and its contribution is the largest in total AFs (Adetunji *et al*., 2014; JECFA, 2010; Yabe & Nakajima, 2004). This indicated the need for immediate intervention for IYC who consumed groundnut and maize-based CFs, to reduce exposure to AFB_1 and the related health effects such as stunting. Ismail *et al*. (2021) argue that African countries require intervention strategies that can mitigate early-life dietary exposure to Afs in foods to reduce the associated health implications.

Most of the CFs studied in the present study were cereal-based which are among the most susceptible foods to mycotoxin contamination (Peraica *et al*., 2014). In a recent study in the same district, samples of several cereal grains intended for porridge preparation were found to be highly contaminated with AFB_1 (mean 38.3 µg/kg; maximum 271 µg/kg) (Anitha *et al*., 2020). These findings are also consistent with those of Kimanya *et al*. (2014) from Rombo district in Kilimanjaro region where the AFB_1 contamination ranged from 0.53 to 364 µg/kg. These results have also been replicated in other studies across Tanzania, for instance, in Rombo district where 58% of 67 maize flour samples consumed by infants under the age of 6 months had detectable Afs (range 0.33 – 69.47 µg/kg; median 6 µg/kg) (Magoha *et al*., 2016), and in Kilosa, Hanang' and Rungwe districts where the levels of Afs in maize samples meant for the preparation of CFs was found to range from 1.0 to 1081 µg/kg (Kamala *et al*., 2017). High levels of contamination (range 150 – 345 µg/kg) have also been established for cooked maize porridge samples collected from several villages in Kilimanjaro, Iringa, and Tabora regions (Geary *et al*., 2016).

Though cereals serves as the main components of IYC foods owing to their excellent energy and nutritional sources (Klerks *et al*., 2019), these ingredients are susceptible to mycotoxins contamination which escalates the risks of exposure to these toxins (Mollay *et al*., 2021). About 86% of the tested samples contained AFB_1 ranging from 0.13 to 316.99 µg/kg. These findings concur with those of Kimanya *et al*. (2014) from the same region where AFB_1 contamination was established to range from 0.53 to 364 µg/kg. Most of the CFs investigated in the present study were cereal-based which are among the most susceptible foods to mycotoxin contamination (Peraica *et al*., 2014).

All groundnut flour and groundnut-based *blended* flours collected from households were also contaminated with AFB_1 and AFs, suggesting that the practice of mixing more than one cereal and/or groundnuts in composite flours may increase the risk of exposure of IYC to AFs. The results also revealed that groundnuts were the major contributors to the exposure of IYC to AFs. In the study by Makori *et al*. (2019), the higher level of AFB_1 in composite flour was also largely attributed to the inclusion of groundnuts as ingredients in the formulations. Similar findings have also been established in other studies conducted elsewhere in Tanzania (Anitha *et al*., 2017; Chen *et al*., 2018; Magembe *et al*., 2016). The susceptibility of groundnuts to contamination by AFs has largely been linked to the geocarpic nature of its pods which favours the growth of fungi (Kachapulula *et al*., 2017; Waliyar *et al*., 2015).

About 78.85% of the maize samples in the present study were contaminated with AFB_1 in the range of 0.28 -7.75 µg/kg. Furthermore, the maize samples had the highest contamination of AFB_1 compared to other cereals with a median of 4.17 µg/kg, followed by pearl millet samples with a median of 1.45 µg/kg and sorghum (median 1.35 µg/kg). Unlike other cereals, maize grains are larger and can promote infestation and subsequent contamination with AFs. According to Benkerroum (2020), maize is the most highly and frequently aflatoxin-contaminated crop in SSA. Although the range of AFB_1 contamination in maize in the present study of 0.28 -7.75 µg/kg with (median 4.17 µg/kg) was lower than that of 0.85 - 55.73 µg/kg (median 3.50 µg/kg) obtained by Magoha *et al.* (2016) in Rombo District, the proportion of maize flour contaminated with AFB_1 in the present study exceeded that established by Magoha *et al*. (2016) who reported that only 50.75% of the samples were contaminated with AFB_1. Unlike the present study that was done in central Tanzania (Kongwa), the study by Magoha *et al*. (2016) was conducted in northern Tanzania (Rombo), thus the climatic differences between the two study locations may account for the differences in contamination of maize with AFB_1.

Only one sample of rice was tested and was contaminated with AFB_1 and total AFs at only 0.27 and 2.34 µg/kg, respectively. This implies that rice is also susceptible to contamination with AFs. Various studies have also reported contamination of AFs in rice, for instance, in countries like Austria (Reiter *et al*., 2010), Iran (Mazaheri, 2009), China (Lai *et al*., 2015; Liu *et al*., 2006), Vietnam (Nguyen *et al*., 2007), Malaysia (Samsudin & Abdullah, 2013), and Pakistan (Iqbal *et al*., 2016; Majeed *et al*., 2013). Compared to maize and groundnuts, low levels of contamination by AFB_1 and total AFs ranging from 1.44 to 1.47 and 2.69 to 7.26 µg/kg respectively were also established for pearl millet. Studies in SSA have also established low levels of AFs in pearl millet, for instance, 0.14 – 6.4 µg/kg in Kenya (Sirma *et al*., 2015), and 1.12 µg/kg in Ethiopia (Chala *et al*., 2014). Although the contamination levels in rice in Tanzania are low and rarely reported, there are indeed a few reports on the same. For example, Kimanya *et al*. (2016) state that the aflatoxin contamination levels in the 101 rice samples from the three main rice-producing districts of Tanzania (Kilosa, Morogoro, Mbarari, Mbeya, and Misungwi, Shinyanga), were ranged from 0.01-3.83 µg/kg. Kuhumba *et al*. (2018) also report that Rice used to make complementary flours in urban markets in Tanzania had a median level of 1.82 µg/kg of total AFs. Nevertheless, these results and results of the present study demonstrate that although such foods are fed to infants in lesser quantities, they are less prone to contamination with AFs, thus their consumption should be encouraged to reduce the risks of exposure of IYC to AFs through the common food ingredients.

About 48.78 and 78.57% of the maize and groundnut samples had higher levels of AFB_1 relative to the ML of 5 µg/kg for AFB_1 set by the EAC (EAC, 2011; TBS, 2004). The samples were also more contaminated than those investigated by Magoha *et al.* (2016) in Rombo District in Northern Tanzania where only about 35% were contaminated with $AFB_1 > 5$ µg/kg. Comparable results have also been established in Dodoma, Tanzania where about 30% of infants were found to be exposed to AFs above the ML through consumption of CFs (Makori *et al*., 2019). Besides, the contamination levels established in the present study are far above the ML of 0.10 µg/kg established by the EU for AFB_1 in baby foods and processed cereal-based foods intended for IYC (European Commission, 2010). Other studies have also found high concentrations of mycotoxins above the MLs in foods meant for IYC in SSA countries like Nigeria (Chilaka *et al*., 2016) and Burkina Faso (Ware *et al*., 2017).

In all the samples where AFB_1 exceeded the ML, 70 and 85% of groundnuts and maize respectively were sourced from markets. This observation contradicts the reports by Lewis *et al.* (2005) which previously established that home-grown foods are more prone to contamination with AFs than market-purchased ones. This is probably because, unlike market-sourced cereals where one can access multiple types of cereals from different sources, reliance on home-grown food may not allow access to multiple types of cereals that are mixed to obtain a composite flour due to different production cycles. The practice of mixing multiple portions of cereal to form composite flour has been found to increase the likelihood of contamination with AFs in the current study as in previous ones (Blankson & Mill-Robertson, 2016; Kimanya *et al*., 2014). In a study done in Kenya, the higher proportion and levels of contamination in market-sourced food samples with AFs compared to homegrown ones were also attributed to the aggregation of cereal stock from different sources which can promote cross-contamination and increase contamination levels (Kang'ethe *et al*., 2017). Moreover, longer storage has previously been associated with a significant increase in contamination of cereals by AFs (Liu *et al*., 2006), as may be the case with market-purchased samples.

The dietary exposure of IYC to AFB_1 estimated in the current study ranged from 0.33 to 1168 ng/kg bw/day with a median of 23.08 ng/kg bw/day. Consequently, the estimated MOE for IYC in the present study ranged from 0.34 to 1212 with a median of 17. This implies that all IYC (100%) were exposed to AFB_1 above 0.04 ng/kg bw/day with a MOE < 10 000, indicating that they are all a cause of public health concern (Barlow *et al.*, 2006; EFSA, 2020). The estimated dietary exposure to AFB_1 for IYC was mainly contributed to by a combination of maize and post-blended flour ingredients for which the highest exposure of 1168 ng/kg bw/day was estimated in this study.

The maximum dietary exposure of AFB_1 was slightly higher for young children (1168 ng/kg bw/day) than for infants (418 ng/kg bw/day) but the difference was not statistically significant ($p = 0.86$). On the other hand, a higher risk of exposure to AFB_1 was determined for cereal-groundnut-based CFs consumed by 60% of children compared to maize only-CFs consumed by 31.40% of the children, and the difference was not statistically significant ($p = 0.33$). More research is, therefore, necessary to inform on the contribution of groundnuts to the exposure of IYC to AFs through CFs. The monotonous consumption of groundnut and maize-based CFs which are susceptible to mycotoxin contamination could have promoted the exposure of IYC to AFs (Ruel, 2003). Dietary exposures of IYC to AFB_1 have also been reported in other parts of SSA, for instance, in the range of 2.5 to 51 192 ng/kg bw/day in Nigeria (Ojuri *et al.*, 2018). The dietary exposure of IYC to AFB_1 in the current study was of public health concern. This could be because the mothers mostly purchased the ingredients for CFs, that may have been stored for long and transported under various weather conditions from various parts of the country, or used their ingredients which may have been stored for long after harvest, both of which may have increased their risk of contamination by AFs, although the difference in exposure between the sources was not statistically significant ($p = 0.60$). The use of freshly harvested cereals for preparing CFs for children as opposed to long-term stored cereals which are more prone to contamination by AFs may minimize the exposure of IYC to AFs. This agrees with the observations by Liu *et al*. (2006) that the risk of exposure to AFs escalates with increased storage of food. According to Temba *et al*. (2017), storage time can affect maize–groundnuts composite flour by increasing AFB_1 contamination The lack of dietary diversity could be another contributing factor to the risk of increased dietary exposure of IYC to AFs.

4.2.3 Acceptability of the Improved Complementary Porridge Flour for Reduction of Exposure to Aflatoxins

The mothers in the present study seem to accept and appreciate the AFSaBF and AFSaGP formulations well. Some of the reasons for the observed acceptability included better feeding and better health of IYC among others. Besides, all mothers reported to enjoyed feeding their IYC with the AFSaGP and AFSaBF. During the feeding of the recommended AFSaBF and AFSaGP to the indexed child, other household members reported having tasted the prepared porridge, and they said it was delicious and the smelt was good. Acceptability is an important aspect that can demonstrate the possible uptake levels of improved formulations as has been demonstrated in other intervention studies involving the formulation of different ingredients of CFs in Tanzania (Boateng *et al*., 2018; Martin *et al*., 2010; Muhimbula *et al*., 2011). According to Babu (2000), the acceptability of food has a crucial role in determining the success of food and nutrition intervention programs. Therefore, our results were fascinating that the mothers in the study area accepted the improved formulated products as ingredients for CFs for their children.

The most important aspect of the AFSaBF and AFSaGP formulations is that they contained lower safe levels of AFs compared to homemade flour. This is because the maize and groundnut ingredients that were used to formulate the pre-blended flour and the groundnut powder were cleaned to minimize the levels of AFs in the final products. This was necessary because, during the first trial of this study, it had been established that 78.57% of groundnuts and 48.78% of maize used to prepare home-made pre- and post-blended flour for IYC in the study area had AFB_1 contamination levels above ML of 5 µg/kg set by TBS. The AFSaBF and AFSaGP formulations had levels of AFB1 below ML 5 µg/kg and were assumed to pose no danger to the IYC. Therefore, unlike what the mothers commonly practice for IYC feeding in the district, the formulated products can certainly be said to be safe for infant feeding. Philips *et al*. (2020) conducted a parallel to the current one and reported that the provision of AFSaBF reduced the prevalence of detectable urinary AFM_1 (a toxic metabolite of AFB_1) by 81% following the provision of this AF-free blended flour for 7–10 days, signifying high acceptability.

These ingredients were sourced in the same locality, winnowed, and then carefully sorted to reduce their contamination by AFs. Sorting, for instance, can reduce AFs contamination due to the removal of damaged or rotten grains (Magoha *et al*., 2014). This is probably because damaged kernels, for example, can increase invasion by molds during storage and consequent accumulation of AFs (Torres *et al*., 2014; Waliyar *et al*., 2015).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The present study was conducted to assess complementary feeding practices and the risk of exposure to AFs among IYC in Kongwa, Tanzania. The study showed that the CFs in Kongwa are mainly prepared in form of stiff or thin porridge whose key ingredients are maize, sorghum, pearl millet, rice, pre-blended flour, and post-blended flour (both pre- and post-blend are composite cereal groundnuts mix differed in formulation process). The study further showed that a higher risk of exposure of the IYC to AFs was largely contributed to by maize and groundnut. The complementary flour consumption ranged from 14.02 to 198.22 g/ child/day with an average of 89.45 g/child/day. Generally, the exposures of IYC to AFB_1 were of public health concern. The mothers showed acceptability of the AFSaBF and AFSaGP which implies that future intervention and recommendation of new complementary feeding practices in this context may be successful. These formative research results suggest multiple interventions points to improve complementary feeding and reduce mycotoxin exposure in this population, including education messages package on feeding practices, mycotoxin control practices, and the formulation of CFs. Perhaps the best way to reduce the risk of exposure to AFs in this context will be through diet diversification and the replacement of maize and groundnuts with locally available foods such as pearl millet and legumes that are less prone to AFs contamination.

5.2 Recommendations

The findings of the present study indicate that IYC in the study area could be exposed to high levels of AFs through complementary feeding which calls for an emphasis on tackling this problem. The exposure of IYC to AFB_1 in this context was likely a result of the repeated consumption of cereal groundnut-blended flours as CFs. The present study thus recommends that:

(i) Owing to the small sample size and the fact that the research was conducted only in Kongwa, further research is necessary to account for the contribution of maize and groundnuts to the exposure of IYC to AFs through CFs in other parts of the country where this has not yet been established.

(ii) An observational study is necessary to test the acceptability of this AFSaBF and AFSaGP to establish the efficacy of this intervention. This can help to know if the provided flour was used for the targeted individuals, the IYC and if mothers trusted the researchers.

(iii) Proposing a trial to investigate the impact of these improved foods on IYC health, and should also pay attention to further AF contamination of these food products at the household level.

(iv) The current efforts to train mothers of IYC and the community on the proper processing of pearl millet and legumes like common beans to remove the anti-nutritional factors should be sustained to make these foods available for the replacement of maize and groundnut flours in CF formulations.

(v) Tanzania to adopt stringent limits of AFs in IYC food and enforce and monitor those limits at both the national and community levels.

(vi) There is a need for the provision of several interventions like education packages on feeding habits, mycotoxin control measures, and preparation of CFs in order to improve complementary feeding practices and lessen mycotoxin exposure in this population.

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APPENDICES

Appendix 1: Check-list questions to guide the FGDs and RTs

Infant Food Safety Project – Formative Research Phase
Nelson Mandela African Institution of Science and Technology
Cornell University

Protocol for Infant and Young Child Feeding Focus Groups and Recipe Trials

Introduction: Hello, my name is _________ and this is ________. I will conduct the discussion today and ________ will observe and take notes. We invited you all to talk about how you feed your babies. I will ask you several questions. Your personal opinions and views are very important for us. There are no right or wrong answers. Please feel welcome to express yourself freely during the discussion. Following our discussion, we will ask you to show us how you cook some foods for you babies.

The discussion will last for about one hour and the cooking session should also last about one to one and half hours. During this time, please give everyone the chance to express their opinions during the conversation.

Is this clear? Do you have any questions? [*Answer all questions participants might have*]. Let's get started.

Data collector name: _______________________________ Date: _____________________________

Village name: _______________________________________ Age group: ________________________

____

____

Section 1: General Lishe Practices and Recipes

1. What foods do you feed your infant in your home?
    a. [Let women list all foods.]
2. *If porridge or lishe came up, say:* You said that you feed your baby porridge or lishe?
   If porridge or lishe did not come up, say: What about lishe? Do you feed your baby porridge or lishe?
    a. If you do feed lishe, how old is your baby now?
        i. How old was your baby when you started feeding him/her lishe?
    b. What ingredients were in the lishe the last time you fed it to your baby?
    c. How much of ___ do you put in compared to ___ and ___.
    d. Is this the recipe (ingredients and quantities of ingredients) you make most frequently?
    e. If you have stopped feeding lishe, how old was your baby when you stopped?
        i. Why did you stop feeding lishe at this age?
3. Is there a lishe recipe that your baby prefers to eat more than others?
    a. If so, what is this recipe?
    b. Do you have those ingredients at home right now or do you need to purchase them at the market?
    c. If purchased, what form are they in? (i.e. unmilled, milled, paste, powder, etc.)
    d. Is this the same all year long or does this change by season?
4. Is there a lishe recipe that your baby does not like to eat?
    a. If so, what is this recipe?
5. Is there a lishe recipe that *you* prefer to prepare for your baby more than others?
    a. Why?

    b. Are these ingredients ones that you have at home right now or do you need to purchase these at the market?
    c. If purchased, what form are these in? (i.e. unmilled, milled, paste, powder, etc.)
    d. Is this the same all year long or does this change by season?
6. Is there a lishe recipe that you do not like to prepare?
    a. Why not?
7. What ingredients make lishe healthy?

Section 2: Steps of lishe preparation, cooking and feeding

1. Can you describe the way you prepare each ingredient for the lishe recipe that you make most often? What is the first step when you start to make lishe? (can include storage procedures, if stored over time)
    a. Are there any other steps you take to prepare lishe before you start to cook it?
    b. [If yes, continue to probe until all steps are complete. If no, continue to question 2].
2. Is there anything you do not like about preparing lishe?
    a. What might make this easier for you?
3. Please describe the way you cook this lishe once you have prepared them?
    a. Are there any other steps you take before you feed the lishe to your baby?
    b. [If yes, continue to probe until all steps are complete. If no, continue to question 4].
4. Is there anything you do not like about cooking or storing lishe?
    a. (Probe if needed: What might make this easier for you?)
5. Is there anything you do not like about feeding lishe to your baby?
    a. (Probe if needed: What might make this easier for you?)
6. How often do you cook lishe each week?
7. How often do you cook lishe each day?
8. How many times per day does your child eat lishe?

Section 3: Consistency and nutrient density

1. Do you prefer to feed your baby a thin or thick style lishe?
    a. Why?
2. If a health professional instructed you to make the lishe you feed your baby thicker, could you agree?
    a. Why/why not?

Section 4: Primary influences and perceptions of groundnut

1. Who provides you advice about how to prepare or feed lishe for your baby?
2. Have you ever had a negative experience feeding your baby lishe, for example diarrhea, a rash or vomiting?
    a. Please describe this/these experience(s).
3. Should groundnuts be fed to children at this age? Why/why not?
4. What have you heard about putting groundnuts in lishe?
5. If someone suggested that you put groundnut in lishe, would you be willing to try?
    a. Why/why not?
6. When does it get difficult to have groundnuts in the house to feed your baby?

Section 5: Perception of provided food

1. Have you heard of projects providing food, such as lishe flours?
    a. If yes, what is your impression of this practice?
2. What kinds of concerns would you have if you received lishe flour from a project?

Is there anything else you would like to tell me about preparation, cooking or feeding lishe to your baby?

Protocol for Recipe Trial

Introduction: Thank you for your participation in our discussion group. If you are able to stay, we would like to invite you to show us how you make porridge for your baby. We have set up a cooking area with some pots. We will ask 2 of you to make porridge just like you made it the last time you prepared porridge in your home. We will ask the others to observe and comment on your preparation.

We will also ask 2 of you to make a specific type of lishe with groundnut and let us know what you think about cooking this lishe.

The purpose of this is to deepen our discussion about porridge/lishe. There are no right or wrong ways to prepare lishe. While some of you will be cooking lishe we shall need the rest of you to observe and give us their comments.

Before we begin, we need to ask each of you two questions:

1. Have you ever fed your baby groundnuts, even if mixed in another food? Yes/No

2. If YES, does your baby ever show any signs of a runny nose, a rash or swelling of his/her skin, diarrhea or vomiting or shortness of breath within 1 hour of eating groundnuts? Yes/No

IF BABY HAS NEVER CONSUMED GROUDNUTS OR HAS SHOWN A SIGN OF POTENTIAL ALLERGY, MOTHER CAN STILL PARTICIPATE IN RECIPE TRIALS, BUT BABY MUST BE OFFERED GROUNDNUT-FREE FOOD.

For the enumerator:
For each ingredient please ask:

1. Why did you put in _____________?
2. Why did you put in that amount of __________?
3. Would you put this in for a younger baby or an older baby?
4. Is this the same all year long or does this change by season?

Consistency

1. *Please note consistency of porridge/lishe when cooked AND when mothers feed to baby Ask mothers:*
    a. Why is this the desired consistency?
    Cooked:

    When fed to baby:

    b. Does this change by age?

2. For the lishe recipe of 3:1 maize and groundnuts, please ask:
    a. Was the cooking time similar to other lishe they make at home?
        i. How so/how not?
    b. Would they be willing to make this at home
        i. Why/why not?
    c. What changes might they try at home?
        i. Why?
    d. How do mothers feel about the appearance, smell, consistency, flavor of 3:1 lishe?
    e. What is the appropriate feeding size and frequency for babies at this age?

Structured Observation Form for Lishe Made as if at home

Time started cooking: *Time ended cooking:*

Ingredient	**Amount used**	**Order**	**Techniques for adding ingredient**	**Notes, including seasonality and reason to add**

Please note mothers' comments, explanations, and reactions to each other's statements or actions during the cooking process

If children are present and consume lishe, please note:
children's responses

style of feeding or encouragement used

estimated amounts served

estimated amounts consumed by the children.

Structured Observation Form for 3:1 Lishe

Time started cooking: *Time ended cooking:*

Ingredient	**Amount used**	**Order**	**Techniques for adding ingredient**	**Notes, including seasonality and reason to add**

Please note mothers' comments, explanations, and reactions to each other's statements or actions during the cooking process

If children are present and consume, please note:
children's responses

style of feeding or encouragement used

estimated amounts served

estimated amounts consumed by the children.

Appendix 2: The 24 –Hour Dietary Recall Protocol

Nelson Mandela African Institution of Science and Technology and Cornell University
Infant Food Safety and Child Growth - Formative Research Phase
24-hour recall and food collection form
Day 1 and Day 15
MT1

No.	**Question**	**Response** (Enter or circle response)
F1	Date of visit	|___|___|/|___|___|/|___|___| DD/MM/YY
F2	Data Collector	1 – 2 – 3 –
F3	Ward	1 – 2 – 3 – 4 – 5 – 6 7 – 8 - ________________________________
F4	Village name	|___|___|___|___|___|___|___|___|___|
F5	Household ID	|___|___|___|/|___|___|___|/|___|___| Ward/Village/HH#
F6	Visit Code	|___|___| 1-6
F7	Baby's name	|___|___|___|___|___|___|___|___|___|___|
F8	Baby's DOB	|___|___|/|___|___|/|___|___| DD/MM/YY
F9	Baby's Sex	01 = Male 02 = Female
F10	Mother's name	|___|___|___|___|___|___|___|___|___|___|

Please note: This Table can be filled out when the other data collector is asking the mother these questions

Intro to participant
*Thank you again for speaking with us. I would now like to ask you more details about the foods that your baby eats. We would also like to collect some of these foods to explore ways to improve on food safety and nutrition for your baby. We will provide payment for these foods, and we will explain this more after our conversation. (*If Day 15 say*: "this is the same process as we did the last time you and I met".) This should take us about 1 hour. Are you ready to begin?*

Part 1 – Breastfeeding

F11	Are you still breastfeeding (name) now?	01 = Yes >> Skip PT 13 02 = No
F12	If no, what age was your baby when you stopped breastfeeding?	|___|___| Age in months
F13	Do you breastfeed your baby 1-10 times a day or more than 10 times a day?	01=1-10 times/day 02=more than 10 times/day
F14	Yesterday, did the baby eat about the same, more, or less amount of food than on a regular day? Why did the baby eat more or less?	01 = Same>>Skip to PT14 02 = More 03 = Less _________________________________ _________________________________
F15	Was yesterday a market day, a fasting day, or a special day in some way?	01 = Market day 02 = Fasting day 03 = Special day 04 = None of the above If special day, please describe: _______ ___________________________________

Part 2 – 24 hour recall

Passes 1 and 2 (Name of food consumed and time)			Pass 3 (if single food)	Pass 3 (if mixed food prepared, record recipe below)				Pass 4 (Quantity consumed by infant)		
Episode (#)	Food/Drink/ Breast milk	Time fed (Swahili time HH:MM and circle time of day)	Description of food (See Table 1)	Ingredient and description (see Table 1)	Amount	Units (Cup; Tbsp; Tsp; Pinch; grams; ml;)	Measure (Actual food/ Water/playdough)	Amount eaten by baby (See Table 2 for desired measurement by food)	Units (Cup; Tbsp; Tsp; Pinch; grams; ml;)	Measure
	________	Asubuhi / Mchana/ Jioni /Usiku	________ ________	________ ________ ________ ________ ________ ________	________ ________ ________ ________ ________ ________	________ ________ ________ ________ ________ ________	________ ________ ________ ________ ________ ________	________	________	Actual food/ Water/playdough
	________	Asubuhi / Mchana/ Jioni /Usiku	________ ________	________ ________ ________ ________ ________ ________	________ ________ ________ ________ ________ ________	________ ________ ________ ________ ________ ________	________ ________ ________ ________ ________ ________	________	________	Actual food/ Water/playdough

F15	Has your baby taken any nutritional supplement in the past 24 hours? *(Can verify supplement with Clinic card if available*): *(Circle all choices that apply)*	00 = No 01 = Mirconutrient sachet 02 = RUTF (i.e. Plumpynut or similar) 03 = Liquid or tablet vitamins (eg. Vitamin A, multivitamins) 04 = Others (specify):______________________
F16	Date of last Vitamin A supplement (only record if clinic card had date recorded)	|___|___|/|___|___|/|___|___| DD/MM/YY 00 = No Vit A
F17	Has your baby taken any medication in the past 24 hours: *(Circle all choices that apply)*	00 = No 01 = Anti-malaria 02 = Deworming tablets 03 = Schistosomiasis treatment 04 = Others (specify):______________________
F18	Date of last deworming tablet *(only record if clinic card had date recorded)*	|___|___|/|___|___|/|___|___| DD/MM/YY 00 = No deworming

Appendix 3: Food collection form

Foods that qualify for food collection include:

1. Maize
2. Groundnuts
3. Lishe flour
4. Millet, finger millet or sorghum ONLY if the baby is not fed any maize

We will not collect other foods consumed by the infant.

Guidelines for Food collection

Step 1: For each food, ask if there is any stored food within the household. If yes, please seek permission to see quantities stored. If that particular food is not available, please note in final column of tables above.

Step 2: With the respondent's permission to access the stored food take a random, representative sample of each food stored in the household (one for each).

Grain and Groundnuts Sampling

Between 250- 500g of each food consumed by a 6–12-month-old in the last 24 hours will be collected; depending on how much is available in stock and the much a household is willing to share.

Where maize stocks are more than 50 kg, take 4 random sub- of approximately 500 g (samples from different sides of the bag and the middle) and aggregate those into a 2 kg mixed sample. Draw a 500g sample from the thoroughly mixed sample. *However, it is not always possible to have a representative sample from each of the stored bags. Make sure to collect a representative sample from the bag that is already open and being consumed by the household currently. Record the quantity of food from which the representative sample was drawn (for examples 500 g of maize out of 80 kg (4 debes), and also note how many more bags are in storage and were not sampled from, in Table 3 below. If there were 6 bags of 120 kg left in the previous example, then record 500 g/ 80 kg under gramu ya sampuli, and then 6 full bags of 120 kg i.e. choice 1: 6 magunia ya kilo 120.*

For quantities between 20 and 50 kg randomly take 4 random sub-samples of approximately 250 grams, mix them thoroughly and draw a 250 g final sample. For quantities below 20 kg, take 3 random sub-samples of 250 g, aggregate and mix them thoroughly, and then draw a final 250 g sample.

In any household where maize is stored in cobs, randomly collect at least 5 cobs from the middles and all sides of the batch. In households where groundnuts are stored in pods, randomly pick enough pods, from the middle and the sides of the batch, to fill in a 1 kg Ziplock bag. Any deviations from this sampling procedure should be carefully documented, especially in cases where taking a representative sample could not be done and reasons why.

Flour Sampling

Preferably, collect milled food whenever these are available. Milling ensures a more homogenous food sample. For quantities less than 1 kg of milled maize, we will request for about 250g (*Robo kilo)* of flour. From past experience groundnuts and Lishe flours were rare and whenever households had stock of such, they were in small quantities. In the event we find limited quantities of these flours we collect a sample of approximately 100 g.

Payment for each of the food sample will be as provided under step 5 below.

Quanitities of each food collected (whole grain or flour) will be recorded in Table 3 below

Table 3: Quantity of food sample

Food	Number of the sample (**HH01-MH-001**) HH number, food type and sample number	Amount of food sample ____g/_____kg	Quantity of cereal/unga 1=___Sacks of 120kg 2=____ Sacks of kilo 90 3=____ Sacks of kilo 50 4=____Bucket of kilo 20 5=____Small bucket of 5 kg 6=____Not easy to estimate
Lishe (LS)			
Mahindi (MH)			
Karanga (KR)			
Finger millet, millet or sorghum ONLY IF baby not fed maize			

Step 3: Place sample in clean Ziplock bag and make sure all the air is squeezed out and the bag is tightly zipped for transport. Do not re-use a bag or combine ingredients in the same bag.

Label each with a unique HH descriptors and food sample ID.

- Unique household descriptors include **Ward and Village name**, **Household Number**. For example Sejeli- followed by village name, and survey round #. The HH number will appear under unique sample ID and will range from 01-60 and survey rounds 1 and 2. We will derive these codes at the beginning of each visit to a village and record appropriately.
- This will be followed by a unique food sample ID. Which will be as follow HH number-sample code-Sample number. For example **HH01-MZ-001**. Other sample codes include GN for groundnuts, LS for lishe. Sample number will range from 001 to 60.
- Date of sample collection

- Date of harvest____________

Record the following information for each food sample collected in the Table below:

Food crop	**Maize**	**Ground nut**	**Lishe**	**Finger millet, millet or sorghum ONLY IF baby not fed maize**
Whole grain or flour? (Circle one) 1= Whole grain 2=Dona (Whole grain flour) 3= *Sembe (dehulled/sifted) flour* *4= Other (specify)* ___________				
Purchased or home grown? (Select and fill in ONE) 1= Purchased; 2= Home grown 3= Received as gift; 4= Mixed				
***If purchased*, w**hen did you purchase this food? ________ weeks/months ago				
***If purchased*: where?** 1= From neighbours/ Within the village; 2= Kibaigwa regional market 3= Other wards *Specify* (_______________)				
If homegrown, how long have you stored this crop/ food? __________ Weeks/ Months				

If homegrown, what was the source of seed for this food/ crop? Source of seed for this food 1= Local/ Previous harvest 2= Purchased from seed company 3= Purchased regular maize 4= unknown 5= others (Specify)				
What type of seed/ variety did you cultivate/ grow for this crop? 1= DK1 2= Stuka 3= TMV 4= Kilima 5= Staha 6= Seedco 7= Pioneer 8= Others (specify)_______________				

Step 4: At the end of every day sampling visit, the team should DOUBLE check all the sample labels to make sure they are consistent and follow the previous day's numbering.

Step 5: Transport samples to lab and record contents and ID numbers in lab-book

Step 6: Payment for food collected

We will pay a flat rate for each sample, depending on the amount:

- Tsh 1,500 for 100-250 g of groundnut, sesame, soybean, Lishe, Rice and other nuts or its flour
- Tsh 1,000 for 100 and 250 g of maize or maize flour sample
- Tsh 1,500 for 500 g of maize or maize flour

Step 7: Closure and future visits

Thank you very much for your participation in the study. We appreciate your participation.

Data collector: Please review forms to make sure they are complete. When you have completed checking the forms, please thank the mother again for her time.

Do you have any questions for us?

(Please record)

____
____
____

RESEARCH OUTPUTS

(i) Publications

Mollay, C., Kassim, N., Stoltzfus, R., & Kimanya, M. (2020). Childhood dietary exposure of aflatoxins and fumonisins in Tanzania: A review. *Cogent Food & Agriculture,* 6(1), 1859047.

Mollay, C., Kassim, N., Stoltzfus, R., & Kimanya, M. (2021). Complementary feeding in Kongwa, Tanzania: Findings to inform a mycotoxin mitigation trial. *Maternal & Child Nutrition*, *17*(4), e13188.

Mollay, C., Kimanya, M., Kassim, N., & Stoltzfus, R. (2022). Main complementary food ingredients contributing to aflatoxin exposure to infants and young children in Kongwa, Tanzania. *Food Control*, *135*, 108709.

(ii) Poster Presentation