AFLATOXIN EXPOSURE OF LACTATING MOTHER-CHILD PAIRS AND NUTRITIONAL STATUS OF BREASTFEEDING CHILDREN 0-6 MONTHS IN MAKUENI COUNTY, KENYA

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DECLARATION

This dissertation is my original work and has not been submitted to any other university.

Signature _____ Date _ 4/5/2022 _____

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DEDICATION

I dedicate this work to my parents Peter Ogalo and Mary Juma for their unrelenting sacrifices and support. I also dedicate this work in the memories of my siblings Linet Adhiambo Ogallo and Evans Obote Ogallo, and thanks to my entire family. I am also forever grateful for the overwhelming support from my wife Loreen Adema, and my children Allan Cody, Kade Keynan, and Tawala Mary Adajuma. Without your constant smile and love, I would not have reached this far. May God's love abound!

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ABBREVIATIONS AND ACRONYMS

AFB1	Aflatoxin B1
AFM1	Aflatoxin M1
ANOVA	Analysis of Variance
BMDL	Benchmark Dose Level
B.w.t	Body weight
CD	Cluster of Differentiation
DNA	Deoxyribonucleic Acid
EBF	Exclusive breastfeeding
EFSA	European Food Safety Authority
ELISA	Enzyme Linked Immuno-Sorbent Assay
EPI	Expanded Program on Immunization
EU	European Union
FAO	Food and Agriculture Organization
FHI	Family Health International
HPLC	High Performance Liquid Chromatography
IAC	Immunoaffinity Column
IARC	International Agency for Research on Cancer
IEBC	Independent Electoral and Boundaries Commission
KDHS	Kenya National Demography Survey
KEBS	Kenya Bureau of Standards
KNBS	Kenya National Bureau of Standards
KNH/UoN	Kenyatta National Hospital/ University of Nairobi
ERC	Ethical Review Committee
KPHC	Kenya Population and Housing Census
MIYCN	Mother, Infant and Young Child Nutrition
MoALF	Ministry of Agriculture, Livestock and Fisheries
MOE	Margin of Exposure
NEBF	Non-exclusive breastfeeding
SID	Society for International Development
SPSS	Statistical Package Software for Social Sciences
UNICEF	United Nations Children's Fund
WDD	Women Dietary Diversity
WDDS	Women Dietary Diversity Score
WFA	Weight-for-age
WHO	World Health Organization

OPERATIONAL TERMS AND DEFINITION

Benchmark dose level	Levels at which quantities of a toxic substance is considered enough to induce an effect for example tumor on an exposed animal or human subject
Complementary feeding	Introducing children to solid or liquid foods after feeding on breast milk for the first six months
Exclusive breastfeeding	Giving only breast milk to newly born babies for the first six months. This does not include solid or liquid foods except oral rehydration solutions, vitamins, syrups, minerals, and medicine when the need arises
Exposure	Intake of a substance by an individual or a population periodically over time
Intake	Quantities of a substance (food/toxins) consumed by an individual expressed per body weight
Margin of exposure	The yardstick for assessing safety or risk levels of a food substance that has genotoxic or carcinogenic potential
Non-exclusive breastfeeding	Giving children breast milk alongside liquid or solid foods during their first six months after birth
Weight-for-age	Growth indicator for determining whether a child has low weight for their age. Determined using z score of <2 SD. It determines underweight
Women dietary diversity	Different number of food groups consumed within a 24-hour period by women collectively deemed sufficient or insufficient for adequate diet

ABSTRACT

Aflatoxins are fungal metabolites, once ingested in food, are detoxified in the liver and transferred into breast milk, urine and tissues. Their accumulation in the body can lead to malnutrition, aflatoxicosis, or cancer which are predominant in sub-Saharan Africa. The southeastern region of Kenya is prone to aflatoxin outbreaks yet exposure levels of the vulnerable population such as breastfeeding children and lactating mothers remain unclear. This study assessed aflatoxin exposure of lactating mother-child pairs, and nutritional status of breastfeeding children aged below six months.

A descriptive cross-sectional study with an analytical component was conducted. Information on socio-demographic characteristics, dietary habits, breastfeeding practices, maize handling and storage practices, and weight of 170 lactating mother-breastfeeding child pairs were collected. A total of 48 breast milk and urine samples were collected from respective lactating mothers whose food samples were picked for analysis. Aflatoxins in the food sample were determined using high-performance liquid chromatography (HPLC). Quantification was done using an enzyme-linked immunosorbent assay (ELISA). Statistical analysis was done using the Statistical Package Software for Social Sciences (SPSS). The level of significance level was set at $p < 0.05$.

Among 170 mothers interviewed, 45.3, 49.4 and 5.3% were from low-, middle- and high-income households, respectively. Of them, 48.2% had not attained basic primary education. Food consumption patterns showed a generally low dietary diversity with the mean women dietary diversity score being 3.4 (*SD*, 1.5), aflatoxin food score being 25%, and 45.9% of lactating mothers eating at least four (4) different foods in the preceding 24-hour period. All lactating mothers (100%) consumed maize and other cereal-based foods per week. The rate of exclusive breastfeeding was 44.1% and at least 45% used cereal-based complementary foods daily. Average breast milk intake was 82.3 (*SD*, 31.7) ml/kg b.w.t/day (31.6 to 157.8). About 50% sourced maize from the market, 50% never treated their maize, and at least 20% stored maize in containers that promote aflatoxin contamination. Aflatoxin was detected in 85.4% (41/48) food samples where over 90% of the positive food samples were above 10 and 2 µg/kg Kenya Bureau of Standards (KEBs) limits for total aflatoxin and aflatoxin B1, respectively. Mean concentration of total aflatoxin was 97.8 µg/kg (*SD,* 57.7; range 2.3 to 210.0), while aflatoxin B1 was 9.0 µg/kg (*SD*, 7.7; range, 0.7 to 32.3). Subsequently, mean dietary intake of

total aflatoxin and aflatoxin B1 were 7.6 μg/kg/b.w.t/day (*SD*, 7.5; range, 0.0 to 23.9) and 0.6 (*SD*, 0.6; range, 0 to 1.9), respectively. Aflatoxin M1 was however detected in 77.1% (37/48) breast milk samples with about 62% exceeding 0.025 μg/kg EU limits. Mean level of aflatoxin was 35 ng/l (*SD*, 0.0; range 5 to 77), while mean intake was 0.47.μg/kg b.w.t/day (*SD*, 0.50; range, 0.0 to 1.7). All urine (100%) had aflatoxin M1 with a mean of 0. 39 ng/ml (*SD*, 0.16; range, 0.15 to 0.82). Total aflatoxin in mothers' diet significantly contributed to levels of aflatoxin M1 in breastmilk ($p = 0.00$), and urine of breastfeeding children ($p = 0.01$). Aflatoxin B1 intake also influenced aflatoxin M1 in breastmilk of exclusively lactating mothers ($p = 0.01$). Education level negatively influenced aflatoxin B1 intake of exclusively lactating mothers ($p = 0.01$), while dietary diversity significantly reduced aflatoxin M1 intake of non-exclusively breastfeeding children ($p = 0.04$). Socioeconomic status was not a significant predictor of aflatoxin even though it showed a positive correlation with aflatoxin B1 intake ($tb = 0.24$, $p = 0.042$ and a negative correlation with aflatoxin M1 in the urine of exclusively breastfeeding children ($tb = -0.35$, $p = 0.041$). No significant correlation was reported between weight-for-age z-scores with aflatoxin exposure ($p_{all} > 0.05$). However, exposure levels of both lactating mothers and breastfeeding children were extremely high with a margin of exposure (MOE) of < 10, 000.

This study concludes that mothers' diets exposed exclusively, and non-exclusively breastfeeding children aged six months and below to high aflatoxin intake in the study area. As a result, knowledge, attitude, and practices that mitigate aflatoxin contamination in diets and breast milk of lactating mothers as well as clear county government policy on the sale and distribution of aflatoxin-contaminated maize should be introduced in the study area.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Aflatoxins are secondary metabolites of fungal origin. They are released as spores that can withstand a range of extreme environmental conditions (Kumar et al., 2021). Their occurrence around the globe depends on geographic, climatic, agronomic, and agricultural factors (Mahato et al., 2019). They can enter foods pre- or post-harvest (Kumar et al. 2021), and subsequently be consumed by humans and animals. Due to their deleterious effects on animal and human health, they are intensively studied (Akbar et al., 2019). The first incidence of aflatoxins' potency was reported in 1960 when 10,000 turkeys and ducklings died in the United Kingdom (Bhat et al., 2010; Blount, 1961). Since then, several incidences of aflatoxin contamination have been reported in the world.

Several studies have been conducted on aflatoxin occurrence in Kenya since fatal cases were reported in 1981 due to aflatoxicosis in lower eastern regions of Kenya (Machakos, Kitui, Mwingi, and Makueni Districts) (Omara et al., 2021). Among studies examined by Omara et al. (2021), maize was given more focus. Other foods that were given attention include sorghum, peanuts, millet and animal milk, and animal feeds. A few studies were also conducted on human serum and urine. Alongside other studies done on aflatoxin M1 and dairy milk, (Bervis et al. 2021) showed that aflatoxin ingested from contaminated feeds could be traced as residues in the milk of dairy animals. This kind of association spurred more research on the occurrence of aflatoxin in human breast milk. However, to the best of my knowledge, only two studies (Kang'ethe et al., 2017; Maxwell et al., 1988) have determined the presence of aflatoxin M1 in the breast milk of lactating mothers in Kenya. Compared to other parts of the world particularly in Asian countries where the occurrence of aflatoxin is also prevalent (Coppa et al., 2019), exposure of exclusively and non-exclusively breastfed children below six months to aflatoxin M1 has not been given more focus in Kenya. This information remains scanty yet the presence of aflatoxin in breastmilk remains a public health concern in areas where aflatoxin occurrence is more prevalent. Breast milk is important during the first six months of life. It is considered safe and acts as the primary source of nutrition for the young ones before they can eat other foods (Boquien, 2018). Older infants may also continue to breastfeed in combination with other foods (Lutter et al., 2021). The exact composition of breast milk, however, varies from day to day and is influenced by the mother's diet (Boquien, 2018).

In Kenya, staple foods are frequently contaminated with aflatoxin at high levels, and the incidence of occurrence is reported almost yearly, particularly in southeastern regions of Kenya (Omara et al., 2021). A study by Nying'uro (2020) predicts an increase in incidences of aflatoxin occurrence in hotspot areas in Kenya due to climate changes. Furthermore, incident rates of cancer including hepatocellular carcinoma which is mostly associated with toxins among children below five years are on the rise in sub-Saharan Africa (Stefan et al., 2017). In 2014, the report by Kenya Demographic Health Survey (KDHS) also showed that most children in the country are likely to become stunted before their second birthday. However, the overall prevalence of stunting (26%), underweight (11%), and wasting (4%) in KDHS (2014) showed a reduction from those reported in previous years. However, these trends are expected to decrease further due to the comprehensive Nutrition Action Plan 2018-2022 (Government of Kenya, 2018). Within this program, Kenya's national and local governments alongside several non-governmental organizations aim to scale up, among others, maternal, infant, and young child nutrition (MIYCN). However, the government's effort to ensure food safety and control measures for aflatoxins, still faces several challenges, especially among small and medium enterprises dealing with maize (Joutsjoki and Korhonen, 2021). These challenges still pose a threat to ensuring that both exclusively and non-exclusively breastfeeding children are not exposed to aflatoxins through breast milk and complementary foods. To reduce the negative impact associated with aflatoxin, it is therefore important to reduce the risk of maternal-to-child aflatoxin exposure, especially in areas where aflatoxin occurrence is more prevalent.

1.2 Statement of the problem

High and persistent prevalence of aflatoxin over the years in Makueni County could imply a high and persistent prevalence of aflatoxin in the breast milk of lactating mothers and consequently higher exposure levels of breastfeeding children to aflatoxin intake compared to other parts of Kenya. Recent comparative results between that of Nabwire et al. (2020a) (100% prevalence of aflatoxin in 338 maize samples in Makueni County) and that of Njeru et al. (2019) (< 20% prevalence of aflatoxin on 367 maize samples picked in four counties in the western region of Kenya) are still indicative of the existence of aforementioned trend of the high prevalence of aflatoxin in Makueni County than other regions in Kenya. Results of a review study by Joutsjoki and Korhonen (2021) show that low implementation of aflatoxin control measures and harsh climatic changes could be the main reasons for elevated levels of aflatoxin in the study area. A study by Kilonzo et al. (2014) also concluded that most households in Makueni County consumed maize-based foods that are highly contaminated with

aflatoxins. While the World Health Organization (WHO) only recommends breast milk as the safest food for children below six months of age, high prevalence and concentration levels of aflatoxin in breast milk could pose a serious food safety and nutrition challenge to newborns in the study area as compared to other parts of Kenya. This is because newborns are considered to have a weak immune system that cannot adequately withstand the effects of high exposure to toxins. However, among children, it is only Maxwell et al. (1988) and Kang'ethe et al. (2017) who have determined the presence of aflatoxin in the breast milk of lactating mothers in Kenya, while a majority of studies have given more focus on aflatoxin contamination in foods. However, several similar studies of aflatoxin in breast milk have been conducted in other countries. A comparison by Coppa et al. (2019) review reveals that studies on aflatoxins in breast milk are not common in Kenya, and thus information regarding their prevalence and levels is lacking in the study area. Moreover, the aforementioned studies conducted in Kenya did not segregate children aged six months and below into those who are exclusively and non-exclusively breastfed. At the time of conducting this study, information regarding their exposure to aflatoxin intake remains scanty yet exposure levels could be an under-evaluated risk factor in Kenya.

1.3 Justification of the study

Recurrent incidences of aflatoxin contamination and outbreaks of aflatoxicosis in the Eastern region of Kenya emphasize the need to quantify and control aflatoxin levels in the diets of both adults and children in the study area. Elucidating maternal and child risk levels to aflatoxin exposure in Makueni County increases research attention on breast milk safety in Kenya. This has a great potential of allowing policymakers to develop adequate interventions that can mitigate exposure levels of lactating mothers and breastfeeding children to high aflatoxin intake. The results can also be used to inform specific existing breastfeeding policies in regions where aflatoxin contamination is more prevalent. As aflatoxin is highly potent, especially in children, its chronic intake even in small doses can lead to deleterious health effects such as acute and chronic aflatoxicosis, malnutrition, and carcinoma among other morbidities. If much focus is not given, this can silently continue to contribute to a high disease burden and ultimately reduce children's future working potential. Therefore, determining levels of aflatoxin in diet and breast milk of lactating mothers, and urine of breastfeeding children and its impact on weight-for-age z-scores remains imperative in Makueni County.

1.4 Aim of the study

The study aims to contribute towards the improvement of food safety as well as maternal and infant and young child health and nutrition during the lactation period.

1.5 Purpose of the study

The purpose of the study is to generate information on the current situation on the levels of aflatoxins in food and breast milk of lactating mothers in Makueni County as a basis for emphasizing the constant need for monitoring aflatoxin occurrence in at-risk regions.

1.6 Main objective

To assess aflatoxin exposure of lactating mothers-child pair and nutritional status of breastfeeding children aged six months and below in Kibwezi West, Makueni County, Kenya

1.6.1 Specific objectives

1. To describe the demographic and socioeconomic status of the lactating mothers in Kibwezi West, Makueni County.
2. To determine food consumption pattern, dietary diversity, post-harvest maize handling, and breastfeeding practices of lactating mothers in Kibwezi West, Makueni County
3. To determine the levels of aflatoxin in breast milk, maize-based foods consumed by lactating mothers, and urine of breastfeeding children in Kibwezi West, Makueni County.
4. To determine the nutrition status of breastfeeding children below six months based on weight-for-age z-scores in Kibwezi West, Makueni County.

1.7 Study Hypotheses

1. There is no association between demographic and socioeconomic characteristics of lactating mothers with the occurrence of aflatoxin in the study
2. There is no association between food consumption pattern, dietary diversity, maize handling, and storage practices, breastfeeding practices of lactating mothers with the occurrence of aflatoxin in the study
3. There are no associations between aflatoxin in food of lactating mothers, aflatoxin in breast milk, and aflatoxin in the urine of exclusive and non-exclusive breastfeeding children aged below six months in the study

4. There is no association between aflatoxin exposure of lactating mothers and breastfeeding children aged six months and below with weight-for-age z-scores outcomes in the study

CHAPTER TWO: LITERATURE REVIEW

2.1 General overview of aflatoxin

Molds or fungi fall are spore-forming plants that are ever-present in the atmosphere (Adejumo and Adejoro, 2014). Their occurrence of foods depends on several factors including climate, type of foods, and pre-and post-harvest practices (Kumar et al. 2017). Due to their versatility, they can thrive in a wide range of temperature, acidity, and moisture content levels (Kumar et al. 2017). Their occurrence is however more prevalent in hot and humid areas. Once they land on foods, they multiply using their spores producing minuscule toxins known as mycotoxins (Adeyeye, 2016). The resilient nature of these secondary metabolites has become a nuisance to human beings for a long time, especially in countries around tropical regions (Ráduly et al., 2020). Most known types of these toxins include aflatoxins, fumonisins, ochratoxins, patulins, zearalenone, and ergot alkaloids among many others (Ráduly et al., 2020). Among the aforementioned secondary metabolites, more study reviews have been done on aflatoxin due to its history of aflatoxicosis, frequent occurrences in a wide variety of foods, and its more chemical types and lethal potency to humans and animals as opposed to its counterparts (Ostry et al., 2017).

2.2 Types of Aflatoxin

Aflatoxins that are renowned are about 20 types and are produced by *Aspergillus* species (Kumar et al., 2017). When aflatoxins are screened using ultra-violet rays, some emit green, while others are blue color. Those that emit blue colors belong to the aflatoxins B group, and green colors belong to the aflatoxins G group. Under the aflatoxin B group, we have B1 and B2, and subsequently, within the G group, there is aflatoxin G1 and G2 (Kensler et al., 2011). Further analysis shows that aflatoxin B1 and B2 can be further broken down to M1 and M2, respectively once they are ingested by humans and animals (Iqbal et al., 2015). All the aflatoxins are detrimental to humans (Rushing and Selim 2019), but aflatoxin B1 is considered more toxic than the others (Ráduly et al., 2020).

2.3 Factors Promoting Occurrence of Aflatoxin

Several factors promote the occurrence of aflatoxins in foods (Kumar et al., 2021). High humidity and hot temperatures have been cited as the most critical factor for aflatoxin occurrence (Diao et al., 2013). For instance, Kumar et al. (2021) report that optimal synthesis of aflatoxins by fungi species commences at 28^0C and progresses to maximum temperatures of about 40^0C. Similarly, Villers (2014) demonstrated an exponential increase in aflatoxins when

fungi species were subjected to the relative humidity of 65% onwards. These results were the same as those reported by Muga et al. (2019) who reported that aflatoxin production was maximum at 30^0C and relative humidity of 90%. These factors, however, according to Negash, (2018), are also tied to the type of environment. The occurrence of aflatoxin in tropical regions is also associated with high temperatures. Similarly, due to climate change, high temperatures and high humidity during summer in temperate regions have lately been shown to be conducive to aflatoxin contamination (Valencia-Quintana et al., 2020). Nonetheless, aflatoxin contamination is also affected by the type of food (Jallow et al., 2021). Tai et al. (2020) reported the occurrence of *Aspergillus flavus* in different foods. Their report showed that varying levels of aflatoxin occurrence are associated with varying levels of food acidity, alkalinity, moisture, and nutrient contents. An increase in food nutrient contents (Liu et al., 2016) and different degrees of acidity, alkalinity, and water activities (Jallow et al., 2021) provide rich substrate media for further growth of fungi and release of aflatoxins. However, mechanical damage due to insect activities, and heat stress during drought also favor the invasion of fungi into foods and crops (Jeyaramraja et al., 2018). For example, weevils and other insects create openings that allow *Aspergillus* species to gain access inside the grains of maize, groundnuts, and other cereals. Once inside the grains, they multiply in numbers as they produce aflatoxin (Jeyaramraja et al., 2018). Poor agronomical practices such as the use of infected seeds, poor timing of planting and harvesting, and poor post-handling of crops also exacerbate the occurrence of aflatoxin (Marete et al., 2020). Lastly, a compilation by Jallow et al. (2021) shows that type of fungi species also determines aflatoxin contamination in foods. In their review, *Aspergillus flavus* affect a wide range of foods including cereals and nuts, while *Aspergillus parasiticus* on the other hand, is highly and only associated with contamination of peanuts and maize. Other species like *Aspergillus nomius, Aspergillus novaparasiticus,* and *Aspergillus arachidichola* among others are also associated with contamination of other types of foods.

2.4 History of aflatoxin outbreak in Kenya

In 1961 aflatoxin was isolated as a result of the deaths of turkeys and ducklings that had fed on animal feeds (Sargeant et al., 1961). Again, it was identified as the cause of human deaths in India (Krishnamachari et al., 1975). Another similar incident happened with children in Malaysia (Chao et al., 1991). From then on, several deaths and food poisoning related to aflatoxins were reported. In Kenya, the first incidence was reported in 1978, followed by 1981 (Ngindu et al., 1982), 1982 (Moturi, 2008), and 1998 in eastern regions of Kenya (Mutegi et

al., 2018). In all these cases, deaths were reported. Later in 2001, 26 cases of aflatoxicosis from consumption of aflatoxin-contaminated maize were reported in Maua; with 16 deaths due to severe liver damage reported (Probst et al., 2007). In 2003, six deaths again from consumption of moldy maize were reported in Thika District (Onsongo, 2004). However, in 2004, the most severe aflatoxicosis outbreak was reported in four districts within the eastern region of Kenya. The highest incidence was reported in Makueni, Kitui, Machakos, and Thika Districts in that order. However, of the 317 cases of aflatoxicosis, 125 deaths were reported (Lewis et al., 2005; Muture and Ogana, 2005). Again in 2005, another aflatoxicosis outbreak in Kenya was reported among 75 people who consumed aflatoxin-contaminated maize. Forty-two (42) death cases were reported in Makueni, Kitui and Machakos districts (Azziz-Baumgartner et al., 2005). In 2006, several cases of acute poisoning occurred in Ndithini (Machakos district), Mutomo (Kitui district), and Matiliku/Kisau (Makueni district) among individuals who consumed aflatoxin-contaminated cereals; with 10 and 11 deaths reported in Mutomo and Makueni district, respectively (Daniel et al., 2011). In May 2007, among four cases of acute aflatoxicosis that occurred in Kasekeu/Makindu (Kibwezi district), two deaths were reported (Wagacha and Muthomi, 2008). In 2008, high levels of aflatoxin were reported in maize samples collected in Embu, Mutomo, and Kibwezi districts where three persons were hospitalized while two deaths were reported (Muthomi et al., 2009). In 2010, 29 districts in the eastern region of Kenya were reported to be at risk of aflatoxin maize contamination (Muthomi et al., 2012). Since then, serious cases of aflatoxicosis outbreaks have never been reported. However, cases of maize contaminated with aflatoxin have always featured in Kenya news almost every year.

2.5 Food and Aflatoxin

As long as favorable conditions exist for the spread and growth of *Aspergillus*, many foods will be susceptible to aflatoxins contamination (Mahato et al., 2019). Unfortunately, cereals which constitute the daily diet of human food, are the ones that are mostly affected by aflatoxins contamination (Achaglinkame et al., 2017). They are contaminated pre- or post-harvest (Filazi and Sireli, 2013). These foods include cereals- sorghum, millet, maize, rice, wheat, oats, rye, and barley; and spices- chili, pepper, and ginger; and nuts-almond, pistachio, hazelnut, Brazil nut, coconut, and walnut (Martinez-Miranda et al., 2019; Rushing and Selim, 2019). Fruits, vegetables as well as meat, and milk are also considered to be susceptible to aflatoxins contamination (Iqbal et al., 2015).

A compilation of studies by (Omara et al., 2021) shows that maize and maize-based foods are the ones that are mostly associated with aflatoxin contamination in Kenya. Maize is the main staple food in Kenya. It is usually consumed in form of stiff solid flour paste known as '*ugali'* which is the main dish that is usually eaten with other foods such as vegetables, fish, meat, and milk among others. It is also consumed as a semiliquid paste known as porridge or *'Uji'*. Maize grains can also be boiled together with legumes (beans, peas, groundnuts) to form *'githeri'*. When *'githeri'* is prepared using dehulled maize it is referred to as *'muthokoi'*. However, consumption of millet and sorghum, peanuts, and animal milk (cow/goat) in form of tea has also been associated with the occurrence of aflatoxin. Other common dishes include mashed plantain (matoke), cassava, sweet potatoes, and rice (Omara et al., 2021). Consumption of these foods by a majority largely increases the risk of exposure to dietary aflatoxin in Kenya.

2.6 Aflatoxin in Milk

2.6.1 Aflatoxin in Dairy Animal Milk

Once aflatoxins are ingested, they are rapidly absorbed in the gut and degraded in the liver into residues (Kumar et al., 2017). Residues are subsequently transferred to body fluids, and tissues while some are eliminated through the urine (Frazzoli et al., 2017). Though the mechanism of transfer to body fluids is still unknown, the process involves enzymes in the liver (Kumar et al., 2017). The enzymes aid in the reduction, epoxidation, hydration, and hydroxylation reactions of aflatoxins leading to the formation of aflatoxicol, a reservoir for aflatoxin in the intercellular fluid (Kumar et al., 2017). Hydroxylation of aflatoxicol further leads to the formation of toxic aflatoxin M1 and M2 which can be detected in body fluids like milk, blood, and urine within 72 to 96 hours post-feeding (Serraino et al., 2019). For instance, Kagera et al. (2019) reported a high prevalence of aflatoxin M1 (98%) in cows' milk among farmers who kept their livestock in free-range and zero-grazing systems in Nairobi County, Kenya. Likewise, Langat et al. (2016), reported a higher prevalence of aflatoxin M1 in milk sampled in Bomet County, Kenya. These results, alongside those compiled in a review of mycotoxins in sub-Saharan Africa (Kemboi et al., 2020), agree with the findings of earlier studies that reported the existence of transfer of aflatoxin in feeds to the milk of dairy animals. Consequently, a direct relationship has been observed between the amount of aflatoxin ingested and the amount of aflatoxin detected in dairy milk (Akbar et al., 2020). The levels, however, have been shown to vary greatly depending on the concentrations of aflatoxin in the feeds, the amount of the feed consumed the duration of the consumption and the prevailing season.

2.6.2 Aflatoxin in Human Breast Milk

Based on the principle of transfer of aflatoxins from animal feeds into the milk of dairy animals, studies have been conducted to determine the presence of aflatoxin in human breast milk. This is because breast milk is the only nutrient source considered to be safe and adequate during exclusive breastfeeding of children less than six months (Boquien, 2018). Older infants may also continue to breastfeed in combination with other foods (Lutter et al., 2021). A comparative study conducted in Austria reported higher levels of aflatoxin (0.071-0.644 ng/ml) in breast milk among lactating mothers (el-Nezami et al., 1995). These levels were reported to be higher than the studies conducted in Zimbabwe (Lamplugh et al., 1988), Gambia (Maxwell et al., 1988), and Ghana (Zarba et al., 1992). (Gürbay et al., 2010) determined the levels of newborns' exposure to aflatoxin B1 and M1 from mothers' breast milk in Ankara, Turkey. The levels of aflatoxin M1 and B1 were found to be in ranges of 60.90 to 299.99 ng/l and 94.50 to 4123.80 ng/l, respectively. The results pointed out the need for further research both in food and biological fluids. In Italy, (Galvano et al., 2008) determined the occurrence of ochratoxin and aflatoxin M1in 82 human mature milk samples drawn from pregnant mothers admitted for delivery. The study too pointed out the need for dietary recommendations during pregnancy and lactation periods. A similar study was done in a human breast milk bank in Sao Paulo, Brazil. Even though one sample tested positive for aflatoxin at levels 0.024 ng/ml, the need to carry out more analyses on other breast milk banks in the city of Sao Paulo was recommended (Navas et al., 2005). A longitudinal study conducted along with a seasonal pattern, January to July, was done in Egypt to determine the levels of aflatoxin M1 in the breast milk of selected Egyptian mothers. The highest mean and range, 64 pg/ml and 6.3-497 pg/ml of aflatoxin, respectively, were recorded in July while the lowest mean and range, 8 pg/ml and 4.2-108 pg/ml, respectively, were recorded in January. The study emphasized the importance of determining toxicant levels in breast milk as a basis for controlling the transfer of chemicals to infants (Polychronaki et al., 2007). Adejumo et al. (2013) determined aflatoxin M1 content in breast milk, dietary exposure to aflatoxin B1, and socioeconomic status of the lactating mothers in Ogun State in Nigeria. Mean aflatoxin B1 levels in food were between 0.16-0.33 g/kg, while that of aflatoxin M1 in breast milk was between 3.49-35 ng/l. The socioeconomic status was found to influence the levels of aflatoxin both in food and breast milk. Some of the recent studies on human breastmilk include (Altun et al., 2017) in Turkey, (Radonić et al., 2017) in Serbia, (Khan et al., 2018) in Pakistan, (Elaridi et al., 2017) in Lebanon, (Azarikia et al., 2018) in Iran, (Mehta et al., 2021) in India. Even though results were varying, all the studies pointed

out the need to monitor levels of aflatoxin exposure in children who are being breastfed. In Kenya, to the best of my knowledge, only two studies (Kang'ethe et al., 2017; Maxwell et al., 1988) have been conducted to determine the presence of aflatoxin M1 in breastmilk of lactating mothers. It is clear that little work has been done in the East Africa region among lactating mothers with children below six months and in particular in Kenya where episodes of aflatoxicosis outbreak have been frequently reported.

2.7 Nutritional and Health Consequences Related to Aflatoxin Contamination

2.7.1 Acute and Chronic Aflatoxicosis

A high concentration of aflatoxin has been associated with acute aflatoxicosis while prolonged intake in small doses has been associated with chronic aflatoxicosis (Marchese et al., 2018). Acute aflatoxicosis is fatal and has often been characterized by hemorrhage, edema, and acute liver damage, while chronic aflatoxicosis on the other hand has been associated with alteration of DNA resulting in the occurrence of cancer, birth abnormalities in fetus, malnutrition, and immune suppression in humans (Kumar et al., 2017). However, with the recategorization of aflatoxin M1 as group 1 human carcinogen (International Agency for Research on Cancer 2012) from group 2B, breastfeeding children are considered to be at more risk of aflatoxin exposure than adults (Kumar et al., 2021). Breastfeeding children's organs are not fully developed to handle toxins as compared to adults.

2.7.2 Effect of Aflatoxin on Nutritional Status

Several studies have determined the impact of dietary aflatoxin exposure and anthropometric indicators (weight-for-age, height-for-age, and weight-for-height) on young children. For instance, a study by Ayelign et al. (2017) in Ethiopia among infants, and Chen et al. (2018) in Tanzania among children aged 24 and 36 months reported no correlation between exposure to aflatoxin in urine and outcome of weight-for-age of children. On the other hand, a study by Magoha et al. (2014) among children below six months in Tanzania, and Mahdavi et al. (2010) reported a negative association between aflatoxin M1 in breastmilk and weight-for-age outcome of breastfed children. Results of Kang'ethe et al. (2017) among breastfed children below 5 years showed that aflatoxin exposure in urine and breastmilk was positively associated with higher malnutrition rates in Makueni County, Kenya. A study by Ahlberg et al. (2018) on aflatoxin and milk consumption based on income levels of participants hypothesized that aflatoxin M1 interferes with the normal growth of exposed children. Similarly, a study by

(Nabwire et al., 2020b) among children between 6 to 12 years in Makueni County, Kenya also showed a negative association between aflatoxin B1-lysine with weight-for-age of children. A review by Rasheed et al. (2021) showed a negative correlation between the degree of aflatoxin exposure and growth and weight gain among children in developing countries. Mahfuz et al. (2021), who did not find a correlation between aflatoxin exposure and stunting in children in Guatemala, also pointed out the possible dose-response relationship between the aforementioned parameters. These results were similar to those of Mitchell et al. (2017) who reported determined aflatoxin exposure in children of Nepal. However, experimental studies conducted on animals fed on aflatoxin-contaminated feeds show consistent results between aflatoxin exposure and growth parameters. For instance, a study by Knipstein et al. (2015) reported stunting in rats exposed to aflatoxin-contaminated feeds. Results of Pu et al. (2021) on the effect of aflatoxin on the growth and biological value of meat in pigs demonstrated that an increase in aflatoxin exposure levels has a profound effect on protein synthesis, and plasma proteins, weight gain, and the biological value of meat. Wang et al. (2017), in a different study, also showed that aflatoxin influenced DNA responsible for synthesizing body proteins. Zhou et al. (2019) study concluded that aflatoxin interferes with the digestion and metabolism of several elements including protein synthesis.

CHAPTER THREE: RESEARCH DESIGN AND METHODOLOGY

3.1 Study Setting

3.1.1 Geographical location of the study area

The study was conducted in Kibwezi Sub-County in Makueni County as shown in Figure 1. Makueni County is among 47 counties located in the Southeastern region of the former Eastern Province (Government of Makueni County 2020). It lies between latitude 1º 35′ S and longitude 37º 10′ and 38º 30′E at an elevation of 800 to 1700 meters. It covers an area of 8008.7 sq Km (Government of Makueni County 2020). Kibwezi West constituency is among the six constituencies making up Kibwezi Sub-County (Government of Makueni County 2020).

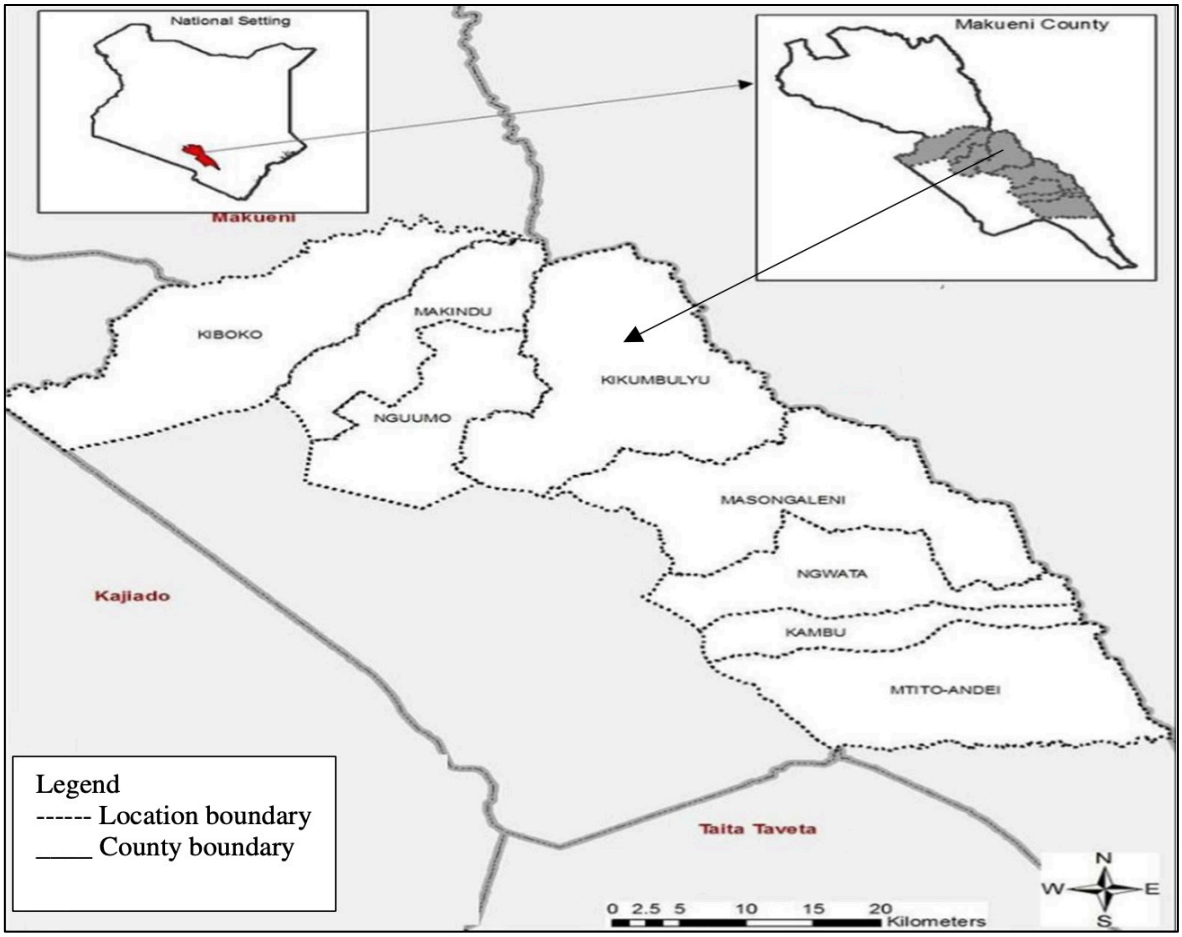

Figure 1: Map of Makueni County, Kenya showing the location of Kikumbulyu in Kibwezi West Constituency (Amwata, 2013)

3.1.4 Population Structure

Makueni County is a home to 987, 653 people (49.6% males and 50.4% females) with a majority (97%) from the Akamba community. Kibwezi Sub-County is the most densely populated sub-county in Makueni County with 197,00 people (50% males and 50% females), 47,912 households (Kenya Population and Housing Census (KPHC), 2019c), and has the highest number of children aged less than six months (KPHC, 2019a). On the other hand, the Kibwezi West constituency has a greater number of village clusters than its counterpart, Kibwezi East (Government of Makueni County, 2020)

3.1.2 Climatic Conditions

Makueni County has two climatic regions, upper and lower regions. The upper region gets between 800-1200mm/ year of precipitation, while the lower region gets between 300-400mm/year of precipitation (Government of Makueni County, 2020). The average temperature in the county is about 23.5ºC with ranges of 17-29 ºC (Government of Makueni County, 2020), but generally, the county experiences high temperatures during the day and low temperatures during the night. Climate change has led to higher temperatures with some areas in Makueni County experiencing prolonged droughts for over four years (MoALF, 2016). Kibwezi sub-County experiences less than 300 mm rainfall per year with higher temperatures of 20.2-35.8 ºC affecting agricultural production in Makueni County (MoALF, 2016).

3.1.3 Socio-economic Profile

The major economic activities in Makueni County include subsistence agriculture, crop farming (coffee, cotton, and horticulture), dairy, and other livestock keeping. Horticulture crops include oranges, mangoes, sugarcane, tomatoes, onions, and vegetables which serve as food and cash crops. Main livestock products are milk, meat, and eggs. Maize, beans, and pigeon peas are the main staple foods for the local community. Other economic activities include beekeeping, trade and manufacturing, transportation and construction, fishing, forestry, charcoal burning, mining, and sand harvesting (Government of Makueni County, 2020).

3.1.5 Health Status

Malnutrition cases in Makueni County have often been among the highest in Kenya. high. As of 2014, approximately 7% of children under five years were severely stunted, 25.1% moderately stunted, 3.1% underweight, and 11.9% wasted (KDHS, 2014). Under-five mortality rates in the Eastern region, of where Makueni County is part, was 45 deaths per 1000 live births (KDHS, 2014). Morbidity among children below five years in Makueni County was recorded

as second highest for the Kibwezi West constituency (Government of Makueni County, 2020). About 11% of the mothers were underweight, while 30% were overweight in the county (KDHS, 2014).

3.2 Study Design

A descriptive and analytical cross-sectional study was conducted among lactating mothers with children aged six months and below. Socio-demographic characteristics, food consumption patterns, breastfeeding practices, anthropometric measurements, and aflatoxin levels in food, breastmilk, and urine of breastfeeding children were determined in the study.

3.3 Study Population

The study focused on lactating mothers and breastfeeding children aged six months and below from different households in Kibwezi West Constituency, Makueni County.

3.4 Sampling

3.4.1 Determination of sample size

The sample size of lactating mothers in the study was determined according to (Fisher et al., 1991) formula ($n = Z^2pq/d^2$) where n is the desired minimum sample size; Z is the standard normal deviation set as 1.96 corresponding to 95% confidence interval; P is the prevalence of aflatoxins maize samples above 10 µg/kg estimated at 87% in Makueni (IFPRI, 2010); q=1-p (proportion of maize sampled without aflatoxins) i.e., 0.13; and d is the degree of accuracy set as 5% i.e., 0.05. A dropout rate of 2% was arbitrarily applied. A sample size of 170 lactating mothers was derived as shown:

$$\begin{aligned} n &= (1.96^2 \times 0.87 \times 0.13) / (0.05 \times 0.05) \\ &= 173.79 \times 0.02 \\ &\approx 170 \end{aligned}$$

The number of foods to be sampled in the study was also determined using (Fisher et al., 1991) formula ($n = Z^2pq/d^2$) where n is the desired minimum sample size; Z is the standard normal deviation set as 1.96 corresponding to 95% confidence interval; P is the prevalence of malnutrition (<-2 SD weight-for-height for children below 5 years, Makueni) estimated at 2.1% (KDHS, 2014); q is 1-p (proportion of children not <-2SD weight-for-height) i.e., 0.979; and d is the degree of accuracy set as 5% i.e., 0.05. An arbitrary attrition rate of 0.2 was added to take into account the anticipated challenges of obtaining cooked maize-based food samples at the

time of the survey. A sample size of 40 foods was generated as shown. However, 48 foods were collected during the survey.

$$n = (1.96^2 \times 0.021 \times 0.979) / (0.05 \times 0.05)$$
$$= 31.6$$
$$= 31.6/ (1-0.2)$$
$$= 39.5$$
$$\approx 40$$

3.4.2 Sampling Procedure

A multistage sampling procedure was used to select the targeted households that have lactating mothers as shown in Figure 2. Makueni County was purposively selected based on several aflatoxin contamination incidences reported in the area, while Kibwezi West Constituency was purposively selected following high results by (Kilonzo et al., 2014) who determined household dietary exposure to aflatoxins from maize and maize products in Kenya. Kikumbulyu Sub-location was preferred because it had more numbers of children aged between 0 to 5 years than other sub-locations in the same constituency (KNBS and SID, 2013). Expanded Program Immunization (EPI) coverage survey random walk method (WHO, 2008) was conducted in all the eight sub-wards until the desired sample size of 170 was achieved. With the assistance of local guides in each sub-ward, a central point was identified, and the starting point was determined by spinning a bottle. However, to avoid leaving out remote dwelling units, access paths were followed as opposed to main roads. In the case of two paths, a coin was flipped once, while in cases where there were more than two paths, a coin was flipped several times until a decision was made. A target of at least 21 lactating mothers was not possible for some of the eight sub-wards. The deficit was offset by recruiting more mothers in other sub-wards. On the other hand, at least six food samples were picked from each of the eight wards to maximize the sample size. However, food samples were picked from households visited during data collection based on the availability of food and willingness of lactating mothers in the study. The process was repeated until the desired sample size was met. Similarly, breast milk and urine were picked from the same lactating mothers whose foods were sampled.

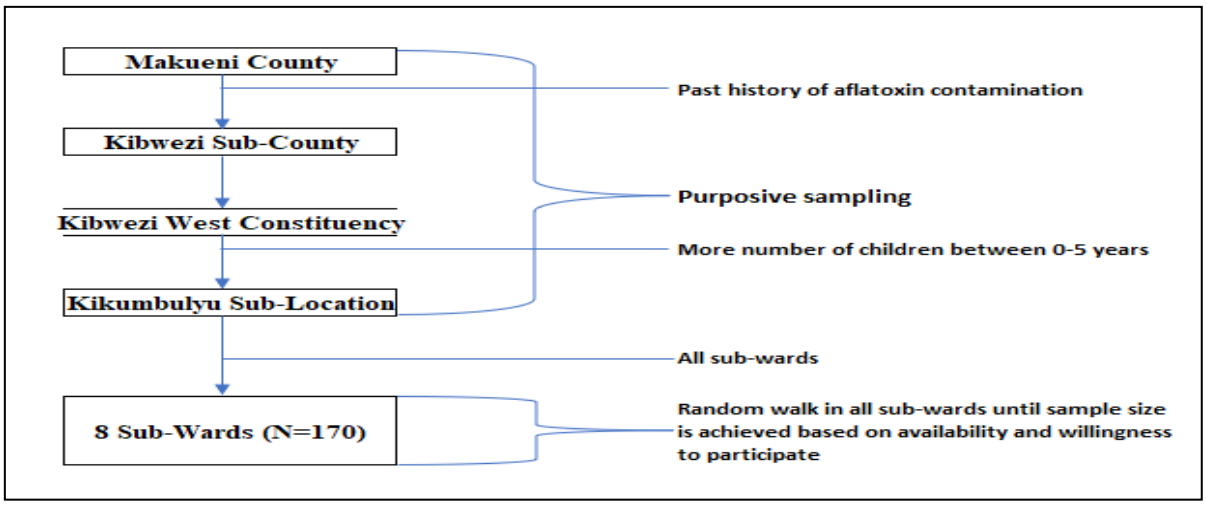

Figure 2: Sampling Procedure Diagram

3.4.3 Inclusion Criteria

Lactating mothers with children between 0 to 6 months were included in the study based on their availability and willingness to participate.

3.4.4 Exclusion Criteria

The study excluded lactating mothers with a disease of the breast or with breast complications. The breast complications were to be reported by the mothers upon inquiry by the community health workers before expressing their breast milk. The complications included breast pain, breast engorgement, nipple pain, milk stasis, mastitis, an overactive letdown, and any other illness that would make lactating mothers uncomfortable to donate breast milk.

3.5 Data Collection Tools and Equipment

The study tools comprised of a semi-structured questionnaire (Appendix 2) with a digital bathroom scale for the field survey. Cooler box, gloves, disinfectants, Ziplock bags, spatula, scooping spoons, cryovial tubes, and food weighing scale were used for collecting breast milk, urine, and food samples. Equipment for aflatoxin analysis was Enzyme-Linked Immuno-Sorbent Assay (ELISA) kit and High-Performance Liquid Chromatography (HPLC).

3.6 Data Collection

3.6.1 Recruitment and training of research assistants

Community health workers conversant with Swahili and Akamba languages administered the questionnaires. They were trained on administering questionnaires and sample collection techniques and matters about field ethics before pretesting the study tools. In addition to the

community health workers, a professional laboratory technologist assisted in the analysis of aflatoxin in food, breast milk, and urine samples collected.

3.6.2 Pretesting of the data collection tools and equipment

A pilot study was conducted among 10 randomly selected households who were not part of the study but with similar characteristics as those of the study households. The purpose of the pilot study was to pretest the questionnaires to be administered and data collection procedures. This helped in identifying ambiguous and difficult questions, estimating the time taken to complete a questionnaire, and assessing whether each question gives an appropriate response. The results for the pre-test were used to standardize and modify the questionnaire appropriately.

3.6.3 Data Collection Procedure

3.6.3.1 Sociodemographic and economic status of lactating mothers

Semi-structured pretested questionnaires were administered to collect information on socio-demographic characteristics of the lactating mothers. Data on variables such as age, marital status, household size, number of children, area of residence, occupation, education level, asset ownership, income, savings, and expenditure were collected from the lactating mothers. The aggregate economic status of lactating mothers in the study was constructed using principal component analysis adopted by KDHS (2014). Economic variables including the main occupational status of lactating mothers, estimated monthly income category, estimated monthly consumption expenditure category, estimated monthly saving category, and asset possession of respective households were ranked and assigned scores accordingly. The total score generated was used as a wealth index for each lactating mother. Lactating mothers were then grouped into lower (total score $\leq$9), middle (total score 10-19), and upper wealth index (total score $\geq$20).

3.6.3.2 Consumption frequency of foods

Food frequency targeting foods that are consumed weekly was determined using a pretested semi-quantitative frequency questionnaire (Appendix 2). Foods that were not consumed weekly were categorized as rarely consumed. Consumption frequency within a normal day was also determined for each food.

3.6.3.3 Estimated daily consumption of foods susceptible to aflatoxin contamination

Consumption quantities of foods that are highly susceptible to aflatoxin contamination were determined using household measures and food atlas compiled by (Ojwang-Ndong, 2013). Daily estimated quantities were arrived at using the formula:

Estimated daily consumption quantities = $(Q \times F1 \times F2)/7$

where Q is the estimated quantities of food consumed per sitting (grams), F1 is the frequency of consumption within a typical day, F2 is the frequency of consumption per week, and **7** is the reference period of consumption frequency. Foods that were rarely consumed, for instance, once a month, were left out. It was also assumed that quantities of food were equally spread within a week in this calculation.

3.6.3.4 Aflatoxin prone foods weekly consumption score of each lactating mother

Weekly consumption frequency score mainly for eighteen selected foods as indicated by asterisks in the food frequency questionnaire (Appendix 2) identified to be commonly consumed by lactating mothers and highly susceptible to aflatoxins contamination in the area of study was determined. Foods that were consumed daily were given a score of seven while those that were consumed twice, thrice, four times in that order until six times per week were given scores of two, three, four, five, and six, respectively. A score of zero was given to foods consumed per two weeks, per month, and never. The scores were multiplied by their respective consumption frequencies reported within a day to generate a total weekly consumption score as shown in the formula:

Total weekly consumption score for aflatoxin prone foods

= Assigned food score × consumption frequency within a day

Weighted aflatoxin consumption score for each lactating mother was subsequently derived by summing up the total weekly consumption score of all aflatoxin categorized food they reported and dividing the summed total consumed with a denominator of 504. The denominator was arrived at by multiplying the expected maximum food score per week (7) of each food by expected maximum frequency consumption within a day (4) by the total number of foods (18) listed as highly susceptible to aflatoxin in this present study, and expressing the result to a percentage as shown below:

Weighted aflatoxin consumption score per lactating mother

$= (\Sigma\ [\text{score food1} + \text{score food 2} + \ldots + \text{score food 18}] \times 100\%)/504$

Lactating mothers were further categorized into 1st, 2nd,3^{rd,} and 4th quartiles according to their percentage scores.

3.6.3.5 Dietary Diversity

Guideline for measuring dietary diversity (FAO, 2011) was used to generate 24-hour dietary diversity scores for lactating mothers. Lactating mothers were asked to mention the foods they consumed in the preceding 24-hour period before data collection. A food score of one was given to the food group whose food was mentioned by lactating mothers, and a score of zero was given to the food group whose food was not mentioned. The score of 13 food groups (cereals, roots and tubers, vitamin A rich vegetables and tubers, dark green leafy vegetable, other vegetables, vitamin A rich fruits, other fruits, organ meat, flesh meats, eggs, fish and sea foods, legume, nuts and seeds, milk and milk products) were aggregated into 9 food groups where cereals and white tubers and roots were combined into starchy staples, other vitamin A rich fruit and vegetables formed one group, other fruits and other vegetables formed another group, and meat and fish formed a single group to generate women's dietary diversity score (WDDS)[1]. Groups of fats and oils, sweets and sugars, spices and condiments were left out. Mean scores between 1 to 3 were considered low, 4 to 6 medium, and 7 to 9 high dietary diversity.

3.6.3.6 Maize source, handling, processing, and storage practices of lactating mothers

Lactating mothers were asked questions regarding where they mainly source their maize from. Responses were mainly categorized into the market, own production, and other sources such as donations, relief, and gifts. Questions regarding what they usually do to maize before storage or cooking and what type of containers they use for storage were also asked.

3.6.3.7 Breastfeeding practices of lactating mothers

Questions on breastfeeding practices were determined by asking lactating mothers the frequency with which they breastfeed their children during the day and night, the time they initiated breastmilk, whether their children were exclusively or non-exclusively breastfeeding, and the type of complementary foods they gave to their non-exclusively breastfeeding children. Breast milk intake was also determined among breastfeeding children in the study. Maternal test weighing method as described by Arcus-Arth et al. (2005) with slight modifications was adopted to suit this study. This was conducted on lactating mothers whose food samples were picked for analysis. Weight measurements for mothers before and after breastfeeding were taken using a two decimal digital bathroom scale. Loss in weight was taken to represent

[1] Dietary diversity score food groups used in this study were as per FAO (2011) at the time of data collection. It was therefore not possible to use the newly adopted Minimum Dietary Diversity for Women as per FAO and FHI 360 (2016) guideline.

quantities of breast milk consumed by breastfeeding children and measurements were made to the nearest 1g. A conversion factor of 1.03 g/ml (breast milk density) was used to express the recorded loss of weight (g) into volume (ml). To get the total quantity of breast milk consumed per day per breastfeeding child, the results were multiplied by the total frequency of breast milk feedings within a typical 24-hour period as reported by the lactating mothers. This is illustrated using the formula:

Total breast milk quantities consumed (ml)/day

= (MWt1- MWt2) x g/ (1.03 ml) x Total breastfeeding frequency/24 h

where MWt1 is the maternal weight before breastfeeding, MWt2 is the maternal weight after breastfeeding, and 1.03g/ml is the density of breast milk. Breast milk intake per child was arrived at by dividing the total breast milk quantities consumed per day by the bodyweight of the breastfeeding child. The result was expressed in volume of breast milk (ml)/ kg b.w.t/day.

3.6.3.8 Weight-for-age z scores of breastfeeding children

The weight of breastfeeding children was determined by first determining the weight of lactating mothers and getting the difference from the second weight of the same mothers measured standing on a scale while carrying the baby. A digital bathroom scale was used, and measurements were done to the nearest 0.01 kg. The age and weight of breastfeeding children were imported into WHO Anthro software (version 3.2.2). The weight-for-age z-score generated was compared against the WHO population standard age group below six months. Weight-for-age z-scores below -2 SD were considered underweight, while z-scores above -2 SD were considered normal in the study.

3.6.4 Sample collection

3.6.4.1 Food samples

Cooked maize-based food samples were collected from selected households with lactating mothers. This was done during data collection based on the availability of food (left-over) and willingness of the lactating mothers. A total of 48 maize-based food samples were collected. Solid foods were sampled by the quartering method in case the food sample was bulk and exceeded the required sample size. A representative sample was drawn from the top, middle, and bottom of a plate, bowl, or cup using a sterilized steel tablespoon for foods that were in small pieces. Semi-liquid foods were stirred to mix, and a representative sample was scooped from the middle. The samples were transferred into a weighed cup until a 60g samples weight

was attained (50g for quantification, and 10g for detection). The samples were transferred into airtight Ziplock bags, labeled, and stored in a cooler box at temperatures of 4˚C. The cooler box was stacked with ice from a deep freezer, and the temperature was monitored using a thermometer. Restocking of ice was done daily at the end of every data collection day. Food samples stayed for a maximum of three days before being taken to the laboratory. Storage was done in a deep freezer at -18ºC before analysis. Food samples collected were solid maize meal (*ugali, n* = 18), semi-liquid maize porridge (*n* = 6), and maize-sorghum porridge (*n* = 9), and mixture of boiled maize and beans (*githeri, n* = 9), and mixture of dehulled maize and beans (*muthokoi, n* = 6). The varying proportion of food samples was as a result of picking foods that remained after the household had had their specific meal at the time of data collection.

3.6.4.2 Breast milk samples

Breast milk samples were collected from the lactating mothers a day after picking food samples. This was done with the help of female community health workers. Mothers first washed their hands, then cleaned and rinsed their breasts before expressing at least 10 ml of breast milk into a cryovial tube fitted with a Teflon cap. The expressed breast milk was transferred into a cooler box and stored at about 4˚C. Samples stayed for a maximum of two days before being taken to the laboratory. Storage was done in a deep freezer at -18ºC before analysis. A total of 48 breast milk samples were collected for analysis.

3.6.4.3 Urine samples

Instructions on how to collect early morning urine were given to lactating mothers a day (during breast milk) before the actual collection of urine samples. However, since it was challenging to collect mainstream urine from children 0 to 6 months, mothers were requested to collect urine from under wrappings (napkins or clothing) used on babies. The use of diapers was discouraged since they retain more urine as compared to napkins and clothing. Lactating mothers were required, just after babies woke up in the morning (as from 5 am onwards), to change the wet wrappings used over the night, clean or dry the babies, wrap them again using a clean dry napkin or clothing, and wait for the babies to pass urine thereafter. Once babies passed urine, mothers wrung the under wrappings, let urine drip into a plastic container provided (at least 10 ml), and transfer them to sterilized cryovials tubes. The collected urine samples were picked by the principal investigator and transferred into a cooler box at about 4˚C for a day. Samples were taken to the laboratory the following day (six hours from the site

of data collection). Storage was done in a deep freezer at -18°C before analysis. A total of 48 urine samples were collected for analysis.

3.7 Analytical methods

3.7.1 Detection of positive aflatoxin cooked maize-based food samples

The method for detecting positive aflatoxin food samples involved liquid-liquid extraction using organic solvent and water, followed by cleaning up using immunoaffinity column (IAC) and derivatization (acylation) of the aflatoxin molecules. Aflatoxin was detected as trifluoracetic derivatives using High-Performance Liquid Chromatography and fluorescence detector (Nexera X2 Model, Shimadzu, Kyoto, Japan). For this process, 5g of the samples were ground to fineness. Extraction was done using 25 ml of 70% methanol. The cleaning up procedure was done using the Romer AflarStar-immunoaffinity column, while aflatoxin derivatization was done using 200 µl trifluoracetic acid. Reverse-phase HPLC column (Lichrospher® RP-18, 250 × 4.0 mm I.D., 5 µm) was used for separation. Identification was done at run time of 30 minutes, velocity 1.0 ml/minute, injection volume 10 µl, column temperature of 35°C, excitation wavelength of 363 nm, emission wavelength of 440 nm, and sloppiness of 10 nm using a fluorescence detector. All the procedure was followed according to the manual provided in the laboratory (Mycotoxin Research Centre Department of Public Health Pharmacology and Toxicology, University of Nairobi).

3.7.2 Determination of total aflatoxin and aflatoxin B1 in positive food samples

Positive samples were quantified for total aflatoxin and aflatoxin B1 using Ridascreen ELISA competitive enzyme immunoassay (r-Biopharm, Darmstadt, Germany) with slight modification. Samples collected (50g) were ground and mixed with 250 ml methanol-water mixture (70%:30%, v/v) and homogenized for three minutes for extraction. The resulting solution was filtered using Whatman filter paper number 1, and 50 µl was used for each standard and sample per well. Provided conjugate and antibody (50 µl each, respectively) were added, and incubation was done for 30 minutes at room temperature (25°C). Wash buffer (Phosphate buffer with tween) of 250 µl was used. Chromogen (100 µl) was added as substrate and incubated again for 15 minutes at room temperature. Acid stop solution was added and reading was done within 15 minutes. The recovery rate was set at 85% for total aflatoxin, 93% for aflatoxin B1, and absorbance was done at 450 nm. The lower detection limit for total aflatoxin and aflatoxin B1 was set at 1.75 and 0.5 µg/kg, respectively.

3.7.3 Determination of dietary aflatoxin intake of lactating mothers

Dietary aflatoxin intake was determined by multiplying the concentration of aflatoxin in each analyzed food with estimated quantities of food consumed in a day by lactating mother. The result was then divided by the bodyweight of a lactating mother as shown in the formula:

Aflatoxin Intake (µg/kg/Kg b.w.t/day) =

$$\frac{\text{Aflatoxin concentration (µg/kg)} \times \text{Estimate quantities of food consumed (g)/day}}{\text{Bodyweight of lactating mother (b.w.t) (Kg)}}$$

3.7.4 Determination of margin of exposure of lactating mothers to dietary aflatoxin intake

The margin of exposure was derived by taking the benchmark dose level (BMDL) of total aflatoxin and aflatoxin B1and dividing by the estimated aflatoxin intake of a lactating mother. Benchmark dose (BMD_{10}) of 0.41 µg/kg/b.w.t/day adequate to increase tumor by 10% in male rats (EFSA CONTAM Panel et al., 2020) was used as illustrated in the formula with a cut-off of greater than or less than 10000 MOE (EFSA, 2005) used to assess the risk levels of lactating mothers in the study area.

$$\text{Margin of Exposure (MOE)} = \frac{\text{(BMDL) for aflatoxin (0.41) (µg/kg/b.w.t/day)}}{\text{Estimated Aflatoxin intake (µg/kg/b.w.t/day)}}$$

3.7.5 Determination of Aflatoxins M1 in the breast milk of lactating mothers

Aflatoxins M1 in breast milk was determined using Ridascreen® Aflatoxin M1 ELISA kit (r-Biopharm, Darmstadt, Germany). A manual procedure was adopted for analysis. Breast milk samples (5ml) were centrifuged for degreasing, and separation with upper-fat layers at 10 min/3500g at 10ºC, and cream removed by aspiration. Samples were then diluted with 35% methanol (1:9) and put in the microwell. The antibody of 100 µL was added to the wells and incubated for 15 minutes. 100 µL of the diluted breast milk sample and standards were used per well and let to incubate for about 30 minutes at room temperature. The wells were washed using a 250 µL buffer solution. Conjugate of 100 µL was added and left to incubate for 15 minutes and washed with 250 µL using phosphate buffer solution. Chromogen of 100 µL was added to the wells and left to incubate for 15 minutes. Stop solution of 100 µL was added to each well and the reading was done within 15 minutes. Absorbance was determined at 450 nm. The lower detection limit was set at 5 ng/l.

3.7.6 Determination of aflatoxin M1 intake in breast milk among breastfeeding children

Dietary aflatoxin intake was determined by multiplying the concentration of aflatoxin M1 in breast milk with estimated quantities of breast milk consumed in a day by breastfeeding

children. The result was then divided by the bodyweight of breastfeeding children as shown in the formula:

$$\text{Aflatoxin M1 intake (μg/kg/b.w.t/day)} = \frac{\text{Aflatoxin concentration (μg/kg) x Breast milk consumed (g)/day}}{\text{Bodyweight of breastfeeding child (b.w.t) (Kg)}}$$

3.7.7 Determination of margin of exposure of breastfeeding children to aflatoxin M1 intake

The margin of exposure was derived by taking the benchmark dose level (BMDL) of aflatoxin M1 and dividing it with the estimated aflatoxin M1 intake of breastfeeding children. Potency factor of 0.1 relative to Aflatoxin B1 $BMDL_{10}$ of 0.17 μg/kg/b.w.t/day was applied for aflatoxin M1 in the breast milk (European Food Safety Authority (EFSA) 2007). As a result, 0.017 μg/kg/b.w.t/day was used to assess the margin of exposure levels of breastfeeding children with a cut-off of greater than or less than 10000 MOE (EFSA, 2005) used to assess the risk levels of breastfeeding children using the formula:

$$\text{Margin of Exposure (MOE)} = \frac{\text{(BMDL) for aflatoxin M1(0.017 μg/kg/b.w.t/day)}}{\text{Estimated aflatoxin M1 intake (μg/kg/b.w.t/day)}}$$

3.7.8 Determination of Aflatoxin M1 in the urine of breastfeeding children

Aflatoxin M1 in urine was determined using Aflatoxin M1 (urine) ELISA kit (Helica Biosystem Inc, California, USA). Urine (10 ml) was mixed with 40 ml deionized water and filtered using a glass microfiber filter paper. Aliquots of urine standards and samples were further diluted with distilled water in the ratio of 1:20. Urine standard and samples (100 μL) were placed in microwells and buffered with 200 μL Phosphate Buffer Saline reconstituted with 0.05% Tween solution. After mixing, an antibody was added to each microwell and left to incubate for 1 hour at about 25°C. Tetramethylbenzidine stop solution was used on hose-radish-phosphate to stop the reaction. Reading was determined at 450 nm with color expected to change from blue to yellow. The detection limit was set at 0.15 ng/ml.

3.9 Data Management and Analysis

3.9.1 Data Quality Assurance

Data quality assurance was ensured across all the stages of sample collection, laboratory, and data analysis. Before sample collection, field assistants (community health workers) recruited in the study were adequately trained on administering questionnaires, collecting food, breast milk, and urine samples. A pretest of the survey was done, and data collection tools, equipment,

and procedures were harmonized. Before collecting breast milk samples into sterilized cryovial tubes, lactating mothers washed their hands using clean water and soap, cleaned their breasts using clean water, and dried them using a clean piece of cloth. For foods, collection tools were cleaned and disinfected every time food samples were picked from a household. The samples collected were immediately stored in an airtight Ziplock bag. The urine of breastfeeding children was also collected into sterilized cryovials tubes. Mothers were also requested to discard urine that mixed with the baby's solid waste and repeat the process with a napkin or under wrapping that is only wet with urine. All the samples collected were labeled and each transferred into their respective cooler box at about 4˚C. During laboratory analysis, quality was ensured by following the standard procedures for HPLC and ELISA techniques A professional laboratory technologist with experience in aflatoxin analysis was consulted to help with analysis. After data collection and data entry, data cleaning was done by exploring data using Statistical Package Software for Science (SPSS version 27).

3.9.2 Ethical Consideration

Ethical issues at all stages were considered. Similarly, ethical approval to conduct the study on lactating mothers and breastfeeding children in the study area was sought by obtaining ethical clearance (P454/08/2013) from Kenyatta National Hospital/the University of Nairobi-Ethical Review Committee (KNH/UoN-ERC). The consenting process also involved meeting administration and community leaders before conducting and collecting samples from participants. Female community health workers from the community with adequate knowledge about the study area were trained to collect food, breast milk, and urine samples. Issues about privacy, anonymity, and confidentiality of the study participant were taken into consideration. No incentives were given to lactating mothers. Mothers who were willing to participate in the study signed informed consent (Appendix 1). However, mothers were also at liberty to discontinue participating in the study even after giving consent.

3.9.3 Statistical Analyses

Relevant data from completed questionnaires and laboratory readings were analyzed using Statistical Package Software for Social Sciences (SPSS version 27). Descriptive statistical analysis was done on socio-demographic variables, dietary diversity, food consumption patterns, aflatoxin food score, consumption levels, breastfeeding practices, and aflatoxin levels in food, breast milk, and urine samples, and weight-for-age z-scores of breastfeeding children. Statistical difference between groups was determined using student t-test (t) for normally

distributed data, and Mann-Whitney *U* for non-normally distributed data. Statistical difference between more than three groups was determined using Analysis of variance (ANOVA) (*F*-test) for normally distributed data, and Kruskal-Wallis H-test for non-normally distributed data. Bonferroni Chi-square post hoc test was used for multiple pair comparisons of ranked variables, while Tukey's b was used for the post hoc ANOVA test. Pearson (*r*), Kendall tau-b (t_b), and Spearman (*rho*) were used to determine the correlation between normal continuous, non-normal continuous, and ranked variables, respectively, while Chi-square (χ^2 test) was used to determine the association between categorical variables. Simple and multiple linear regressions were used for determining significant predictors of aflatoxin concentration levels in analyzed foods, breastmilk of lactating mothers, urine of breastfeeding children, and outcome of weight-for-age z-scores. A significant level was set at $p<0.05$. @Risk software version 8.2 was used to determine the regression coefficient of each food on total aflatoxin and aflatoxin B1 intake in the study.

CHAPTER FOUR: RESULTS

4.1 Sociodemographic and economic status of lactating mothers in Kibwezi West

4.1.1 Sociodemographic characteristics

Table 1a and 1b show sociodemographic characteristics of 170 lactating mothers recruited in the study. Out of 170 households, 22.4% were from Kathyaka, 29.4% from Ngandani, 30% from Mukuyuni, and 18.2% from Ndetani sub-locations. The mean household size in the area was 6.2 (*SD*, 1.3) with a range of between 4 to 8 persons. However, the mode was six persons per household. The proportion of household size between exclusively and non-exclusively breastfeeding mothers was insignificant (Mann-Whitney *U*, $p = 0.203$). The average number of children per lactating mother was 3.0 (*SD*, 1.7), the mode was three children, while the range was from one to seven (1 to 7). Again, the proportion of children per lactating mother between exclusively and non-exclusively lactating mothers was insignificant (Mann-Whitney *U*, $p = 0.442$). Of the lactating mothers, 46.5 and 42.4% were in the age category of 20-29 and 30-39 years, respectively, while only a smaller percentage, 4.7 and 6.5%, were in the age category of 15-19 and 40-49 years, respectively. However, the mean, mode, and range were 29.5 (*SD*, 5.9), 29, and 18 to 43 years, respectively. No significant difference in mean age was reported between mothers exclusively and non-exclusively breastfeeding their children (Mann-Whitney *U,* $p = 0.858$). Likewise, no significant association between the age category of mothers and breastfeeding status was reported (Fisher's exact test, sig. 2-sided, $p = 0.591$). Again, at the time of the study, 13.5% of lactating mothers had no formal education, 35.3% had attempted primary education, while the rest (51.2%) had satisfactorily completed basic education level. However, education was statistically associated with the breastfeeding status of lactating mothers (Fisher's exact test, sig. (2-sided), $p = 0.001$). Bonferroni Chi-square post hoc test for multiple pair comparisons showed that the number of exclusively lactating mothers (53.3%) who attempted primary education was statistically higher than those of non-exclusively lactating mothers (21.1%) ($p = 0.00$).

Table 1a: Sociodemographic characteristics of lactating mothers in Kibwezi West

Characteristics	Median	Mode	Range	Mean (*SD*)	EBF*NEBF (*U* test)
Household size					
EBF mothers (*n* = 75)	6.0	6.0	4-8	6.1(1.3)	0.203
NEBF mothers (*n* = 95)	6.0	8.0	4-8	6.3(1.3)	
All mothers (*n* = 170)	6.0	6.0	4-8	6.2(1.3)	
Number of children					
EBF mothers (*n* = 75)	3.0	2.0	1-7	2.9(1.5)	0.442
NEBF mothers (*n* = 95)	3.0	3.0	1-7	3.1(1.6)	
All mothers (*n* = 170)	3.0	3.0	1-7	3.0(1.7)	
Age of lactating mothers					
EBF (*n* = 75)	29.0	32.0	18-40	29.6(5.5)	0.858
NEBF (*n* = 95)	30.0	28.0	18-43	29.4(6.2)	
All mothers (*n* = 170)	29.0	32.0	18-43	29.5(5.9)	

EBF: Exclusively lactating mothers; NEBF: Non-exclusively lactating mothers

Table 1b: Age and education categories of lactating mothers in Kibwezi West

Characteristics	(N = 170) (%)	(EBF, n = 75) (%)	(NEBF, n = 95) (%)	Sig. (χ^2)
Age categories (Years) of lactating mothers				0.591
15-19	4.7	4.0	5.3	
20-29	46.5	45.3	47.4	
30-39	42.4	46.7	42.4	
40-49	6.5	4.0	6.5	
Educational level of lactating mothers				
No formal education	13.5	6.7	18.9	0.001*
Attempted primary education	35.3	53.3	21.1	
Completed primary education	28.2	21.3	33.7	
Attempted secondary education	10.6	9.3	11.6	
Completed secondary education	10.0	6.7	12.6	
College/University	2.4	2.7	2.1	

EBF: Exclusively lactating mothers; NEBF: Non-exclusively lactating mothers, *Significant at p<0.05

4.1.2 Economic status of lactating mothers

Over half (52.4%) of the lactating mothers in the study were housewives, 19.4% casual laborers, and 15.9% self-employed. Those who depended on farming were 15.9%, while the rest (2.4%) depended on salaried employment as their main occupational status (Table 2). However, occupational status was significantly different between breastfeeding groups of lactating mothers (Fisher exact, sig. 2-sided = 11.629, $p = 0.018$). Post hoc analysis using Bonferroni

Chi-square for pair comparison showed that the number of exclusively lactating mothers (30.7%) working as casual laborers was thrice that of non-exclusively breastfeeding mothers (10.5%) ($p = 0.00$). Nonetheless, over half (53.6%) of lactating mothers had a monthly income of ≤ USD 75, while the rest (46.4%) had a monthly income of >USD 75 (1 US Dollar ≈ 100 Kenya shillings). The mean monthly income was USD 70.04 (*SD*, 18.64), ranging from USD 25 to 110. The mode was USD 80. An insignificant difference, however, was reported between the two breastfeeding groups (Mann-Whitney *U*, $p = 0.907$). Consumption expenditure of 59.4% of lactating mothers was ≤ USD 34.40, while for the rest (40.6%) was > USD 34.40. Mean and median consumption expenditure (in USD) were 32.42 (*SD*, 8.99), and 30.00, respectively. A range of between USD 20 to 56 was reported. However, Mann-Whitney *U* showed no significant difference in consumption expenditure between the two breastfeeding groups of lactating mothers ($p = 0.520$). Monthly saving of <USD 10 was reported for 72.4%, USD 10 to 20 reported for 22.9%, and > USD reported for 4.7%. Slightly over a quarter (25.9%) of lactating mothers were from households that owned productive land (agricultural). Among other assets, 57.6% owned at least a mobile phone, 44.7% owned a functional media accessory (television and/or radio), and 34.1% owned at least a means of transport (bicycle, motorbike, animal-drawn cart, car). Among 55.3% who own livestock, 41.2% kept poultry, 28.2% goats while 15.9% and 11.2% owned cattle and sheep, respectively. Consequently, aggregate economic status constructed using principal component analysis showed that 49.4% of lactating mothers were in the lower wealth index, 45.3% in the medium wealth index, and only 5.3% were in the upper wealth index.

Table 2: Economic status of lactating mothers in the study indicating a comparison between exclusive and non-exclusive breastfeeding mothers in Kibwezi West

Characteristics	Total (N= 170) %	EBF (n=75) %	NEBF (n=85) %	(χ^2) Sig.
Main Occupation				
Salaried employed	2.4	1.3	3.2	0.018*
Farmer	10.0	9.3	10.5	
Self-employed	15.9	12.0	18.9	
Casual laborer	19.4	30.7	10.5	
Housewife	52.4	46.7	52.4	
Monthly income categories (USD)				
0-25	0.6	1.3	0	0.363
>25-50	20.6	19.2	22.0	
>50-75	32.4	28.0	36.8	
>75-105	41.2	44.7	37.7	
>105-130	3.0	2.8	3.2	
>130-155	2.4	4.0	0.3	
Consumption expenditure (USD)				
≤ 34.40	59.4	62.7	58.8	0.530
>34.40	40.6	37.3	43.2	
Monthly savings categories (USD)				
<10	72.4	66.7	76.8	0.317
10-20	22.9	28.0	18.9	
>2000	4.7	5.3	4.2	
Asset Possession				
Productive land	25.9	22.7	28.4	0.481
Own livestock	55.3	58.7	52.6	0.169
At least a mobile phone	57.6	58.7	56.8	0.876
Media Accessory	44.7	42.7	46.3	0.645
Any means of transport	34.1	25.3	41.1	0.35
Wealth Index Categories				
<9 (Lower Wealth Index)	45.3	37.3	51.6	0.099
10-19 (Medium Wealth Index)	49.4	58.7	42.1	
≥20 (Upper Wealth Index)	5.3	4.0	6.3	

EBF: Exclusively breastfeeding; NEBF: Non-exclusively breastfeeding; * Significant p-value <0.05

4.2 Food consumption pattern, Dietary diversity, Maize source, handling and storage practices, and Breastfeeding practices of lactating mothers in Kibwezi West

4.2.1 Consumption frequency of foods likely to be contaminated with aflatoxins

Stiff solid maize flour paste *'ugali'* and porridge were the most frequently consumed foods at least once per week by all (100%) lactating mothers. Almost 90% consumed both groundnuts and maize grains boiled together with legumes such as beans and peas *'githeri'* at least once per week (Table 3). More than a half (>50%) on the other side, consumed milk tea, rice, and dehulled maize grains boiled together with legumes such as beans and peas '*muthokoi'* at least once per week. Only 8.2 and 11.2% of lactating mothers consumed finger millet and cassava at least once per week, respectively. On the other hand, 22.9% consumed plain sorghum flour while 14.1% consumed mixed flour porridge at least once per week. Approximately 8.8% of lactating mothers consumed fish, 11.2% chicken while 34.1% consumed eggs at least once per week. Using chi-square, no significant association was observed between food consumption frequency of each food and breastfeeding status of lactating mothers (Table 3), except for groundnut ($p = 0.01$), sorghum porridge ($p = 0.03$), and fish ($p = 0.03$).

The frequency of consumption within a typical day showed that all the foods were mostly consumed once a day (Table 4). Among these foods, maize ugali, porridge (regardless of type), groundnuts, and milk (cow/goat) were consumed up to four times within a typical day by 1 to 11% of lactating mothers. *'Githeri'* rice, millet, meat, and eggs consumption were in the ranges of between one to three times (1 to 3) a typical day, while *'muthokoi'* and cassava, and fish were consumed at most twice on a typical day. Chicken was eaten at most once on a typical day. Frequency percentages were presented in terms of all lactating mothers, exclusive and non-exclusive lactating mothers in Table 4. Chi-square showed no significant difference in consumption frequency within a day for each food between exclusively and non-exclusively lactating mothers ($p_{all} > 0.05$) except for '*ugali'* ($p = 0.01$).

Table 3: Consumption frequency per week of foods likely to be contaminated with aflatoxins

Food		% Frequency per week EBF [a] (n=75), NEBF [b] (n=95), all (n=170)								χ^2
		1	2	3	4	5	6	7	Rarely	
Maize *ugali* [c]	***EBF***	5.3	4.0	20.0	14.7	8.0	13.3	34.7	-	0.15
	NEBF	0.0	2.1	14.7	16.8	13.7	8.4	44.2	-	-
	All	2.4	2.9	17.1	15.9	11.2	10.6	40.0	-	-
Maize porridge	***EBF***	9.3	4.0	21.3	18.7	14.7	10.7	21.3	-	0.66
	NEBF	3.2	5.3	23.2	21.1	14.7	15.8	16.8	-	-
	All	5.9	4.7	22.4	20.0	14.7	13.5	18.8	-	-
Sorghum	***EBF***	2.7	5.3	1.3	6.7	1.3	4.0	1.3	77.3	0.03*
	NEBF	10.5	2.1	3.2	2.1	0.0	0.0	5.3	76.8	-
	All	7.1	3.5	2.4	4.1	0.6	1.8	3.5	77.1	-
Mixed porridge	***EBF***	1.3	2.7	2.7	4.0	2.7	1.3	-	85.3	0.94
	NEBF	0.0	2.1	3.2	2.1	3.2	3.2	-	86.3	-
	All	0.6	2.4	2.9	2.9	2.9	2.4	-	85.9	-
Githeri [d]	***EBF***	29.3	20.0	20.0	4.0	2.7	2.7	5.3	16.0	0.80
	NEBF	34.7	24.2	14.7	5.3	5.3	2.1	4.2	9.5	-
	All	32.4	22.4	17.1	4.7	4.1	2.4	4.7	12.4	
Muthokoi [e]	***EBF***	26.7	10.7	5.3	2.7	2.7	1.3	-	50.7	0.59
	NEBF	27.4	13.7	9.5	2.1	0.0	0.0	-	47.4	-
	All	27.1	12.4	7.6	2.4	1.2	0.6	-	48.8	-
Rice	***EBF***	28.0	18.7	8.0	2.7	2.7	0.0	-	40.0	0.11
	NEBF	31.6	7.4	4.2	1.1	1.1	1.1	-	53.7	-
	All	30.0	12.4	5.9	1.8	1.8	0.6	-	47.6	-
Finger millet	***EBF***	4.0	1.3	1.3	1.3	-	-	4.0	88.0	0.18
	NEBF	3.2	2.1	0.0	0.0	-	-	0.0	94.7	-
	All	3.5	1.8	0.6	0.6	-	-	1.8	91.8	-
Cassava	***EBF***	16.0	2.7	1.3	1.3	1.3	-	-	77.3	0.91
	NEBF	15.8	1.1	2.1	1.1	0.0	-	-	80.0	-
	All	15.9	1.8	1.8	1.2	0.6	-	-	78.8	-
Groundnut	***EBF***	24.0	28.0	12.0	10.7	8.0	8.0	-	9.3	0.01*
	NEBF	26.3	18.9	8.4	0.0	20.0	12.6	-	13.7	-
	All	25.3	22.9	10.0	4.7	14.7	10.6	-	11.8	-
Beef	***EBF***	30.7	4.0	2.7	5.3	2.7	-	-	54.7	0.12
	NEBF	31.6	7.4	2.1	0.0	0.0	-	-	58.9	-
	All	31.2	5.9	2.4	2.4	1.2	-	-	57.1	-
Chicken	***EBF***	22.7	1.3	0.0	0.0	-	-	-	76.0	0.96
	NEBF	18.9	2.1	1.1	1.1	-	-	-	76.8	-
	All	20.6	1.8	0.6	0.6	-	-	-	76.5	-
Eggs	***EBF***	30.7	4.0	1.3	0.0	-	-	-	64.0	0.60
	NEBF	22.1	7.4	2.1	1.1	-	-	-	67.4	-
	All	25.9	5.9	1.8	0.6	-	-	-	65.9	-
Fish	***EBF***	13.3	1.3	-	-	-	-	-	85.3	0.03*
	NEBF	4.2	0.0	-	-	-	-	-	95.8	-
	All	8.2	0.6	-	-	-	-	-	91.2	-
Milk	***EBF***	26.7	6.7	4.0	4.0	5.3	0.0	10.7	42.7	0.88
	NEBF	28.4	5.3	5.3	5.3	3.2	1.1	5.3	46.3	-
	All	27.6	5.9	4.7	4.7	4.1	0.6	7.6	44.7	-

[a] EBF: exclusive breastfeeding mothers, [b] NEBF: non-exclusive breastfeeding mothers, [c] *Ugali:* stiff solid maize flour paste; [d] *Githeri*: maize grains boiled together with either beans or peas; [e]*Muthokoi*: dehulled maize boiled together with either beans or peas, -: no frequency

Table 4: Consumption frequency within a day of foods likely to be contaminated with aflatoxin in Kibwezi West among lactating mothers who reported weekly consumption

Food		Consumption frequency within a day (%)				χ^2
		1	2	3	4	
Maize '*ugali*'[a]	*EBF (n=75)*	58.7	29.3	2.7	9.3	0.01
	NEBF (n=95)	47.4	23.2	20.0	9.5	-
	ALL (n=170)	52.4	25.9	12.4	9.4	-
Porridge	*EBF (n=75)*	33.3	34.7	21.3	10.7	0.81
	NEBF (n=95)	28.4	41.1	22.1	8.4	-
	ALL (n=170)	30.6	38.2	21.8	9.4	-
Sorghum	*EBF (n=17)*	35.3	35.3	29.4	0.0	0.28
	NEBF (n=22)	54.5	13.6	27.3	4.5	-
	ALL (n=39)	46.2	23.1	28.2	2.6	-
'Githeri'[b]	*EBF (n=63)*	65.1	25.4	9.5	-	0.20
	NEBF (n=86)	50.0	37.2	12.8	-	-
	ALL (n=149)	56.4	32.2	11.4	-	-
'Muthokoi'[c]	*EBF (n=39)*	74.4	25.6	-	-	0.11
	NEBF (n=50)	58.0	42.0	-	-	-
	ALL (n=89)	65.2	34.8	-	-	-
Rice	*EBF (n=47)*	70.2	21.3	8.5	-	0.41
	NEBF (n=44)	63.6	31.8	4.5	-	-
	ALL (n=91)	67.0	26.4	6.6	-	-
Millet	*EBF (n=9)*	66.7	22.2	11.1	-	0.91
	NEBF (n=5)	60.0	20.0	20.0	-	-
	ALL (n=14)	64.3	21.4	14.3	-	-
Cassava	*EBF (n=6)*	64.7	35.3	-	-	0.51
	NEBF (n=10)	50.0	50.0	-	-	-
	ALL (n=16)	56.8	43.2	-	-	-
Groundnut	*EBF (n=68)*	45.6	35.3	8.8	10.3	0.77
	NEBF (n=82)	40.2	37.8	13.4	8.5	-
	ALL (n=150)	42.7	36.7	11.3	9.3	-
Milk	*EBF (n=43)*	74.4	20.9	4.7	0.0	0.50
	NEBF (n=51)	64.7	21.6	11.8	2.0	-
	ALL (n=94)	69.1	21.3	8.5	1.1	-
Meat	*EBF (n=34)*	79.4	17.6	2.9	-	0.91
	NEBF (n=39)	74.4	20.5	5.1	-	-
	ALL (n=73)	76.7	19.2	4.1	-	-
Chicken	*EBF (n=18)*	100	-	-	-	-
	NEBF (n=22)	100	-	-	-	-
	ALL (n=50)	100	-	-	-	-
Eggs	*EBF (n=27)*	77.8	18.5	3.7	-	0.77
	NEBF (n=31)	67.7	29.0	3.2	-	-
	ALL (n=58)	72.4	24.1	3.4	-	-
Fish	*EBF (n=11)*	90.9	9.1	-	-	0.53
	NEBF (n=4)	100.0	-	-	-	-
	ALL (n=15)	93.3	6.7	-	-	-

EBF: exclusive breastfeeding mothers, NEBF: non-exclusive breastfeeding mothers, [a] *Ugali:* stiff solid maize flour paste; [b] *Githeri*: maize grains boiled together with legumes such as beans and peas; [c]*Muthokoi*: dehulled maize grains boiled together with legumes such as beans and peas, -: no frequency

4.2.2 Consumption frequency of foods least susceptible to aflatoxin contamination

Among foods that are least susceptible to aflatoxin contamination, kales, cowpea leaves, and cabbage were consumed every week by 93.9, 66.5, and 58.8% of lactating mothers, respectively, while amaranth, and *'managu'* (*Solanum nigrum*) were consumed every week by 22.4, and 23.4% lactating mothers, respectively. Beans were consumed every week by 72.9%, pigeon peas by 20.6%, and green grams by 31.2%. Bananas were consumed every week by 21.8%, sweet potatoes by 10.6%, and potatoes by 27.1%. Mangoes, oranges carrots, and pawpaw were consumed weekly by 47.1, 46.5, 19.4, and 7.1% of lactating mothers, respectively. Chi-square showed no significant difference in consumption frequency of each food least susceptible to aflatoxin contamination between exclusively and non-exclusively lactating mothers ($p_{all} > 0.05$) (Table 5).

The frequency of consumption within a day for foods that are least susceptible to aflatoxin contamination showed that carrots were the only food consumed at least four times by 5.9% of lactating mothers. Potatoes, beans, pigeon peas, green grams, and oranges were consumed at most three times on a usual day by 4.5, 12.1, 28.6, 1.9, and 9.7% lactating mothers, respectively. All the remaining foods were consumed at most twice on a typical day. This included kales by 54.4%, cabbage by 29%, cowpea leaves by 55.8%, amaranth by 50%, *'managu'* by 31.6%. Fruits included bananas by 8.1%, pawpaw by 33.3%, mangoes by 62.5%. The rest are summarized in Table 6. There was no significant difference between exclusive and non-exclusive lactating mothers ($p_{all} > 0.05$).

Table 5: Consumption frequency per week of foods unlikely to be contaminated by aflatoxin among lactating mothers in Kibwezi West

		% Frequency per week EBF [a] (n=75), NEBF [b] (n=95), all (n=170)								
Food		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Rarely**	χ^2
Irish potatoes	*EBF*	17.3	13.3	4.0	-	-	-	-	65.3	0.18
	NEBF	12.6	5.3	3.2	-	-	-	-	78.9	-
	All	14.7	8.8	3.5	-	-	-	-	72.9	-
Sweet potatoes	*EBF*	13.3	1.3	100	-	-	-	-	85.3	0.76
	NEB	4.2	3.2	100	-	-	-	-	92.6	-
	All	8.2	2.4	100	-	-	-	-	89.4	-
Bananas	*EBF*	16.0	8.0	1.3	-	-	-	-	74.7	0.72
	NEBF	10.5	7.4	1.1	-	-	-	-	81.1	-
	All	12.9	7.6	1.2	-	-	-	-	78.2	-
Pawpaw	*EBF*	6.7	-	-	-	-	-	-	93.3	0.86
	NEBF	7.4	-	-	-	-	-	-	92.6	-
	All	7.1	-	-	-	-	-	-	92.9	-
Mangoes	*EBF*	21.3	16.0	5.3	2.7	4.0	-	-	50.7	0.97
	NEBF	20.0	13.7	6.3	3.2	2.1	-	-	54.7	-
	All	20.6	14.7	5.9	2.9	2.9	-	-	52.9	-
Oranges	*EBF*	24.0	9.3	5.3	1.3	-	-	-	60.0	0.78
	NEBF	22.1	7.4	2.1	2.1	-	-	-	66.3	-
	All	22.9	8.2	3.5	1.8	-	-	-	63.5	-
Kales	*EBF*	8.0	16.0	12.0	13.3	22.7	10.7	10.7	6.7	0.94
	NEBF	6.3	15.8	14.7	20.0	18.9	9.5	7.4	7.4	-
	All	7.1	15.9	13.5	17.1	20.6	10	8.8	7.1	-
Cabbage	*EBF*	25.3	10.7	2.7	5.3	4.0	1.3	5.3	45.3	0.17
	NEBF	28.4	11.6	10.5	4.2	6.3	1.1	0.0	37.9	-
	All	27.1	11.2	7.1	4.7	5.3	1.2	2.4	41.2	-
Carrots	*EBF*	18.7	2.7	0.0	0.0	-	-	-	78.7	0.49
	NEBF	11.6	3.2	2.1	1.1	-	-	-	82.1	-
	All	14.7	2.9	1.2	0.6	-	-	-	80.6	-
Cow pea leaves	*EBF*	8.0	2.7	5.3	13.3	16.0	10.7	4.0	40.0	0.58
	NEBF	10.5	4.2	8.4	16.8	15.8	6.3	9.5	28.4	-
	All	9.4	3.5	7.1	15.3	15.9	8.2	7.1	33.5	-
Amaranth	*EBF*	8.0	2.7	2.7	2.7	1.3	-	-	82.7	0.23
	NEBF	10.5	10.5	6.3	3.2	2.1	-	-	67.4	-
	All	9.4	7.1	4.7	2.9	1.8	-	-	74.1	-
Managu[c]	*EBF*	9.3	5.3	4.0	2.7	-	-	-	78.7	0.95
	NEBF	11.6	6.3	2.1	3.2	-	-	-	76.8	-
	All	10.6	5.9	2.9	2.9	-	-	-	77.6	-
Beans	*EBF*	34.7	17.3	6.7	8.0	5.3	-	-	28.0	0.72
	NEBF	30.5	16.8	14.7	7.4	4.2	-	-	26.3	-
	All	32.4	17.1	11.2	7.6	4.7	-	-	27.1	-
Pigeon peas	*EBF*	6.7	1.3	1.3	5.3	4.0	-	-	81.3	0.45
	NEBF	9.5	6.3	1.1	4.2	1.1	-	-	77.9	-
	All	8.2	4.1	1.2	4.7	2.4	-	-	79.4	-
Green grams	*EBF*	9.3	12.0	8.0	2.7	0.0	-	-	68.0	0.09
	NEBF	18.9	4.2	4.2	1.1	2.1	-	-	69.5	-
	All	14.7	7.6	5.9	1.8	1.2	-	-	68.8	-

[a] EBF: exclusive breastfeeding mothers, [b] NEBF: non-exclusive breastfeeding mothers, [c] *Managu*: (*Solanum nigrum*), -: no frequency

Table 6: Consumption frequency within a day of foods unlikely to be contaminated with aflatoxin among lactating mothers who reported weekly consumption of foods in Kibwezi West

Food		Consumption frequency within a day (%) 1	2	3	4	χ^2
Potatoes	[a] EBF(n=25)	64.0	36.0	-	-	0.204
	[b] NEBF (n=19)	68.4	21.1	10.5	-	-
	ALL(n=44)	65.9	29.5	4.5	-	-
Sweet potatoes	EBF (n=11)	81.8	18.2	-	-	0.605
	NEBF (n=7)	71.4	28.6	-	-	-
	ALL (n=18)	77.8	22.2	-	-	-
Bananas	EBF (n=19)	94.7	5.3	-	-	0.604
	NEBF (n=18)	88.9	11.1	-	-	-
	ALL (n=37)	91.9	8.1	-	-	-
Pawpaw	EBF (n=5)	100.0	-	-	-	0.071
	NEBF (n=7)	42.9	57.1	-	-	
	ALL(n=12)	66.7	33.3	-	-	
Mangoes	EBF(n=37)	45.9	54.1	-	-	0.148
	NEBF(n=43)	30.2	69.8	-	-	-
	ALL(n=80)	37.5	62.5	-	-	-
Oranges	EBF (n=30)	46.7	40.0	13.3	-	0.391
	NEBF(n=32)	62.5	31.3	6.3	-	-
	ALL (n=62)	54.8	35.5	9.7	-	-
Kales	EBF(n=69)	43.5	56.5	-	-	0.642
	NEBF(n=89)	47.2	52.8	-	-	-
	ALL(n=158)	45.6	54.4	-	-	-
Cabbage	EBF(n=41)	82.9	17.1	-	-	0.043
	NEBF(n=59)	62.7	37.3	-	-	-
	ALL(n=100)	71.0	29.0	-	-	-
Carrots	EBF(n=16)	68.8	18.8	6.3	6.3	0.995
	NEBF(n=18)	66.7	22.2	5.6	5.6	-
	ALL(n=34)	67.6	20.6	5.9	5.9	-
Cowpea leaves	EBF(n=45)	46.7	53.3	-	-	0.702
	NEBF(n=68)	42.6	57.4	-	-	-
	ALL (n=113)	44.2	55.8	-	-	-
Amaranth	EBF(n=13)	38.5	61.5	-	-	0.51
	NEBF(n=31)	54.8	45.2	-	-	-
	ALL(n=44)	50.0	50.0	-	-	-
Managu[c]	EBF(n=16)	62.5	37.5	-	-	-
	NEBF(n=22)	72.7	27.3	-	-	-
	ALL(n=38)	68.4	31.6	-	-	-
Beans	EBF(n=54)	61.1	25.9	13.0	-	0.931
	NEBF(n=70)	60.0	28.6	11.4	-	-
	ALL(n=124)	60.5	27.4	12.1	-	-
Pigeon peas	EBF(n=14)	35.7	42.9	21.4	-	0.469
	NEBF(n=21)	42.9	23.8	33.3	-	-
	ALL(n=35)	40.0	31.4	28.6	-	-
Green grams	EBF(n=24)	58.3	37.5	4.2	-	0.84
	NEBF(n=29)	82.8	17.2	0.0	-	-
	ALL (n=43)	71.7	26.4	1.9	-	-

[a] EBF: exclusive breastfeeding mothers, [b] NEBF: non-exclusive breastfeeding mothers, [c] *Managu*: Traditional leafy vegetable (*Solanum nigrum*), -: no frequency

4.2.3 Weekly aflatoxin consumption score of lactating mothers in Kibwezi West

The mean weekly percentage aflatoxin consumption score for all lactating mothers (n = 170) in the study was 8.0% (*SD,* 3.3; range, 1.8 to 20%). Based on breastfeeding status, a percentage mean score of 7.8% (*SD*, 3.5; range, 8 to 18.7%) and 8.2% (*SD* 3.1; range, 2.6 to 20%) was reported for exclusively (n = 75) and non-exclusively (n = 95) lactating mothers, respectively. Consequently, all lactating mothers were categorized under the 1st quartile group (0 to <25% score). However, out of a possible maximum consumption score of 28 (maximum consumption per week [7] × maximum consumption within a day [4]), a mean score of 9.4 (*SD,* 6.3; range, 1 to 28) was reported for maize ugali, 9.2 (*SD* 5.1; range, 1 to 24) was reported for maize porridge, while a mean score of 5.0 (*SD,* 4.8; range 1 to 20) was reported for groundnuts. *'Githeri's'* mean score was 3.6 (*SD,* 3.9; range, range 0 to 21). Consumption scores for the remaining foods were less than 2.1 recorded for animal milk (cow/goat) (Table 7), with the least being reported for fish ($\bar{x}$ = 0.1, *SD* = 0.3; range 1 to 2). A statistical difference was only observed in the weekly consumption score of maize '*ugali'* (Mann Whitney U, p = 0.01), rice (Mann Whitney U, p = 0.02), and fish (Mann Whitney U, p = 0.02) between exclusively and non-exclusively lactating mothers.

Table 7: Weekly aflatoxin consumption score of lactating mothers in Kibwezi West

	All (N=170)		(EBF [a]) (n=95)		(NEBF [b]) (n=75)		[g] M-W *U*-test EBF*NEBF
Food	**$\bar{x}$ (*SD*)**	**Range**	**$\bar{x}$ (*SD*)**	**Range**	**$\bar{x}$ (*SD*)**	**Range**	***p*-value**
Sorghum *ugali*[c]	1.2(3.7)	0-28	1.1(3.9)	0-28	1.3(3.6)	0-21	0.55
Maize *ugali*	9.4(6.3)	1-28	7.8 (5.0)	1-28	10.7(7.1)	2-28	0.01*
Mixed *ugali*	0.9(2.5)	0-14	0.6(1.8)	0-7	1.1(2.9)	0-14	0.48
Githeri[d]	3.6(3.9)	0-21	3.2(3.5)	0-21	3.8 (4.1)	0-21	0.31
Muthokoi[e]	1.3(1.8)	0-8	1.3 (1.8)	0-8	1.4 (1.8)	0-8	0.87
Maize porridge	9.2(5.1)	1-24	9.0 (5.3)	1-21	9.4 (5.0)	1-24	0.49
Mixed porridge	1.5(4.1)	0-21	1.3 (3.5)	0-16	1.7(4.6)	0-21	0.99
Cassava porridge	0.8(3.5)	0-24	1.4 (4.6)	0-24	0.4(2.4)	0-20	0.16
Finger millet	0.4(2.3)	0-21	0.8(3.3)	0-21	0.1(0.6)	0-6	0.10
Groundnuts	5.0(4.8)	0-20	4.5(4.2)	0-20	5.3(5.3)	0-20	0.80
Sorghum porridge	1.4(3.2)	0-18	1.6 (3.7)	0-18	1.1 (2.8)	0-14	0.87
Rice	1.4(2.2)	0-15	1.8 (2.5)	0-15	1.1 (1.9)	0-12	0.02*
Cassava tuber	0.5(1.2)	0-8	0.5 (1.4)	0-8	0.5 (1.1)	0-6	0.84
Beef	0.8(1.2)	0-6	0.9(1.4)	0-6	0.7(0.9)	0-4	0.42
Chicken	0.3(0.6)	0-4	0.3 (0.5)	0-2	0.3 (0.7)	0-4	0.99
Eggs	0.6(1.3)	0-9	0.5(1.2)	0-6	0.7(1.4)	0-9	0.88
Fish	0.1(0.3)	0-2	0.1(0.4)	0-2	0.04(0.2)	0-1	0.02*
Dairy Milk	2.1(3.5)	1-21	2.3(3.6)	0-15	2.0(3.4)	0-21	0.65

[a] EBF: exclusive breastfeeding mothers, [b] NEBF: non-exclusive breastfeeding mothers, [c] *Ugali:* stiff solid maize flour paste; [d] *Githeri*: maize grains boiled together with beans or peas; [e]*Muthokoi*: dehulled maize grains boiled together with beans or peas, [g] M-W: Mann Whitney *Significant p<0.0

4.2.4 Consumption estimates of foods likely to be contaminated with aflatoxin in the study

Mean consumption quantity (g/day) of maize porridge (*n* = 170) was 412.3 (*SD*, 172.5; range, 105.4 to 861.4), while that of maize ugali (*n* =170) was 340.5 (*SD*, 154.4; range, 107.1 to 720.0). Consumption quantities of maize-sorghum porridge, '*githeri',* '*muthokoi'*, cassava, finger millet, rice, and groundnuts were less than 100 g/day (Table 8). Consumption quantities of animal-based foods were less than 20 g/day with consumption of milk in form of milk tea being 19.5 g/day (*SD*, 27.3; range, 0.0 to 85.7). The lowest consumption was reported for both beef and fish at 0.0 g/day (Table 8). Consumption estimates for each food between mothers who exclusively and non-exclusively breastfed their children were not statistically different (Mann Whitney *U*, $p_{all} > 0.05$).

Table 8: Consumption of foods likely to be contaminated with aflatoxin in Kibwezi West

Foods	All $\bar{x}$ (*SD*) g/day (*Range*)	EBF [a] $\bar{x}$ (*SD*) g/day (*Range*)	NEBF [b] $\bar{x}$ (*SD*) g/day (*Range*)	Mann Whitney-*U* EBF*NEBF
Maize porridge	412.3(172.5) $^{n=170}$ (105.4-861.4)	404.5(172.6) $^{n=75}$ (115.7-790.7)	418.5(173.0) $^{n=95}$ (105.4-861.4)	0.58
Maize *Ugali* [c]	340.5(154.4) $^{n=170}$ (107.1-720.0)	319.7(130.4) $^{n=75}$ (107.1-720.0)	356.9(169.9) $^{n=95}$ (107.1-720)	0.30
Maize sorghum porridge	59.0(146.3) $^{n=170}$ (0.0-698.1)	67.7(155.4) $^{n=75}$ (0.0-655.7)	52.2(139.1) $^{n=73}$ (0.0-698.1)	0.90
Githeri [d]	93.6(52.7) $^{n=111}$ (23.1-215.2)	90.0(48.5) $^{n=48}$ (23.1-211.8)	96.3(55.9) $^{n=63}$ (23.1-215.2)	0.70
Muthokoi [e]	36.2(45.9) $^{n=154}$ (0.0-151.0)	33.9(41.3) $^{n=69}$ (0.0-149.3)	38.0(49.4) $^{n=85}$ (0-151.0)	0.90
Finger millet	15.9(65.7) $^{n=170}$ (0.0-500.0)	26.4(90.3) $^{n=75}$ (0.0-500.0)	7.6(34.6) $^{n=95}$ (0-200.0)	0.11
Cassava	38.0(85.5) $^{n=170}$ (0.0-494.0)	37.5(74.1) $^{n=75}$ (0.0-247.0)	38.4(94.0) $^{n=95}$ (0-494.0)	0.78
Rice	30.6(53.8) $^{n=170}$ (0.0-327.9)	40.9(67.3) $^{n=75}$ (0.0-327.9)	22.5(38.5) $^{n=95}$ (0-229.5)	0.07
Groundnuts	30.9(23.8) $^{n=130}$ (0.0-85.7)	30.9(20.9) $^{n=62}$ (0.0-85.7)	31.0(26.3) $^{n=68}$ (0-85.7)	0.67
Beef	3.4(5.2) $^{n=147}$ (0.0-17.1)	3.7(5.5) $^{n=63}$ (0.0-17.1)	3.2(5.0) $^{n=84}$ 0.0-17.1	0.63
Chicken	0.0 $^{n=130}$ (0.0-0.0)	0.0 $^{n=57}$ (0.0-0.0)	0.0 $^{n=73}$ (0.0-0.0)	1.00
Eggs	1.7(3.0) $^{n=151}$ (0.0-7.8)	2.1(3.2) $^{n=69}$ (0.0-7.8)	1.4(2.7) $^{n=82}$ (0-7.81)	0.22
Fish	0.0 $^{n=155}$ (0.0-0.0)	0.0 $^{n=64}$ (0.0-0.0)	0.0(0.0) $^{n=91}$ (0.0-0.0)	1.00
Milk Tea	19.5(27.3) $^{n=132}$ (0.0-85.7)	19.8(27.0) $^{n=53}$ (0.0-85.7)	19.3(27.6) $^{n=70}$ (0-85.7)	0.79

[a] EBF: exclusive breastfeeding mothers, [b] NEBF: non-exclusive breastfeeding mothers, [c] *Ugali:* stiff solid maize flour paste; [d] *Githeri*: maize grains boiled together with beans or peas; [e]*Muthokoi*: dehulled maize grains boiled together with beans or peas

4.2.5 Typical number of meals taken by lactating mothers in the study

The mean usual number of meals among lactating mothers in the study was three meals, the least being one meal while the most being five meals in a day. One meal was reported by 1.8% of mothers, two meals by 18.2%, three meals by 57.1%, and five meals by 1.8%. Breakfast, lunch, and supper were the most consumed types of meals by 80.6, 86.5, and 98.8% of the mothers, respectively. Ten percent (10%) of lactating mothers took light breakfast before consuming the main breakfast with other household members. Approximately 17.1% took midmorning snacks, 5.9% afternoon snacks, and only 4.1% took a snack before sleeping. The total daily number of meals per day for exclusively and non-exclusively breastfeeding mothers was similar (Mann-Whitney U, $p = 0.087$).

4.2.6 Dietary diversity of lactating mothers

The mean dietary diversity score for the lactating mothers in the study was 3.4 (*SD*, 1.5), with a range of 1 to 6, and a mode and median of two and three food groups, respectively. More than half (54.1%) of lactating mothers were within lower dietary diversity (1-3), while 45.9% were within the medium dietary diversity (4-6). None of the mothers interviewed were within the high dietary diversity category. A significant difference was reported between the dietary diversity of exclusively lactating mothers (mean = 3.9, *SD* 1.5) and non-exclusively lactating mothers (3.0, *SD* 1.3) (Mann-Whitney *U*, $p = 0.00$). Further analysis showed that starchy staple food groups were consumed in the preceding day by all (100%) lactating mothers in the study, followed by milk (57.1%), other fruits and vegetables (54.7%). Legume food group was consumed by 36.5% of the lactating mothers. Vitamin A-rich fruits and vegetables, and fish and meat food groups were both consumed by 30.6% of the mothers. Eggs were consumed by 14.1% while organ meat was the least consumed by 2.9%.

4.2.7 Source, storage, and processing of cereals by lactating mothers

Slightly more than half (52.4%) of the lactating mothers obtained their cereals mainly from the market, 40.0% cultivated, while 7.6% depended on other sources (gifts/donations/reliefs). Almost half (44.7%) of the mothers did nothing to their grains during storage, while a few (5.3%) dried their cereals in the sun. Only 7.6% sorted out discolored and disfigured grains before storage. Of those who stored their grains for a longer time, 20.6% reported applying chemicals, while 12.4% mixed grains with ash before storage. The main storage containers used by mothers included sacks (37.1%), buckets (24.7%), granaries (22.4%), and other forms of bags (15.9%). Before cooking grains, all mothers reported sorting out damaged grains, while

53.5 and 46.5% washed in normal and ash-diluted water, respectively. Those who dry in the sun before cooking were 25.3%. All the mothers mentioned sorting out particularly maize before milling. There was no difference between exclusive and non-exclusive breastfeeding mothers on different storage and cooking processes subjected to cereals (Mann-Whitney *U*, source of cereals, $p = 0.63$, washing grains in normal water, $p = 0.06$, washing grains in ash diluted water, $p = 0.06$, drying in the sun, $p = 0.08$).

4.2.8 Breastfeeding practices of lactating mothers in the study

4.2.8.1 Breastfeeding practices of lactating mothers

The mean age of breastfeeding children was 3.8 months (*SD*, 1.5) ranging between 0.49-5.75 months. Less than a half (44.1%) were exclusively breastfeeding at the time of conducting the study. The average age for introducing other foods/or liquids alongside breast milk was 3.34 months (*SD*, 1.3). About a half (48.4%) of non-exclusive breastfeeding mothers introduced complementary foods/liquids at an age of four months, 15.8% at the first month, 13.7% at the third month, and 10.5% for both second and fifth month. Only 1.1% of the mothers introduced complementary feeds less than a month after delivery. All non-exclusively breastfeeding mothers mentioned animal milk as one of the most commonly used complementary foods, followed by 90.5% millet porridge, 87.4% maize porridge, 78.9% mixed flour porridge, 53.7% mashed ugali, and 46.3% sorghum porridge. However, maize porridge and animal milk (cow/goat) were the most frequently consumed complementary foods per week by 100% of non-exclusively breastfed children, followed by 73.7% mashed maize ugali. The least frequently consumed complementary food per week by non-exclusively breastfeeding children, was millet porridge (11.5%), followed by sorghum porridge (16.8%), and mixed porridge (28.4%).

All breastfeeding children in the study were introduced to breast milk not more than 24 hours after delivery. Exclusively breastfeeding children averagely consumed breast milk 17.2 times per 24-hour period with a range of 9-26 times. The mode frequency was 13 times. Non-exclusive breastfeeding children on the other hand averagely consumed breast milk 13.9 times per 24-hour period. The range was 7 to 25 times, while mode frequency was 13 times. Overall mean frequency over a typical 24-hour period was 15.3 times. However, the frequency of breastfeeding per day for exclusively breastfeeding children was higher than that of non-exclusively breastfeeding counterparts (Mann-Whitney *U*, $p = 0.00$).

Results showed that 73.3 and 68.4% of exclusively and non-exclusively breastfeeding mothers, respectively desired to continue breastfeeding their children up to two years, while only 5.3% of exclusive and 4.2% of non-exclusive breastfeeding mothers desired to breastfeed their children up to six months. Those who desired to continue breastfeeding their children up to a year were 14.7% exclusively and 23.2% for non-exclusively breastfeeding mothers. A few exclusively (6.7%) and non-exclusively (4.2%) breastfeeding mothers desired to continue breastfeeding their children up to three years.

Frequency consumption of complementary foods per typical day showed that 46.3% of the mothers fed their children maize porridge five times on a typical day, 23.2% thrice, 14.7% four times, and 15.8% twice. Animal milk was mostly consumed twice on a typical day by (49.5%) non-exclusively breastfed children., thrice by 28.4%, once by 14.7%, four times by 6.3%, and five times by 1.1%. On the other hand, 40.0% consumed mashed ugali twice on a typical day, 25.37% once, and 9.5% thrice. Sorghum porridge was mostly used once on a typical day (8.4%), followed by 4.2 thrice, 3.2% twice, and 1.1% four times. Millet porridge was consumed once per typical day by 6.3%, twice by 4.2%, and thrice by 3.2%. Mixed was consumed mostly twice by 13.7% of children, once by 6.3%, thrice by only 5.3%, and four times by 3.2%.

4.2.8.2 Quantities of breast milk consumed per day by breastfed children in the study

The average quantity of breast milk consumed by breastfeeding children (n = 48) in the study was 543.3 ml/day (*SD*, 184.2; range, 233.0 to 922.3). Children aged between 0 to 1 month averagely consumed 583.6 (*SD*, 189) ml/day of breast milk, while those aged between >1 to 2 months averagely consumed 528.6 (*SD*, 203) ml/day. Children between >3 to 4 months averagely consumed 586.7 (*SD*, 207) ml/day, while those between >4 to 5 and >5 to 6 months consumed 491.3 (*SD*, 121) and 527.5 (*SD*, 176) ml/day, respectively. No significant difference was reported (One-way ANOVA, $F_{4,43}$ = 0.0330, p = 0.856) (Table 9a). Further analysis showed that exclusively breastfeeding children (n = 20) averagely consumed 588.4 (*SD*, 156.9) ml/day of breastmilk, while non-exclusively breastfeeding children (n = 28) averagely consumed 511.1 (*SD*, 197.9) ml/day. However, no significant difference was observed between them (t_{46} = 1.449, p = 0.154).

Table 9a: Quantities of breastmilk consumed by breastfeeding children per age group in Kibwezi West

Months	$\bar{x}$ (*SD*)(ml/day)	95% Confidence Interval		F-value	P-value
		Lower Bound	Upper Bound		
0-1 (n=9)	583.6(189)	438.5	728.7	0.33	0.856
>1-2 (n=18)	528.6(203)	427.7	629.4	df (4,43)	
>3-4 (n=7)	586.7(207)	395.2	778.2		
>4-5 (n=5)	491.3(121)	340.8	641.7		
>5-6 (n=9)	527.5(176)	392.5	662.5		

Further details (Table 9b) shows that average quantities of breast milk consumed by exclusively breastfeeding children aged between 0 to1, >1 to 2 and >4 to 5 months was not statistically different (One-way ANOVA, $F_{1,17} = 0.009$, $p = 0.991$). Likewise, average quantities of breast milk consumed by non-exclusively breastfeeding children aged between >1 to 2, >3 to 4, >4 to 5, and >5 to 6 months was not statistically different (One-way ANOVA, $F_{3,24} = 0.67$, $p = 0.579$). Correlation between age of breastfeeding children and quantities (volume) of breastmilk consumed per day (ml/day) was insignificant ($r = 0.038$, $p = 0.798$, $n = 48$) for all breastfeeding children; $r = -0.186$, $p = 0.434$, $n = 20$ for exclusively breastfeeding children; and $r = 0.121$, $p = 0.539$, $n = 28$ for non-exclusively breastfeeding children).

Table 9b: Quantities of breastmilk consumed by breastfeeding children per age group per breastfeeding status in Kibwezi West

Month	$\bar{x}$ (*SD*)(ml/day)	95% Confidence Interval		F-value	P-value
		Lower Bound	Upper Bound		
Exclusively breastfeeding children					
0-1 (n=9)	583.6(188.8)	438.5	728.7	0.01	0.991
>1-2 (n=10)	593.2(142.4)	491.4	695	df,1,17	
>4-5 (n=1)	582.5	-	-		
Non-exclusively breastfeeding children					
>1-2 (n=8)	447.8(245.8)	242.3	653.3	0.67	0.579
>3-4 (n=7)	586.7(207.1)	395.2	778.2	df,3,24	
>4-5 (n=4)	468.4(126.9)	266.5	670.4		
>5-6 (n=9)	527.5(175.6)	392.5	662.5		

4.2.8.3 Breast milk intake of breastfeeding children in Kibwezi West

Mean breastmilk intake among breastfeeding children (n = 45) in the study was 82.3 (*SD*, 31.7) ml/kg b.w.t/day with a range of 31.6 to 157.8. The mean intake for exclusively breastfeeding children (n = 18) was 91.4 (*SD*, 34.1), while that of non-exclusively breastfeeding children (n =27) was 76.3 (*SD*, 28.9) ml/kg b.w.t/day. The means between the two breastfeeding groups were, however, not significantly different (t_{43} =1.598, p = 0.117). On the other hand, a statistical significance (One-way ANOVA, $F_{4,40}$ = 3.650, p = 0.013) in breast milk intake was observed between age groups of all breastfeeding children (0-1, >1-2, >2-3, >3-4, >4-5, and >5-6 months) (Table 10a). Post hoc analysis (Tukey's b) showed statistical significance was between children aged 0 to 1 month and those aged 5 to 6 months (p = 0.035).

Table 10a: Breast milk intake by age group of breastfeeding children in Kibwezi West

		95% Confidence Interval			
Month	**$\bar{x}$ (*SD*) (ml/kg b.w.t/day)**	**Lower Bound**	**Upper Bound**	**F-value**	**p-value**
0-1 (n=7)	107.6(38)	72.9	142.3	3.650	0.013
>1-2 (n=17)	78.0(28)	63.2	92.1		
>3-4 (n=7)	102.6(31)	73.5	131.7		
>4-5 (n=5)	66.6(20)	42.1	91		
>5-6 (n=9)	64.6(22)	47.9	81.3		

Based on breastfeeding status (Table 10b), a significant difference in breast milk intake was reported among age groups of non-exclusively breastfeeding children (One-way ANOVA, $F_{3,23}$ = 3.628, p = 0.028). However, no significant difference in breast milk intake was reported between age groups of exclusively breastfeeding children (One-way ANOVA, $F_{1,17}$ = 1.359, p = 0.287). Post hoc analysis among non-exclusively breastfeeding children (Tukey's b) showed that statistical significance was between children aged >3 to 4 months ($\bar{x}$ = 102.6, *SD* = 31.5) and those aged >5 to 6 months ($\bar{x}$ = 64.6, *SD* = 22) (p = 0.032). However, correlation between age of children (regardless of breastfeeding status) and breast milk intake was insignificant (r = -0.074, p = 0.631, n = 45). Based on breastfeeding status, a negative significant correlation was reported for exclusively breastfeeding children (r = -0.484, p = 0.042, n = 18) as opposed to non-exclusively breastfeeding children (r = 0.114, p = 0.571, n = 27).

Table 10b: Breast milk intake by age group and breastfeeding status of children in Kibwezi West

Months	$\bar{x}$ (*SD*)(ml/kg b.w.t/day)	95% Confidence Interval		F-value	P-value
		Lower.Bound	Upper.Bound		
Exclusively breastfeeding children					
0-1 (n=7)	107.6(37.5)	72.9	142.3	1.359	0.297
>1-2 (n=10)	80.5(30.4)	58.7	102.3		
>4-5 (n=1)	86.9	-	-		
Non-exclusively breastfeeding children					
>1-2 (n=7)	73.5(25.9)	49.5	97.5	3.628	0.028
>3-4 (n=7)	102.6(31.5)	73.5	131.7		
>4-5 (n=4)	61.5(18.5)	32	91		
>5-6 (n=9)	64.6(21.7)	47.9	81.3		

4.3 Weight-for-age z-score of breastfeeding children below six months in the study

The mean weight for children recruited in the study was 6.6 (*SD*, 1.9) kg. The mode was 5 kg, and range was between 2.3 to 11.0 kg. The mean weight for non-exclusively breastfeeding children ($\bar{x}$ = 7.1, *SD* = 1.8 kg; *n* = 95) was significantly greater than the mean that of exclusively breastfeeding children ($\bar{x}$ = 5.9, *SD* =1.8 kg, *n* = 75) at (t_{168} = -4.271, *p* = 0.00). Nutritional status based on weight-for-age z scores showed that 87.6% children had normal weight (z-score >-2), while the rest 12.4% (5.9% exclusively, and 6.5% non-exclusively breastfeeding) were underweight (z-score <-2 SD). Mean weight-for-age z-scores for all breastfeeding children in the study was -0.1 (*SD*, 1.6), that of exclusively breastfeeding children was -0.4 (*SD*, 1.6; *n* = 75), while that of non-exclusive breastfeeding children was 0.1 (*SD*, 1.7; *n* = 95). A significant difference was observed between the two breastfeeding groups (t_{168} = -2.049, *p* = 0.042). When children were categorized into age groups, a statistical difference in weight-for-age z-score was noted among all children (*n* = 48) regardless of their breastfeeding status (one-way ANOVA, $F_{5,164}$ = 9.632, *p* = 0.00). A statistical difference was also observed between age groups of exclusively breastfeeding children (one-way ANOVA $F_{5,69}$ = 5.692, *p* = 0.00), and that of non-exclusively breastfeeding children ($F_{5,89}$ = 4.099, *p* = 0.002) (Table 11). Underweight of 12.4% was reported in the study (13.3% for exclusively, and 11.6% for non-exclusively breastfeeding children).

Table 11: Weight-for-age z-scores of breastfeeding children by age group and breastfeeding status in Kibwezi West

	All Breastfeeding children			Exclusively Breastfeeding children			Non-exclusively Breastfeeding children		
Age	**n**	**$\bar{x}$ (SD)**	**WFA<-2[a] (%)**	**n**	**$\bar{x}$ (SD)**	**WFA<-2 (%)**	**n**	**$\bar{x}$ (SD)**	**WFA<-2[a] (%)**
>0-1	8	-1.6(1.4)	2.4	7	-1.4(1.4)	4.0	1	-2.9	1.1
>1-2	21	-1.4(0.9)	2.9	12	-1.5(0.8)	2.7	9	-1.4(1.0)	3.2
>2-3	27	-0.6(1.8)	2.9	18	-0.8(1.4)	4.0	9	-0.1(2.4)	2.1
>3-4	37	-0.1(1.5)	2.4	20	-0.1(1.2)	1.3	17	-0.2(1.8)	3.2
>4-5	38	0.3(1.7)	1.2	15	0.8(1.7)	1.3	23	0.1(1.6)	1.1
>5-6	39	0.8(1.2)	0.6	3	0.9(1.5)	0.0	36	0.8(1.2)	1.1
Total	170	-0.1(1.6)	12.4	75	-0.4(1.6)	13.3	95	0.1(1.7)	11.6

[a] WFA <-2: underweight

4.4 Concentration levels of total aflatoxin and aflatoxin B1 in the study

Aflatoxin was detected in 85.4% (41/48) food samples with an overall mean concentration of 97.8 µg/kg (*SD,* 57.7; range, 2.3 to 210.0) and 9.0 µg/kg (*SD*, 7.7; range, 0.7 to 32.3) being reported for total aflatoxin and aflatoxin B1, respectively (Table 12). Based on breastfeeding status, a prevalence of 95% (n = 20), mean concentration of 114.8 µg/kg (SD, 40.9; range 45.5 to 195.2) for total aflatoxin and 9.2 µg/kg (SD, 7.9; 1.0 to 29.9) for aflatoxin B1 was reported for exclusively breastfeeding mothers. On the other hand, a prevalence of 78.6% (n = 28), and a mean concentration of 83.1 µg/kg (SD, 66.5; range 2.3 to 210.0) for total aflatoxin and 8.8 µg/kg (SD, 7.7; range 2.1 to 32.3) for aflatoxin B1 was reported for non-exclusively breastfeeding mothers. No statistical difference was reported between the mean concentration of total aflatoxin (Mann Whitney *U*, p = 0.07), and aflatoxin B1(Mann Whitney *U,* p = 0.97) of lactating mothers. Prevalence of aflatoxin in foods of exclusively breastfeeding mothers was 1.2 times that of non-exclusively breastfeeding mothers [Prevalence Ratio, 95% C.I, 0.97 to 1.50] even though not statistically different (Fisher's exact sig. 2-sided, p = 0.214). Of the positive food samples (n = 41), 90.2% (100% for exclusively [n =19] and 81.8% for non-exclusively breastfeeding mothers [n = 22]) were above 10 µg/kg Kenya Bureau of Standards (KEBS) maximum tolerable limit set for total aflatoxin. Similarly, 92.7% of the same samples (84.2% for exclusively [n = 19] and 100% for non-exclusively breastfeeding mothers [n = 22]) were above 2 µg/kg KEBS maximum tolerable limit set for aflatoxin B1. Prevalence of aflatoxin in maize porridge (n = 6) was 100% with a mean concentration of total aflatoxin and aflatoxin B1 being 48.0 µg/kg (*SD,* 64.2; range 2.33 to 172.9) and 8.89 µg/kg (*SD*, 10.4; range 2.7-29.9), respectively. Prevalence of aflatoxin in '*muthokoi'* (n = 6) was also 100% with a

mean concentration being 102.9 µg/kg (*SD*, 47.6; range, 52.5 to 195.2) for total aflatoxin and 6.92 µg/kg (SD, 6.3, range 0.7 to 17.4) for aflatoxin B1. For '*githeri'* (*n* = 9), a prevalence of 88.9%, and a mean concentration of 130.1µg/kg (SD, 57.9; range 60.8 to 210) and 7.09 µg/kg (*SD*, 3.5; range 1.1 to 12) was reported for total aflatoxin and aflatoxin B1, respectively. Similarly, a prevalence of 77.8% was reported for both maize ugali (*n* = 18) and maize-sorghum porridge (*n* = 9). Their mean concentration for total aflatoxin were 109.3 µg/kg (*SD*, 47.6; range 38.4 to 168.3) and 75.9 µg/kg (SD, 53.4; range, 2.7 to 139.4), respectively, while that of aflatoxin B1 were 12.1µg/kg (SD, 8.6; range, 2.9 to 32.3) and 6.85 µg/kg;(SD, 7.9; range 1.1 to 24.2), respectively (Table 12).

Table 12: Concentration levels of total aflatoxin and aflatoxin B1 in maize-based cooked food samples in Kibwezi West

Total aflatoxin concentration (µg/kg)						
	All mothers		**[a] EBF mothers**		**[b] NEBF mothers**	
Food	**x̄ (*SD*)**	**Range**	**x̄ (*SD*)**	**Range**	**x̄ (*SD*)**	**Range**
Maize *ugali* [c]	109.3(47.6) *n=14*	38.4-168	126.4(37.7) *n=8*	49.5-168.3	86.7(53.1) *n=6*	38.4-162.8
M.porridge [d]	48.0(64.3) *n=6*	2.3-173	45.5 *n=1*	45.5	48.5(71.9) *n=5*	2.3-172.9
M.s.porridge [e]	75.9(53.4) *n=7*	2.7-139	127.5(16.9) *n=2*	115.5-139.4	55.2(48.4) *n=5*	2.7-112.0
Githeri [f]	130.1(57.9) *n=8*	60.8-210	92.7(31.7) *n=5*	60.8-129.5	192.4(20.4) *n=3*	170-210.0
Muthokoi [g]	102.9(52.9) *n=6*	52.5-195	135.2(54.6) *n=3*	88.4-195.2	70.6(29.8) *n=3*	52.5-105.0
All foods	97.8(57.7) *n=41*	2.3-210.0	114.8(40.9) *n=19*	45.5-195.2	83.1(66.5) *n=22*	2.3-210.0
Aflatoxin B1 concentration (µg/kg)						
	All mothers		**EBF mothers**		**NEBF mothers**	
Food	**x̄ (*SD*)**	**Range**	**x̄ (*SD*)**	**Range**	**x̄ (*SD*)**	**Range**
Maize *ugali*	12.1(8.6) *n=14*	2.9-32.3	10.1(6.9) *n=8*	2.9-21.0	14.7(10.5) *n=6*	4.2-32.3
M.porridge	8.9(10.4) *n=6*	2.7-29.9	29.9 *n=1*	29.9	4.7(1.9) *n=5*	2.7-7.4
M.s. porridge	6.9(7.9) *n=7*	1.1-24.2	2.9(2.6) *n=2*	1.1-4.7	8.4(9.0) *n=5*	2.8-24.2
Githeri	7.1(3.5) *n=8*	1.1-12.0	7.5(4.4) *n=5*	1.1-12.0	6.3(1.3) *n=3*	5.3-7.8
Muthokoi	6.9(6.3 1) *n=6*	1.0-17.4	7.0(9.0) *n=3*	1.0-17.4	6.8(4.1) *n=3*	2.1-9.4
All foods	9.0 (7.7) *n=41*	1.0-32.3	9.2(8.0) *n=19*	1.0-29.9	8.8(7.7) *n=22*	2.1-32.3

[a] EBF: Exclusive breastfeeding; [b] NEBF: Non-exclusive breastfeeding; [c] *Ugali:* stiff solid maize flour paste; [d] M. porridge: Maize porridge; [e] M. s. porridge: Maize-sorghum porridge; [f] *Githeri*: maize grains boiled together with beans or peas, [g] *Muthokoi*: De-hulled maize grains boiled together with beans or peas

4.4.1 Dietary intake of total aflatoxin and aflatoxin B1 of lactating mothers in the study

The overall mean dietary intake of total aflatoxin and aflatoxin B1 among lactating mothers regardless of breastfeeding was 7.6 µg/kg/b.w.t/day (*SD*, 7.5; range, 0.0 to 23.9) and 0.6 (*SD*, 0.6; range, 0 to 1.9), respectively (Table 13). Moreover, based on breastfeeding status, an overall mean intake of 9.4 µg/kg/b.w.t/day (*SD*, 8.6; range, 0 to 23.9), and 0.6 (*SD*, 0.6; 0 to 1.9) for total aflatoxin and aflatoxin B1, respectively, were reported for exclusively breastfeeding mothers (*n* = 19). On the other hand, an overall mean intake of 5.7 µg/kg/b.w.t/day (SD, 6.4; 0 to 17.6), and 0.5 (SD, 0.7; range, 0 to 1.9) for total aflatoxin and aflatoxin B1, respectively, were reported for non-exclusively breastfeeding mothers (*n* = 22). However, no significant difference was reported in dietary cumulative intake of total aflatoxin (Mann-Whitney *U*, *p* = 0.233), and aflatoxin B1 (Mann-Whitney *U*, *p* = 0.642) between exclusively and non-exclusively breastfeeding mothers in the study. Among food samples, the highest and lowest mean dietary intake of total aflatoxin (µg/kg/b.w.t/day) was reported for maize '*ugali'* ($\overline{x}$ = 14.4, *SD* = 6.2; range, 3.6 to 23.9), and maize-sorghum porridge ($\overline{x}$ = 0.1, SD = 0.2; range, 0 to 0.2), respectively. Likewise, highest and lowest mean intake of aflatoxin B1 was reported for maize '*ugali'* ($\overline{x}$ = 1.2, *SD* = 0.5; range, range 0.4 to 1.9), and *'muthokoi'* ($\overline{x}$ = 0.0, *SD* = 0.0; range 0.0 to 0.0), respectively (Table 13).

Table 13: Dietary intake of aflatoxin among lactating mothers in Kibwezi West

	Total aflatoxin intake (µg/kg b.w.t/day)					
	All mothers		EBF mothers [a]		NEBF mothers [b]	
Food	**x̄ (*SD*)**	**Range**	**x̄ (*SD*)**	**Range**	**x̄ (*SD*)**	**Range**
Maize *ugali* [c]	14.4(6.2) *n=14*	3.6-23.9	16.7(6.1) *n=8*	3.6-23.9	11.2(5.3) *n=6*	4.7-17.6
Maize porridge	4.0(6.2) *n=6*	0.3-16.1	1.68 *n=1*	1.68	4.5(6.7) *n=5*	0.3-16.1
Maize sorghum porridge	0.1(0.2) *n=7*	0-0.5	0(0) *n=2*	0-0.0	0.1(0.2) *n=5*	0-0.5
Githeri [d]	7.8(5.3) *n=8*	0-14.2	6.1(6.2) *n=5*	0-14.2	10.7(1.2) *n=3*	9.6-12.0
Muthokoi [e]	0.5(1.3) *n=6*	0-3.0	1.0(1.7) *n=3*	0-3.0	0.0(0.0) *n=3*	0.0-0.0
All	7.6(7.5) *n=41*	0-23.9	9.4(8.6) *n=19*	0-23.9	5.7(6.4) *n=22*	0-17.6
	Aflatoxin B1 intake (µg/kg b.w.t/day)					
	All mothers		EBF mothers		NEBF mothers	
	x̄ (*SD*)	**Range**	**x̄ (*SD*)**	**Range**	**x̄ (*SD*)**	**Range**
Maize ugali	1.2(0.5) *n=14*	0.4-1.9	1.0(0.5) *n=8*	0.4-1.9	1.4(0.5) *n=6*	0.8-1.9
Maize porridge	0.7(0.4) *n=6*	0.3-1.1	1.1 *n=1*	-	0.4(0.2) *n=5*	0.3-0.8
Maize sorghum porridge	0.0(0.1) *n=7*	0-0.2	0(0.0) *n=2*	0(0.0)	0.0(0.1) *n=5*	0-0.2
Githeri	0.4(0.4) *n=8*	0-1.2	0.5(0.6) *n=5*	0.5(0.6)	0.4(0.1) *n=3*	0.3-0.5
Muthokoi	0.0(0.0) *n=6*	0.0-0.0	0.0(0.0) *n=3*	0.0(0.0)	0.0(0.0) *n=3*	0.0-0.0
All	0.6(0.6) *n=41*	0-1.9	0.6(0.6) *n=19*	0-1.9	0.5(0.7) *n=22*	0-1.9

[a] EBF: Exclusively breastfeeding mothers; [b] NEBF: Non-exclusively breastfeeding mothers; [c] *Ugali:* stiff solid maize flour paste; [d] *Githeri*: Maize grains boiled with either beans and peas, [e] *Muthokoi*: De-hulled maize grains boiled with either beans and peas

4.4.2 Margin of exposure to dietary aflatoxin in lactating mothers in the study

Dietary total aflatoxin intake (µg/kg/b.w.t/day) of 65.9% (*n* = 41) lactating mothers (73.7% exclusively [n = 19] and 59.1% non-exclusively breastfeeding [*n* = 22]) were above benchmark does levels of 0.41µg/kg/b.w.t/day used in this study. Similarly, aflatoxin B1 intake of 48.8 % (*n* = 41) of lactating mothers (57.8% exclusively [*n* = 19] and 40.9% non-exclusively breastfeeding [n = 22]) were above the same benchmark does level (0.41µg/kg/b.w.t/day) used in this study. However, results showed that margin of exposure (MOE) of lactating mothers based on mean and 95th percentile levels of total aflatoxin and aflatoxin B1 dietary intake were lower than 10, 000 (European Food Safety Authority (EFSA) 2005) cut-off point (Table 14).

Table 14: Margin of exposure to dietary aflatoxin in lactating mothers in Kibwezi West

	AFT Intake [a] (µg/kg b.w.t/day)	MOE [b]	AFB1 [c] (µg/kg b.w.t/day)	MOE [d]
All lactating mothers (n=41)				
Mean	7.6	0.05	0.6	0.68
p95	20.9	0.02	1.9	0.22
EBF [e] mothers (n=19)				
Mean	9.4	0.04	0.6	0.68
p95	23.5	0.02	1.9	0.22
NEBF [f] mothers (n=22)				
Mean	5.6	0.07	0.5	0.82
p95 [g]	17.4	0.02	1.9	0.21

[a] AFT: Total aflatoxin; [b] MOE: Margin of exposure based on total aflatoxin intake; [c]AFB1: Aflatoxin B1; [d] MOE: margin of exposure based on aflatoxin B1 intake; [e] EBF: Exclusive breastfeeding; [f] NEBF: Non-exclusive breastfeeding; [g] p95: 95th percentile

4.5 Aflatoxin M1 in the breast milk of lactating mothers in the study

Aflatoxin M1 was detected in 77.1% (n = 48) breast milk samples of lactating mothers (90% for exclusively breastfeeding mothers [n = 20], and 67.9% for non-exclusively breastfeeding mothers [n = 28]). Prevalence ratio (PR) of 1.32 [95% C.I, 0.99 to 1.78] with an insignificant prevalence difference (Fisher's exact sig. 2-sided, p = 0.09) was reported between presence of aflatoxin M1 in breast milk of exclusively and non-exclusively breastfeeding mothers in the study. However, of the positive samples (n = 37), slightly over three-fifth (61.8% [77.8% for exclusively (n = 18) and 52.6% for non-exclusively breastfeeding mothers (n = 19)]) were above 25 ng/l EU recommended limits set for infant milk. Overall mean concentration levels of aflatoxin M1 reported in the study, after correcting for normality using Shapiro-Wilk (p > 0.05), was 35 ng/l (*SD*, 0.0; range 5 to 77, n = 34), while that of exclusively and non-lactating mother were 38 ng/l (*SD* = 0.2; range 5 to 77, n = 16) and 32 ng/l (*SD* = 0.2; range 5 to 68, n = 18), respectively. No significant difference was reported between mean concentration levels of aflatoxin M1 in breast milk of the two breastfeeding groups of lactating mothers (t_{32} = 0.906, and p = 0.372).

4.5.1 Aflatoxin M1 intake through breast milk among breastfeeding children in the study

Overall mean intake of aflatoxin M1 through breast milk was 0.47.µg/kg b.w.t/day (*SD*, 0.50; range, 0.0 to 1.7, *n* = 48). Highest mean intake was reported for children between >2 to 3 months ($\bar{x}$ = 0.9 µg/kg b.w.t/day, *SD* = 0.57; range, 0.2 to 1.7, *n* = 6), while the lowest intake was reported for one child aged between >5 to 6 months (0.2 µg/kg b.w.t/day) (Table 15a).

Table 15a: Aflatoxin M1 intake in breastmilk by age group of breastfeeding children in Kibwezi West

Month	Frequency	$\bar{x}$ (*SD*) (µg/kg b.w.t/day)	Range (µg/kg b.w.t/day)
0-1	4	0.6(0.46)	0.2-1.2
>1-2	5	0.6(0.29)	0.3-1.0
>2-3	6	0.9(0.57)	0.2-1.7
>3-4	6	0.6(0.48)	0.1-1.4
>4-5	12	0.7(0.50)	0.04-1.7
>5-6	1	0.2	-
All	34	0.7(0.47)	0.04-1.7

Based on breastfeeding status, mean aflatoxin M1 intake of exclusively breastfeeding children (*n* = 16) was 0.8 µg/kg b.w.t/day (*SD*, 0.50; range, 0.1 to 1.7). Highest intake (1.2 µg/kg b.w.t/day) in this group was reported for one child aged between 0 to 1 month, while the lowest ($\bar{x}$ = 0.1 µg/kg b.w.t/day, *SD* = 0.09; range, 0.1 to 0.2) was reported for children aged between >3 to 4 months (*n* = 5) (Table 15b). On the other hand, overall mean aflatoxin M1 intake of 0.6 µg/kg b.w.t/day (*SD*, 0.44; range, 0.0 to 1.7) was reported for non-exclusively breastfeeding children (*n* = 18). However, within this group, highest mean aflatoxin M1 intake ($\bar{x}$ = 0.8 µg/kg b.w.t/day, *SD* = 0.5; range 0.4 to 1.4) was reported for children aged between >3 to 4 months (*n* = 4), while the lowest intake (0.2 µg/kg b.w.t/day) was reported for a child aged between >5 to 6 months (Table 15b).

Table 15b: Aflatoxin M1 intake in breast milk by breastfeeding status and age group of breastfeeding children in Kibwezi West

Age group (month)	Exclusively breastfeeding children			Non-exclusively breastfeeding children		
	n	$\bar{x}$ (*SD*) (µg/kg b.w.t/day)	Range	*n*	$\bar{x}$ (*SD*) (µg/kg b.w.t/day)	Range
0-1	1	1.2	1.2	3	0.4(0.2)	0.2-0.6
>1-2	2	0.7(0.18)	0.6-0.9	3	0.6(0.4)	0.4-1.0
>2-3	5	1.0(0.56)	0.2-1.8	1	0.4	0.4
>3-4	2	0.1(0.08)	0.1-0.2	4	0.8(0.5)	0.4-1.4
>4-5	6	0.8(0.45)	0.1-1.3	6	0.6(0.6)	0.1-1.7
>5-6	-	-	-	1	0.2	-
Overall	16	0.8(0.50)	0.1-1.7	18	0.6(0.4)	0.05-1.7

Generally, aflatoxin M1 intake between exclusively (n = 16) and non-exclusively breastfeeding children (n = 18) were not significantly different in the study (Mann-Whitney U, p = 0.198). No significant difference (Kruskal-Wallis H-test, $\chi^2(5) = 3.467$, p = 0.628) was observed in aflatoxin M1 intake between age groups of all breastfeeding children (n = 34). Similar results were noted between age groups of exclusively breastfeeding children (Kruskal-Wallis H-test, $\chi^2(4) = 5.8$, p = 0.198, n =16). This was also the case between age groups of non-exclusively breastfeeding children (Kruskal-Wallis H-test, $\chi^2(5) = 4.289$, p = 0.509, n =18).

4.5.2 Margin of exposure of breastfeeding children to aflatoxin M1 in breast milk

Aflatoxin M1 intakes (µg/kg/b.w.t/day) derived for positive breast milk samples showed that daily intakes of breastfeeding children (n = 34) were above 0.017 µg/kg/b.w.t/day benchmark level used in the study. Consequently, the margin of exposure (MOE) of all children regardless of breastfeeding status in the study, based on mean and 95th percentile aflatoxin M1 intake through breastmilk, was lower than 10, 000 (European Food Safety Authority (EFSA) 2005) cut-off point (Table 16).

Table 16: Margin of exposure of breastfeeding children to aflatoxin M1 in breast milk of lactating mothers Kibwezi West

	Aflatoxin M1 Intake (µg/kg b.w.t/day)	MOE [a]
Breastfeeding children (n=34)		
Mean	0.7	0.02
p95	1.6	0.01
EBF [b] children (n=19)		
Mean	0.8	0.05
p95	1.7	0.01
NEBF [c] children (n=22)		
Mean	0.6	0.03
p95 [d]	1.7	0.02

[a] MOE: Margin of exposure based on aflatoxin M1, [b] EBF: Exclusive breastfeeding; [c] NEBF: Non-exclusive breastfeeding; [d] p95: 95th percentile

4.6 Aflatoxin M1 levels in urine of children aged six months and below in the study

Overall mean of aflatoxin M1 in urine of breastfeeding children (n = 48) was 0. 39 ng/ml (*SD*, 0.16) with a range of between 0.15 to 0.82 ng/ml. That of exclusively breastfeeding children (n = 20) was 0.35 ng/ml (*SD*, 0.13; range 0.15 to 0.61), while that of non-exclusively breastfeeding children was 0.42 ng/ml (*SD*, 0.18; range 0.18 to 0.82). However, no significant difference was reported between aflatoxin M1 in urine of exclusively and non-exclusively breastfeeding children (t_{46} = -1.520, p = 0.135).

Within age groups of exclusively breastfeeding children (n = 20), the highest mean (0.43 ng/ml, *SD,* 0.16), and median (0.46 ng/ml) was reported for children aged between >4 to 5 months, while the lowest mean (0.16 ng/ml, *SD*, 0.01), and median (0.16 ng/ml) was reported for children aged between >1 to 2 months (Table 17). On the other hand, within age groups of non-exclusively breastfeeding children (n = 28), the highest mean (0.54 ng/ml, *SD*, 0.19) and median (0.56 ng/ml) was reported for children aged between >1 to 2 months, while the lowest mean (0.22 ng/ml, *SD*, 0.49) and median (0.22 ng/ml) was reported for children aged between >2 to 3 months. Aflatoxin M1 levels in urine were not statistically different between age groups of all breastfeeding children (regardless of breastfeeding status) (one-way ANOVA, $F_{5,42}$ =2.10, p = 0.84). The same was also observed between age groups of exclusively breastfeeding children (one-way ANOVA, $F_{4,15}$ =2.015, p =0.144). However, a significant difference was reported between age groups of non-exclusively breastfeeding children (one-way ANOVA, $F_{5,22}$ =3.099, p =0.029).

Table 17: Aflatoxin M1 in the urine of exclusively and non-exclusively breastfeeding children in Kibwezi West

Age (Months)	Aflatoxin M1 EBF children (ng/ml)				Aflatoxin NEBF breastfed children (ng/ml)			
	n	Mean (SD)	Median	Range	n	Mean (SD)	Median	Range
0-1	1	0.35	0.35	0.35	3	0.34 (0.23)	0.22	0.2-0.6
>1-2	2	0.16 (0.01)	0.16	0.15-0.17	8	0.54 (0.19)	0.56	0.32-0.82
>2-3	7	0.34(0.08)	0.37	0.19-0.41	2	0.22 (0.49)	0.22	0.18-0.25
>3-4	4	0.36 (0.12)	0.39	0.19-0.47	5	0.29 (0.13)	0.24	0.18-0.5
>4-5	6	0.43 (0.16)	0.46	0.15-0.61	9	0.48 (0.94)	0.47	0.34-0.59
>5-6	-	-	-	-	1	0.3	0.3	0.3
Overall	20	0.35 (0.13)	0.38	0.15-0.61	28	0.42 (0.18)	0.40	0.18-0.82

4.7 Correlation of variables with aflatoxin occurrence in the study

4.7.1 Correlation of variables with total aflatoxin and aflatoxin B1 levels

No significant correlation was reported between concentration levels of total aflatoxin and age of the lactating mothers (r = 0.26, p = 0.106), education level (t_b = -0.19, p = 0.105), and aggregate socioeconomic status (t_b = -0.09, p = 0.454) (Table 18a). Neither was there a significant correlation between breastfeeding status and presence or absence of aflatoxin in food samples (Fisher's exact, p = 0.214). On the other hand, no correlation was reported between concentration level of total aflatoxin with mothers who clean maize grains before storage (t_b = 0.07, p = 0.604), apply ash before storage (t_b = -0.25, p = 0.061), treat maize with chemicals before storage (t_b = -0.204, p = 0.119), dry maize in the sun (t_b = 0.118, p = 0.367) and store maize without any treatment (t_b = 0.11, p =0.388). Similarly, no significant relationship was reported between concentration levels of total aflatoxin in the analyzed foods and places where lactating mothers sourced their cereals (market, t_b = -0.05, p = 0.750; own production, t_b = -0.11, p = 0.448, and other sources, donations, gifts, relief, t_b = 0.11, p = 0.458). Furthermore, no relationship was reported between levels of total aflatoxin with storage of maize grains in granary (t_b = -0.12, p = 0.345), in home buckets (tb = -0.17, p = 0.190) and in sacks (tb =0.21, p =0.115)**.** Finally, on the basis of breastfeeding status, no significant correlation was reported between variables in the study and total aflatoxin levels among exclusively breastfeeding mothers. However, among non-exclusively breastfeeding mothers, no significant correlation was reported except between household size and total aflatoxin levels in the study (t_b = 0.35, p = 0.04) (Table 18a).

Table 18a: Correlation of variables with total aflatoxin levels in Kibwezi West

	Concentration level of total aflatoxin (μg/kg)					
	All mothers (n=41)		EBF(n=19)		NEBF (n=22)	
Variables	***r***	***p-value***	***r***	***p-value***	**r**	***p-value***
Sociodemographic						
Age	0.26^{r}	0.116	0.28^{tb}	0.105	0.15^{tb}	0.335
Education level	-0.19^{rho}	0.105	-0.22^{rho}	0.367	-0.17^{rho}	0.294
socioeconomic status	-0.09^{r}	0.454	-0.17^{tb}	0.340	-0.07^{tb}	0.668
Household size	0.09^{r}	0.457	-0.12^{tb}	0.494	0.35^{tb}	0.040*
Breastfeeding status	-	0.214[a]	-	-	-	-
Storage practices						
Cleaning/sorting maize	0.07^{tb}	0.604	-0.03^{tb}	0.894	0.11^{tb}	0.534
Applying ash	-0.25^{tb}	0.061	-	-	-0.25^{tb}	0.171
Chemical treatment	-0.20^{tb}	0.119	0.13^{tb}	0.507	-0.29^{tb}	0.108
Drying in the sun	0.12^{tb}	0.367	-0.04^{tb}	0.855	0.21^{tb}	0.253
No treatment	0.11^{tb}	0.388	-0.06^{tb}	0.781	0.09^{tb}	0.633
Sources of maize						
Market	0.06^{tb}	0.636	-0.03^{tb}	0.870	0.08^{tb}	0.664
own production	0.11^{tb}	0.448	0.03^{tb}	0.894	-0.22^{tb}	0.237
other sources	-0.04^{tb}	0.754	0.02^{tb}	0.934	0.01^{tb}	0.947
Place of storage						
Granary	-0.12^{tb}	0.345	-0.13^{tb}	0.502	-0.12^{tb}	0.507
Home buckets	-0.17^{tb}	0.190	-0.17^{tb}	0.380	-0.25^{tb}	0.170
Sacks	0.21^{tb}	0.115	0.25^{tb}	0.215	0.24^{tb}	0.195

[a] Fisher's exact, test between breastfeeding status and presence/absence of aflatoxin in food samples
^{r} Pearson correlation, ^{tb} Kendall's tau-b, ^{rho} Spearman rho; EBF-Exclusively breastfeeding mothers, NEBF- Non-exclusively breastfeeding mothers; *Significant *p*-value (<0.05)

Equally, correlation of the same variables with aflatoxin B1 levels in the study (Table 18b) showed no statistical significance with socioeconomic status (t_b = 0.14, p =0.267), education level (t_b = -0.06, p =0.657), and age of lactating mothers *(*t_b = 0.13, p = 0.450). No significant correlation was reported between aflatoxin B1 and cleaning of maize before storage (t_b = 0.02, p = 0.915), treating of maize with ash before storage (t_b = 0.05, p = 0.714), spraying of maize with chemical before storage (t_b = 0.07, p = 0.655), drying of maize in the sun before storage (t_b = -0.09, p =0.534), and storing maize without any treatment (t_b = -0.057, p = 0.691). Similarly, no significant correlation was reported between sources of cereals with concentration levels of aflatoxin B1 (own production, t_b = -0.04, p = 0.764; market, t_b = 0.06, p =0.64; and other sources t_b = -0.04, p = 0.754). On the basis of breastfeeding status, no

significant correlation was reported between the variables with aflatoxin B1 levels for both exclusively and non-exclusively breastfeeding mothers (Table 18b).

Table 18b: Correlation of variables with aflatoxin B1 levels in Kibwezi West

	Concentration level of aflatoxin B1 (μg/kg)					
	All mothers (n=34)		EBF (n=19)		NEBF (n=22)	
Variables	***r***	***p-value***	***r***	***p-value***	**r**	***p-value***
Sociodemographic						
Age	0.13^{r}	0.450	0.23^{tb}	0.251	-0.05^{tb}	0.778
Education level	0.06^{rho}	0.657	0.03^{rho}	0.928	0.19^{rho}	0.293
socioeconomic status	0.14^{r}	0.267	0.23^{tb}	0.248	0.03^{tb}	0.859
Household size	0.88	0.506	0.05^{tb}	0.797	0.11^{tb}	0.572
Storage practices						
Cleaning/sorting maize	0.02^{tb}	0.915	-0.04^{tb}	0.865	0.08^{tb}	0.690
Applying ash	0.05^{tb}	0.714	-	-	0.05^{tb}	0.790
Chemical treatment	0.07^{tb}	0.655	0.19^{tb}	0.396	0.02^{tb}	0.926
Drying in the sun	0.09^{tb}	0.534	-0.31^{tb}	0.165	0.13^{tb}	0.523
No treatment	-0.06^{tb}	0.691	0.06^{tb}	0.806	-0.17^{tb}	0.386
Sources of maize						
Market	0.06^{tb}	0.636	-0.03^{tb}	0.906	-0.06^{tb}	0.767
own production	-0.04^{tb}	0.764	-0.04^{tb}	0.865	-0.25^{tb}	0.201
other sources	-0.04^{tb}	0.754	0.05^{tb}	0.817	0.17^{tb}	0.386
Place of storage						
Granary	-0.19^{tb}	0.190	-0.31^{tb}	0.174	-0.12^{tb}	0.548
Home buckets	0.06^{tb}	0.699	0.11^{tb}	0.637	0.03^{tb}	0.890
Sacks	0.08^{tb}	0.583	0.17^{tb}	0.462	-0.03^{tb}	0.901

^{r} Pearson correlation, ^{tb} Kendall's tau-b, ^{rho} Spearman rho; EBF:Exclusively breastfeeding mothers, NEBF:Non-exclusively breastfeeding mothers

4.7.1.1 Correlation of variables with dietary intake of total aflatoxin and aflatoxin B1 of lactating mothers in Kibwezi West

No significant correlation was reported between relevant socioeconomic variables with the intake of total aflatoxin among lactating mothers in the study (p_{all} >0.05) (Table 19a). But based on breastfeeding status, a significant negative correlation was reported between the education level of exclusively breastfeeding mothers and intake of total aflatoxin in the study (*rho* = -0.47, $p = 0.040$). For food consumption, a negative significant correlation was reported between dietary diversity of non-exclusively breastfeeding mothers ($t_b = -0.36$, $p = 0.03$) as opposed to exclusively ($t_b = 0.23$, $p = 0.225$) and all breastfeeding mothers ($t_b = -0.02$, $p = 0.843$). No significant correlation was reported between consumption score of foods that are highly susceptible to aflatoxin contamination and intake of total aflatoxin among lactating mothers in the study ($t_b = 0.10$, $p = 0.407$ for all mothers; $t_b = 0.11$, $p = 0.533$; and $t_b = 0.17$, $p = 0.309$).

Despite cleaning or sorting maize before storage, a positive significant correlation was reported with intake of total aflatoxin among non-exclusively breastfeeding mothers (t_b = 0.39, p = 0.037). This was not the case for exclusively breastfeeding mothers (t_b = 0.14, p = 0.39). No significant correlation was reported between where lactating mothers do source and store their maize and other cereals with the intake of total aflatoxin reported in the study (Table 19a).

Table 19a: Correlation of variables with dietary intake of total aflatoxin of lactating mothers in Kibwezi West

	Intake of total aflatoxin (µg/kg/b.w.t/day)					
	All mothers (n=41)		**EBF mothers (n=19)**		**NEBF mothers (n=22)**	
Variables	***t_b***	***p-value***	***t_b***	***p-value***	***t_b***	***p-value***
Sociodemographic						
Age	0.15	0.174	0.27	0.117	0.13	0.435
Education level	-0.27^{rho}	0.920	-0.47^{rho}	0.040*	-0.43^{rho}	0.848
Household size	-0.17	0.154	-0.12	0.512	-0.16	0.347
Socioeconomic status	0.11	0.352	0.08	0.642	0.06	0.706
Dietary pattern						
WDD score	-0.02	0.843	0.23	0.225	-0.36	0.030*
Consumption score [a]	0.10	0.407	0.11	0.533	0.17	0.309
Storage practices						
Cleaning	0.06	0.643	-0.30	0.140	0.39	0.037*
Applying ash	-0.02	0.854	-	-	0.02	0.908
Chemical treatment	0.07	0.587	0.34	0.094	-0.07	0.720
Drying	-0.05	0.704	-0.26	0.197	0.13	0.486
No treatment	-0.04	0.791	0.10	0.607	-0.30	0.111
Maize source						
Market	0.05	0.700	0.03	0.902	0.08	0.659
Shamba	-0.07	0.594	-0.14	0.503	-0.05	0.810
Other source	-0.01	0.916	0.06	0.771	-0.06	0.738
Place of storage						
Granary	-0.03	0.836	-0.09	0.652	0.07	0.708
Sacks	-0.07	0.598	-0.08	0.677	-0.08	0.677
Buckets	-0.12	0.379	-0.04	0.825	-0.23	0.217

[a] consumption score for foods susceptible to aflatoxin contamination (n=38 for all mothers, n=17 for EBF (exclusively breastfeeding) mothers, and n= 21 for non-exclusive breastfeeding (NEBF) mothers
^{rho} Spearman rho correlation, t_b = Kendall's tau-b correlation

On the other hand, socioeconomic status was the only sociodemographic variable significantly associated with intake of aflatoxin B1 among all lactating mothers in the study (t_b = 0.24, p = 0.042). However, this was not the case for exclusively (t_b = 0.3, p = 0.086) and non-exclusively breastfeeding mothers (t_b = 0.18, p = 0.282) (Table 19b). Education level was statistically correlated to aflatoxin B1 among exclusively breastfeeding mothers (rho = -0.56, p = 0.012),

while the household size was statistically correlated to aflatoxin B1 among non-exclusively breastfeeding mothers in the study (t_b = -0.39, p = 0.027). No significant correlation was reported between other variables with the intake of aflatoxin B1 among lactating mothers as summarized in Table 19b.

Table 19b: Correlation of variables with dietary intake of aflatoxin B1 of lactating mothers in Kibwezi West

	Intake of aflatoxin B1 (µg/kg/b.w.t/day)					
	All mothers (n=41)		EBF mothers (n=19)		NEBF mothers (n=22)	
Variables	t_b	*p-value*	t_b	*p-value*	t_b	*p-value*
Social demographic						
Age	0.13	0.249	0.26	0.135	0.08	0.623
Education level	-0.10^{rho}	0.529	-0.56^{rho}	0.012*	-0.43^{rho}	0.848
Household size	-0.21	0.084	-0.07	0.689	-0.39	0.027*
Socioeconomic status	0.24	0.042*	0.30	0.086	0.18	0.282
Dietary pattern						
WDD score	-0.08	0.491	0.14	0.462	-0.34	0.049*
Consumption score [a]	0.01	0.919	0.02	0.901	0.09	0.600
Storage practices						
Cleaning	0.06	0.672	-0.24	0.228	0.34	0.072
Applying ash	0.12	0.391	-	-	0.13	0.486
Chemical treatment	0.02	0.861	0.16	0.421	-0.07	0.720
Drying	0.04	0.780	-0.26	0.197	0.26	0.164
No treatment	-0.08	0.560	0.19	0.350	-0.35	0.061
Maize source						
Market	0.08	0.576	0.02	0.934	0.13	0.476
Shamba	-0.04	0.780	0.00		-0.11	0.575
Other source	-0.06	0.682	-0.02	0.934	-0.09	0.639
Place of storage						
Granary	0.00	0.987	-0.02	0.910	0.00	1.000
Sacks	-0.09	0.499	0.14	0.479	-0.22	0.238
Buckets	-0.09	0.503	-0.15	0.452	-0.07	0.720

[a] consumption score for foods susceptible to aflatoxin contamination (n=38 for all mothers, n=17 for EBF (exclusively breastfeeding) mothers, and n= 21 for non-exclusive breastfeeding (NEBF) mothers
^{rho} Spearman rho correlation, t_b = Kendall's tau-b correlation

4.7.2 Correlation of variables with aflatoxin M1 levels in the breast milk of lactating mothers in Kibwezi West

A significant correlation was reported between total aflatoxin levels and aflatoxin M1 in breastmilk of lactating mothers in the study (r = 0.71, p = 0.00 for all mothers, n = 33; t_b = 0.52, p = 0.005 for exclusively breastfeeding mothers, n = 16; and t_b = 0.75, p = 0.00 for non-exclusively breastfeeding mothers, n = 17). Estimated intake of aflatoxin B1 statistically correlated with aflatoxin M1 in breastmilk of exclusively lactating mothers in the study (t_b = -

0.56, p = 0.003) as opposed to that of non-exclusively breastfeeding mothers (t_b = -0.07, p = 0.742). Estimates of total aflatoxin intake did not statistically correlate with the levels of aflatoxin M1 in breastmilk of lactating mothers in the study (t_b = 0.10, p = 0.443 for all mothers, n = 33; t_b = 0.75, p = 0.00 for exclusively breastfeeding mothers, n = 16: and t_b = 0.27, p = 0. 139 for non-exclusively breastfeeding mothers, n = 17). In summary (Table 20), no socioeconomic and dietary consumption pattern variables were statistically correlated with aflatoxin M1 levels in breastmilk of lactating mothers in the study. Similarly, no statistically significant correlation was reported between variables that focused on where lactating mothers frequently sourced, where they stored, and how they handled maize before storage with levels of aflatoxin M1 found in breastmilk in the study.

Table 20: Correlation of variables with aflatoxin M1 levels in the breastmilk of lactating mothers in Kibwezi West

	Aflatoxin M1 levels in breastmilk					
	All mothers (n=34)		EBF mothers (n=16)		NEBF mothers (n=18)	
Variables	**Correlation**	**p-value**	**Correlation**	**p-value**	**Correlation**	**p-value**
Sociodemographic						
Education level	0.02^{rho}	0.893	0.32^{tb}	0.225	-0.16^{tb}	0.518
Age (mothers)	0.04^{tb}	0.721	0.01^{tb}	0.964	0.04^{tb}	0.818
Household size	0.03^{tb}	0.818	-0.04^{tb}	0.853	0.25^{tb}	0.182
SES	-0.07^{tb}	0.808	-0.39^{tb}	0.044	0.08^{tb}	0.673
Dietary pattern						
Meals/day	-0.16^{tb}	0.275	-0.31^{tb}	0.159	-0.04^{tb}	0.828
Food score	-0.18^{r}	0.307	0.03^{tb}	0.882	0.24^{tb}	0.160
WDD score	-0.03^{tb}	0.80	0.04^{tb}	0.851	-0.19^{tb}	0.323
Source of maize						
Market	-0.17^{tb}	0.241	-0.09^{tb}	0.674	-0.33^{tb}	0.110
Own cultivation	0.10^{tb}	0.508	0.07^{tb}	0.745	-	-
Other sources	0.14^{tb}	0.344	0.06^{tb}	0.791	0.30^{tb}	0.100
Storage practices						
Cleaning	0.05^{tb}	0.718	0.07^{tb}	0.751	-0.02^{tb}	0.915
Applying ash	-0.14^{tb}	0.333	-	-	-0.18^{tb}	0.386
Chemical	0.03^{tb}	0.827	0.21^{tb}	0.341	-0.04^{tb}	0.859
Drying	-0.03^{tb}	0.608	0.07^{tb}	0.745	-0.11^{tb}	0.574
Nothing	-0.03^{tb}	0.832	-0.23^{tb}	0.282	0.11^{tb}	0.574
Place of maize storage						
Granary	-0.03^{tb}	0.823	-0.25^{tb}	0.253	0.09^{tb}	0.671
Sacks	-0.02^{tb}	0.815	-0.10^{tb}	0.634	0.18^{tb}	0.390
Buckets	-0.04^{tb}	0.889	0.21^{tb}	0.332	-0.32^{tb}	0.111
Aflatoxin						
AFB1 level [a]	-0.21^{r}	0.13	-0.27^{tb}	0.217	-0.07^{tb}	0.742
AFT [b]	0.71^{r}	0.000*	0.52^{tb}	0.005*	0.75^{tb}	0.000*
AFB1 intake [b]	-0.3^{tb}	0.143	-0.56^{tb}	0.003*	-0.09^{tb}	0.642
AFT intake [b]	0.10^{tb}	0.443	-0.10^{tb}	0.587	0.27^{tb}	0.139

[a] (n= 26 for all mothers; n=12 for EBF; n=14 for NEBF), [b] (n = 33 for all mothers; n=16 for EBF mothers; n=17 for NEBF mothers), EBF: exclusively breastfeeding, NEBF: Non-exclusively breastfeeding, AFB1: Aflatoxin B1, AFT: Total aflatoxin, ^{rho} Spearman's rho correlation, ^{tb} Kendall's tau-b correlation, ^{r} Pearson correlation

4.7.2.1 Correlation of variables with aflatoxin M1 intake through breast milk of breastfeeding children in Kibwezi West

Age of lactating mothers did not statistically correlate with the intake of aflatoxin M1 through breastmilk among breastfeeding children in the study ($t_b = 0.01, p = 0.929$). Likewise, education level (rho = 0.05, $p = 0.687$), household size ($t_b = 0.03$, $p = 0.794$), socioeconomic status ($t_b = -0.13$, $p = 0.315$), and age of breastfeed children ($t_b = 0.004$, $p = 0.976$) did not correlate with

aflatoxin M1 intake in breast milk among breastfeeding children. Among dietary consumption variables, the consumption score of foods highly susceptible to aflatoxin contamination significantly and positively correlated with aflatoxin M1 intake of non-exclusively breastfeeding children (t_b = 0.38, p = 0.031). On the other hand, women's dietary diversity scores significantly and negatively correlated with intake of aflatoxin M1 among non-exclusively breastfeeding children (tb = -0.48, p = 0.010). Consumption scores of foods that are highly susceptible to aflatoxin contamination also positively correlated with aflatoxin M1 intake among non-exclusively breastfeeding children (tb = 0.38, p = 0.031). No significant correlation was reported between aflatoxin M1 intake among breastfeeding children with total numbers of meals consumed per day by lactating mothers (t_b = -0.007, p = 0.959) and time for initiating breastmilk with (tb = -0.10, p = 0.588). Total aflatoxin concentration levels in analyzed foods were significantly correlated with intake of aflatoxin M1 in breastmilk among breastfeeding children (tb = 0.31, p = 0.011).

Based on breastfeeding status, a statistically significant correlation was reported between total aflatoxin concentration levels and aflatoxin M1 intake of non-exclusively breastfeeding children in the study (tb = 0.38, p = 0.032) as opposed to non-exclusively breastfeeding children (tb = 0.02, p = 0.928). On the other hand, no significant intake was reported between aflatoxin B1 concentration levels with aflatoxin M1 intake among breastfeeding children (t_b = -0.12, p = 0.378). However, the estimate of aflatoxin B1 intake of exclusively breastfeeding mothers significantly correlated with aflatoxin M1 intake of their breastfeeding children (t_b = -0.39, p = 0.037). This was not the case for non-exclusively breastfeeding mothers (t_b = 0.16, p = 0.375). Conversely, the estimate of total aflatoxin intake of non-exclusively breastfeeding mothers significantly correlated with aflatoxin M1 intake of their breastfeeding children (t_b = 0.49, p = 0.008) as opposed to exclusively breastfeeding mothers (t_b = -0.14, p = 0.49). However, no statistically significant correlation was reported between intake of aflatoxin M1 in breastmilk with other variables in the study as summarized in Table 21.

Table 21: Correlation of variables with aflatoxin M1 intake through breast milk among breastfeeding children in Kibwezi West

Variables	AFM1 intake (μg/kg/b.w.t/day)					
	All (n=34)		EBF children (n=16)		NEBF children (n=18)	
	Correlation (t_b)	P-value	Correlation (t_b)	P-value	Correlation (t_b)	P-value
Sociodemographic						
Age (mother)	0.01	0.929	-0.13	0.495	-0.03	0.878
Education level	0.05^{rho}	0.687	-0.15^{rho}	0.955	0.24^{rho}	0.339
Household size	0.03	0.794	0.18	0.354	0.08	0.656
SES	-0.13	0.315	-0.49	0.110	0.12	0.514
Age (children)	0.004	0.976	-0.13	0.496	0.14	0.423
Dietary variables						
Meals/day	-0.007	0.959	-0.21	0.329	0.22	0.276
Time of initiating breastmilk	-0.10	0.588	0.15	0.445	-0.21	0.253
Food score	0.09	0.466	-0.14	0.458	0.38	0.031^{*}
WDD score	-0.06	0.636	0.09	0.638	-0.47	0.010^{*}
Source of maize						
Market	-0.14	0.334	-0.25	0.248	-0.09	0.657
Shamba	0.28	0.530	0.22	0.315	0.17	0.396
Other sources	0.07	0.642	0.10	0.634	0.09	0.657
Storage practices						
Cleaning	0.13	0.366	0.10	0.634	0.24	0.243
Applying ash	0.02	0.878	-	-	0.14	0.500
Apply chemical	0.004	0.981	0.21	0.341	-0.21	0.314
Drying	0.02	0.879	0.31	0.159	-0.11	0.574
No treatment	-0.08	0.570	-0.38	0.079	0.00	-
Place for storing maize						
Granary	0.003	0.983	-0.10	0.638	0.17	0.396
Sacks	-0.09	0.552	-0.29	0.186	0.10	0.618
Buckets	0.06	0.685	0.26	0.225	-0.22	0.288
Aflatoxin						
AFB1 level [a]	-0.12	0.378	0.03	0.891	0.00	-
AFT level [b]	0.31	0.011^{*}	0.02	0.928	0.38	0.032^{*}
AFB1 intake [b]	-0.06	0.616	-0.39	0.037^{*}	0.16	0.375
AFT intake [b]	0.17	0.183	-0.14	0.469	0.49	0.008^{*}

[a] (n=26 for all children, n=12 for EBF children and n=14 for NEBF children); [b] (n=33 for all children, n=16 for EBF children, and n=17 for NEBF children); EBF: Exclusively breastfeeding, NEBF: Non-exclusively breastfeeding; SES: Socioeconomic status; ^{rho} Spearman's rho correlation; ^{tb} Kendall's tau-b correlation; * significant $p<0.05$

4.7.3 Correlation of variables with aflatoxin M1 in urine of breastfeeding children in Kibwezi West

No statistically significant correlation was reported between aflatoxin M1 in urine of breastfeeding children (regardless of breastfeeding status) with age of lactating mothers (r = 0.18, p = 0.211), education level (rho = 0.00, p = 0.984), household size (t_b = -0.01, p = 0.949), and socioeconomic status (r = -0.20, p = 0.173). No significant correlation was observed for these variables even within exclusively and non-exclusively breastfeeding mothers. However, the age of exclusively breastfeeding children was positively and significantly correlated with the aflatoxin M1 in the urine of breastfeeding children (t_b = 0.41, p = 0.017) as opposed to that of non-exclusively breastfeeding children (t_b = 0.08, p = 0.953). Similarly, the socioeconomic status of lactating mothers was negatively correlated with aflatoxin M in the urine of their respective exclusively breastfeeding children (t_b = -0.35, p = 0.041). However, no significant correlation was observed between dietary consumption variables of lactating mothers with aflatoxin M1 in the urine of breastfeeding children. Correlation with women dietary diversity score was t_b = -0.06, p = 0.595, with consumption score of foods highly susceptible to aflatoxin contamination was r = -0.05, p = 0.761, and association with total number of meals per day was t_b = 0.02, p = 0.854. Also, the age of introducing complementary foods was not statistically associated with aflatoxin M1 in the urine of non-exclusively breastfeeding children in the study (t_b = 0.08, p = 0.588). A significant correlation was observed between the concentration of total aflatoxin in the study with aflatoxin M1 in the urine of breastfeeding children (r = 0.39, p = 0. 013).

Based on breastfeeding status, a significant correlation was noted between the concentration of total aflatoxin and aflatoxin M1 in the urine of exclusively breastfeeding children in the study (r = 0.817, p = 0.00). Conversely, this was not the case for the non-exclusively breastfeeding children (r = 0.35, p = 0.115). No significant correlation was reported between aflatoxin B1 levels in the study with aflatoxin M1 in the urine of breastfeeding children (r = 0.27, p = 0.128). No statistical association was reported between aflatoxin intake in the study with aflatoxin M1 in urine of breastfeeding children (total aflatoxin intake, t_b = 0.13, p = 0.241; aflatoxin B1 intake, t_b = -0.05, p = 0.673; and aflatoxin M1 intake, t_b = 0.12, p = 0.320). No significant correlations were reported between aflatoxin M1 in urine with the maize source, maize storage practices, and place of storage variables as summarized in Table 22.

Table 22: Correlation of variables with aflatoxin M1 in urine of breastfeeding children in Kibwezi West

	Aflatoxin M1 in urine								
	All children			EBF children			NEBF children		
Variables	*n*	t_b	*P-value*	*n*	t_b	*P-value*	*n*	t_b	*P-value*
Sociodemographic									
Age (mothers)	48	0.18[r]	0.211	20	0.38	0.026	28	-0.02	0.905
Education level	48	0.00[rho]	0.984	20	-0.25[rho]	0.170	28	0.15[rho]	0.288
Household size	48	-0.01	0.949	20	-0.18	0.297	28	0.09	0.562
SES	48	-0.20[r]	0.173	20	-0.35	0.041[*]	28	-0.01	0.921
Age (children)	48	0.130	0.207	20	0.41	0.017[*]	28	0.08	0.953
Dietary Pattern									
WDD score	48	-0.06	0.595	20	0.07	0.711	28	-0.06	0.685
Food score	44	-0.05[r]	0.761	17	0.23	0.199	27	-0.14	0.297
Meals/day	48	0.02	0.854	20	-0.15	0.457	28	0.12	0.425
Age complementary	-	-	-	-	-	-	26	0.08	0.588
Aflatoxin									
AFT level	41	0.39[r]	0.013[*]	19	0.82[r]	0.00[*]	22	0.35[r]	0.115
AFB1 level	34	0.12[r]	0.491	15	-0.15[r]	0.60	19	0.34[r]	0.157
AFM1 level (Breastmilk)	34	0.27[r]	0.128	16	0.49[r]	0.055	18	0.19[r]	0.457
AFT Intake	41	0.13	0.241	19	0.2	0.232	22	0.10	0.514
AFB1 Intake	41	-0.05	0.673	19	0.15	0.523	22	-0.12	0.455
AFM1 intake	34	0.12	0.320	16	0.20	0.278	18	0.98	0.570
Maize source									
Market	48	0.02	0.876	20	0.10	0.620	28	-0.02	0.889
Shamba	48	-0.16	0.199	20	-0.17	0.382	28	-0.09	0.555
Other sources	48	-0.03	0.811	20	-0.05	0.816	28	-0.02	0.908
Maize storage practices									
Cleaning	48	0.04	0.715	20	-0.04	0.85	28	0.09	0.589
Applying ash	48	-0.01	0.963	20	-	-	28	-0.05	0.758
Chemical treatment	48	0.1	0.422	20	0.30	0.129	28	-0.04	0.810
Drying	48	0.01	0.966	20	-0.12	0.129	28	0.04	0.789
No treatment	48	-0.09	0.464	20	-0.12	0.54	28	0.03	0.859
Place of storage									
Granary	48	-0.10	0.404	20	-0.24	0.222	28	-0.09	0.576
Sacks	48	0.12	0.311	20	0.01	0.970	28	0.24	0.143
Buckets	48	0.03	0.821	20	0.15	0.457	28	-0.01	0.955

[tb] Kendall's tau-b, [r] Pearson r, and [rho] Spearman correlation, [*] Significant p<0.05

4.7.4 Correlation of variables, and aflatoxin occurrence with weight-for-age outcome of breastfeeding children in Kibwezi West

Age of children was positive and significantly correlated with weight-for-age z-scores ($r = 0.62$, $p = 0.00$). On the basis of breastfeeding status, age was positively and significantly correlated with weight-for-age among non-exclusively breastfeeding mothers ($t_b = 0.56$, $p = 0.00$) as

opposed to their counterpart (t_b = 0.28, p = 0.093). No significant correlation was observed between correlation of weight-for-age z-scores of breastfeeding children with age of lactating mothers (r = 0.13, p = 0.381), household size (t_b = 0.04, p = 0.693), education level (*rho* = -0.06, p = 0.67), and socioeconomic status (r = 0.03, p = 0.840) (Table 23). Similarly, total number of meals consumed by lactating mothers (t_b = -0.06, p = 0706), consumption score of foods highly susceptible to aflatoxin contamination (t_b = 0.07, p = 0.649), and women dietary diversity score (t_b = -0.12, p = 0.260) were not statistically correlated with weight-for-age of breastfeeding children in the study. No significant correlation was observed between breastfeeding practices with weight-for-age z-score of breastfeeding children in the study. Correlation with time for initiating breastmilk was t_b = 0.01, p = 0.940, age of introducing complementary foods was (t_b = 0.22, p = 0.137), while correlation with breastfeeding status of children was χ^2 (1) = 0.09, p = 0.76. Correlations between weight-for-age z-score with aflatoxin B1 (r = 0.15, p = 0.405) and total aflatoxin levels (t_b = 0.00, p = 0.993) in the study were not significant. Likewise, aflatoxin M1 in breastmilk of lactating mothers (r = 0.03, p = 0.887) and urine of breastfeeding children (r = 0.09, p = 0.350) were not statistically associated with weight-for-age z-scores in the study. Estimates of aflatoxin intake among lactating mothers did not correlate with weight-for-age of breastfeeding children. A weak nonsignificant correlation was observed for aflatoxin B1 (t_b = -0.07, p = 0.546), total aflatoxin intake (t_b = -0.07, p = 0.509) and aflatoxin M1 intake through breastmilk (t_b = 0.00, p = 0.796) (Table 23).

Table 23: Correlation of variables with weight-for-age z-score of breastfeeding children in Kibwezi West

	Weight-for-age z-scores								
	All children			EBF children			NEBF children		
Variables	n	t_b	P value	n	t_b	P value	n	t_b	P value
Sociodemographic									
Age (children)	48	0.62^{r}	0.00^{*}	20	0.28	0.093	28	0.56	0.000^{*}
Age (mother)	48	0.13^{r}	0.381	20	0.09	0.578	28	0.07	0.620
Household size	48	0.04	0.693	20	0.01	0.973	28	0.07	0.620
Education level	48	-0.06^{rho}	0.67	20	0.22^{rho}	0.353	28	-0.25^{rho}	0.201
SES [a]	48	0.03^{r}	0.840	20	0.08	0.622	28	-0.03	0.811
Dietary consumption									
Meals/day	48	-0.04	0.706	20	0.02	0.934	28	-0.08	0.622
Food score	44	0.07^{r}	0.649	17	0.03	0.869	27	0.13	0.327
WDDS score [b]	48	-0.12	0.260	20	0.13	0.440	28	-0.12	0.405
Breastfeeding practices									
Initiating BM [c]	48	0.01	0.940	20	-0.29	0.098	28	0.17	0.244
Age (complementary foods)	-	-	-	-	-	-	83	0.22	0.137
BF Status [d]	48	$0.09^{\chi 2}$	0.760	-	-	-	-	-	-
Aflatoxin levels									
AFB1 conc [e]	34	0.15^{r}	0.405	15	0.28	0.305	19	0.03	0.894
AFT conc [f]	41	0.00^{r}	0.993	19	-0.04^{r}	0.860	22	0.09^{r}	0.695
AFM1 conc BM [g]	34	0.03^{r}	0.887	16	0.21^{r}	0.445	18	-0.02^{r}	0.943
AFM1 Urine conc [h]	48	0.09	0.350	20	0.10	0.536	28	0.06	0.649
AFB1 Intake	41	-0.07	0.546	19	0.01	0.944	22	-0.09	0.566
AFT Intake	41	-0.07	0.509	19	-0.04	0.832	22	-0.07	0.646
AFM1 intake	34	0.00	0.796	16	0.05	0.787	18	0.05	0.791

[a] SES: socioeconomic status; [b] WDDS: women dietary diversity score: [c] Initiating BM: Time for initiating breastmilk, [d] BF status: Breastfeeding status; [e] AFB1 conc: aflatoxin B1 concentration; [f] Total aflatoxin concentration; [g] AFM1 conc BM: aflatoxin M1 concentration in breastmilk; [h] AFM1 urine conc: aflatoxin M1 concentration in urine; ^{tb} Kendall's tau-b correlation; ^{r} Pearson correlation; ^{rho} Spearman correlation; $^{\chi 2}$ Chi-square correlation * Significant $p < 0.05$

4.7.5 Predictors of aflatoxin, and weight-for-age z-scores of breastfeeding children in Kibwezi West

Household size in a simple linear regression model (Adjusted R^2=0.134, $F_{1,20} = 4.250$, and $p = 0.052$, enter method) was not a significant predictor of total aflatoxin in foods consumed by non-exclusively lactating mothers in the study (β= 2.06, $p = 0.052$). Also, women dietary diversity score (β= -0.27, $p = 0.22$), and sorting out of maize before storage (β= 0.36, $p = 0.11$) were not significant predictor of concentration of total aflatoxin in the study despite generating a significant predictor model (Adjusted $R^2 = 0.214$, $F_{2,19} = 3.853$, $p = 0.039$) (Table 24).

Similarly, socioeconomic status was found not to be a significant contributor to aflatoxin B1intake among lactating mothers in the study (β = 0.296, p = 0.06). However, level of education significantly and negatively influenced estimates of aflatoxin B1 intake among exclusively lactating mothers in the study (β = -0.56, p = 0.01). On the other hand, women dietary diversity scores negatively influenced estimates of aflatoxin B1 intake among non-exclusively lactating mothers (β = -0.43, p = 0.04). Moreover, aflatoxin concentration in analyzed foods significantly contributed to levels of aflatoxin M1 in breastmilk of lactating mothers (β = 0.71, p = 0.00), while based on breastfeeding status, estimates of aflatoxin B1 intake in the study significantly influenced the levels of aflatoxin M1 in breastmilk of exclusively lactating mothers (β = -0.63, p = 0.01). The study also found that levels of total aflatoxin was a significant predictor of aflatoxin M1 estimate intake through breastmilk among breastfeeding children in the study (β = 0.49, p = 0.00). The study also showed that the regression model containing women's dietary diversity and consumption score of foods that are highly susceptible to aflatoxin contamination in regard to aflatoxin M1 intake was significant **(**Adjusted R^2= 0.023, $F_{(2,15)}$ = 4.912, p = 0.023). However, women's dietary diversity score was a significant predictor of aflatoxin M1 intake among non-exclusively breastfeeding children (β = -0.47, p = 0.04) as opposed to consumption score of foods that are highly susceptible to aflatoxin contamination in the model (β = 0.36, p = 0.10). Furthermore, the level of total aflatoxin was found to be the major contributor of aflatoxin M1 in the urine of breastfeeding children in the study (β = 0.39, p = 0.01). On the basis of breastfeeding, regression model (Adjusted R^2 = 0.698, $F_{3,15}$ = 14.872, p = 0.000) showed that total aflatoxin (β = 0.70, p = 0.00) was a significant predictor of aflatoxin M1 in urine of exclusively breastfeeding children, as opposed to age of children (β = 0.26, p = 0.08) and socioeconomic status of mothers (β = -0.16, p = 0.27). Finally, age was the only significant predictor of weight-for-age z-score outcome among breastfeeding children in the study (β = 0.39, p = 0.01 for all children, and β = 0.73, p = 0.00 for exclusively breastfeeding children). The rest are summarized in Table 24.

Table 24: Predictors of aflatoxin occurrence and weight-for-age z-scores of breastfeeding children in Kibwezi West

Predictors	Unstandardized Coefficients B	Unstandardized Coefficients Std. Error	Standardized Coefficients Beta	t	Sig.
AFT conc (NEBF mothers)					
Household size	27.68	13.43	0.42	2.06	0.052
AFT intake (NEBF mothers)					
Women Dietary Diversity score	-1.35	1.05	-0.27	-1.28	0.22
Cleaning maize before storage	6.55	3.85	0.36	1.70	0.11
AFB1 intake (All mothers)					
Socioeconomic Status	0.06	0.031	0.296	1.938	0.06
AFB1intake (EBF mothers)					
Mother Level of Education	-0.23	0.08	-0.56	-2.81	0.01*
AFB1 intake (NEBF mothers)					
Household size	-0.25	0.13	-0.38	-1.93	0.69
Women Dietary Diversity score	-0.22	0.10	-0.43	-2.21	0.04*
AFM1 conc BM (all mothers)					
AFT concentration	0.00	0.00	0.71	5.64	0.00*
AFM1 conc BM (EBF mothers)					
AFB1 Intake	-0.02	0.01	-0.63	-4.09	0.01*
AFT concentration	0.00	0.00	0.49	3.18	0.07
AFM1 intake (All children)					
AFT concentration	0.00	0.00	0.49	3.16	0.00*
AFM1 intake (EBF children)					
AFB1 Intake	-0.38	0.19	-0.47	-2.00	0.07
AFM1 intake (NEBF children)					
Women dietary diversity	-0.18	0.08	-0.47	-2.31	0.04*
Food score	0.13	0.07	0.36	1.78	0.10
AFM1 urine (all children)					
AFT concentration	0.00	0.00	0.39	2.61	0.01*
AFM1 urine (EBF children)					
Age (children)	0.04	0.02	0.26	1.89	0.08
Socioeconomic status	-0.01	0.06	-0.16	-1.14	0.27
AFT concentration	0.00	0.00	0.70	4.91	0.00*
AFM1 urine (NEBF children)					
AFT concentration	0.00	0.00	0.46	2.68	0.02*
AFT Intake	0.02	0.01	0.25	1.34	0.20
Women Dietary Diversity score	-0.12	0.06	-0.32	-1.97	0.72
Food score	0.01	0.01	0.18	1.25	0.24
WFA z-scores (all children)					
Age (children)	0.78	0.14	0.62	5.41	0.00*
WFA z-scores (EBF children)					
Age (NEBF children)	0.88	0.16	0.73	5.50	0.00*

AFT: total aflatoxin; AFB1: Aflatoxin B1; EBF: Exclusively breastfeeding; AFM1: Aflatoxin M1; NEBF: Non-exclusively breastfeeding; WFA: weight-for-age z-scores, * significant p-value<0.05

Further analysis using the simulation model (@Risk software version 8.2, iteration 100,00, mean and range of aflatoxin intake for each food, and Risk Beta General for distribution) showed that maize ugali was the greatest contributor to cumulative intake of aflatoxin in the study among lactating mothers with a regression coefficient of 0.69, and 0.70 for total aflatoxin and aflatoxin B1 intake, respectively. The least contributor to cumulative intake of aflatoxin in the study was '*muthokoi'* (*b* = 0.02) (Figure 3).

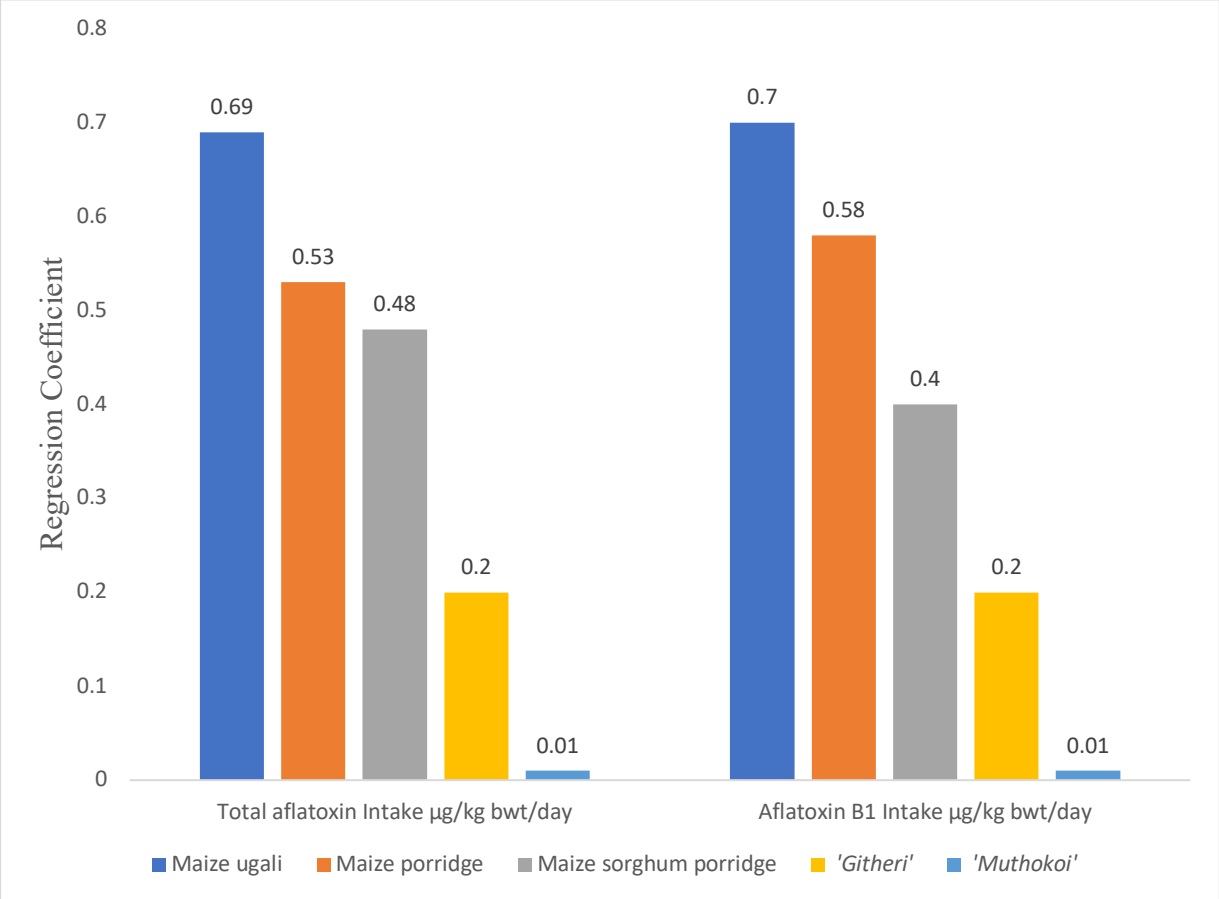

Figure 3: Regression coefficient of dietary aflatoxin intake of each food on cumulative intake of aflatoxin (µg/kg/b.w.t/day) among lactating mothers in the study

CHAPTER 5: DISCUSSION

5.1 Socio-demographic and economic status of lactating mothers in Kibwezi West

Lactating mothers' socio-demographic and economic status were compared with those reported in Kenya Population and Housing Census survey (Kenya Population and Housing Census (KPHC), 2019a, 2019b). The mean household size of lactating mothers was greater than the national mean. This was expected as rural areas in Kenya are generally characterized by larger household sizes (KPHC, 2019b). The majority of mothers interviewed were multiparous mothers of whom studies including Mohamed et al. (2018) have associated with exclusive and prolonged breastfeeding. The number of children per lactating mother (mean, 3.1) however, was identical to those reported in Makueni County (mean of 3.3) (KDHS, 2014). The percentage of lactating mothers (51.2%) who had attained basic education was lower than the national rate (67.3%) (KDHS, 2014). This was indicative of low educational status among lactating mothers in the study area. Age demography of lactating mothers, on the other hand, was similar to those reported by KDHS (2014). They both showed that lactating mothers aged between 20-29 and 30-39 years were more than those aged between 15-19 and 40-49 years in the country. Low to modest wealth index, income levels, and occupation status reported is a pointer to low economic status. In fact, consumption expenditure of less than a dollar per day by almost 60% indicates low disposable income in the study area. This might be one of the factors that deter mothers from accessing quality and diverse diets in the study area. However, most socioeconomic and demographic variables between exclusively and non-exclusively lactating mothers were similar. This was expected as the study area population is almost homogenous.

5.2 Food consumption pattern, Dietary diversity, Maize source, handling and storage practices, and Breastfeeding practices of lactating mothers in Kibwezi West

5.2.1 Food consumption and dietary diversity of lactating mothers in Kibwezi West

According to weekly consumption frequency, '*ugali'*, maize porridge, *'githeri'*, and groundnut would put lactating mothers at a higher risk of aflatoxin exposure. This did not come as a surprise as these foods constitute staple foods mostly consumed in Kenya. Other foods that could easily contribute to aflatoxin exposure include animal milk (mostly cow's milk) in form of milk tea and '*muthokoi'*. However, their frequency of consumption was modest per week in the study. Since fish, chicken, cassava, finger millet, eggs, plain sorghum flour, and mixed flour porridge were rarely consumed per week in the study, they were considered to contribute

less to dietary aflatoxin exposure among lactating mothers. Overall, the food frequency result of this study was similar to those of Kilonzo et al. (2014) in Makueni, and Magoha et al. (2014) in Tanzania. However, the aflatoxin consumption score of <25% out of 18 foods shows that dietary exposure in the study area likely comes from a limited range of foods. This sentiment is in parallel with the results of this study which showed low consumption frequency of foods that are least susceptible to aflatoxin contamination. In fact, out of sixteen foods listed in the food frequency questionnaire, kales, cowpea leaves, cabbage, beans, and pigeon peas were the most consumed every week. Comparatively, women's dietary diversity score in this study (3.4) was lower than the mean score of 5.5 reported in Tanzania (Magoha et al., 2014), and 4.2 reported in Nepal (Andrews-Trevino et al., 2020). From these results, it is clear that the dietary diversity of lactating mothers in this study is considerably low and is similar to those of (Nabwire et al., 2020b) in Makueni. These results are indicative of low food availability and accessibility in the study area. In fact, the low socioeconomic status reported in this study is a pointer that low purchasing power could be a barrier to meeting adequate dietary diversity and quality food in the area. However, it has also been noted that a hot harsh climate does not favor agricultural activities in this study area. Based on consumption quantities, the mean consumption of maize '*ugali'* (340.5 g/day) and maize porridge (412.3 g/day) were higher than 195.5 and 38.6 g/day, respectively, reported by Kilonzo et al. (2014) in Makueni. However, estimates of *'githeri'* (93.6 g/day), and '*muthokoi'* (36.2 g/day) in this study were comparable to the estimates of the same study by Kilonzo et al. (2014) (*'githeri'* [103.3 g/day], and *'muthokoi'* [28.6 g/day]). Consumption of maize '*ugali'* was again similar to 360 g/day reported by Kang'ethe et al. (2017) also in Makueni. Conversely, consumption of animal milk (150 g/day) and rice (250 g/day) among lactating mothers in northern India was higher than those reported in this study (19.5 and 30.6 g/day, respectively). Similarly, the consumption of eggs in this study (1.7 g/day) was lower than those reported among mothers in Iran (50 g/day) (Azarikia et al., 2018). From these results, it is evident that maize-based foods constitute a larger portion of mothers' dietary intake in the study region than other foods. Finally, the absence of significant differences in food frequency, dietary diversity, and consumption quantities between breastfeeding groups shows that all lactating mothers in the study area have similar food consumption patterns.

5.2.2 Source, storage, and processing of cereals by lactating mothers in Kibwezi West

This study agrees with Koskei et al. (2020) and Malusha et al (2016) that metal and plastic buckets, sacks, and granaries are the most commonly used maize storage containers in Makueni.

Okoth and Ohingo (2004) further showed that storage in plastics, polythene bags, metal buckets, and sacks leads to a moisture content of at least 13.6% in maize. In the environment of a hot and humid temperature, it is highly possible that storage practices could be a cause of the high prevalence of aflatoxin in Makueni. Just as the study by Nii et al. (2019), storage of maize products for a long time in these containers could exacerbate the occurrence of aflatoxin in the area. Findings of this study showed that most households in Makueni bought maize from the market, followed by their cultivation and other sources (gifts/donations/reliefs). These findings were similar to those of Daniel et al. (2011). However, varying results linking aflatoxin contamination and source have been reported in the study area. For instance, Daniel et al. (2011) showed that maize sourced from cultivation was most contaminated by aflatoxin. On the other hand, Mwihia et al. (2008) showed that maize produced at home and those bought from the market were all highly contaminated with aflatoxin. This study, however, did not categorize the food samples into sources, and thus, could not link the source of maize and aflatoxin occurrence in the study. Lastly, the proportion of lactating mothers applying methods that could reduce aflatoxin occurrence in maize was found to be lower than those reported by Koskei et al. (2020). It is thus clear that maize handling and storage practices among lactating mothers are still low to mitigate the occurrence of aflatoxin in the study area.

5.2.3 Breastfeeding practices of lactating mothers in Kibwezi West

Overall exclusive breastfeeding rate (44.1%) in this study was lower than the national rate (61%) (KDHS, 2014). This was not surprising as Makueni County falls under arid and semi-arid areas (ASAL) in Kenya. ASAL areas are presumed to face several constraints to exclusive breastfeeding compared to non-ASAL areas. In fact, the exclusive breastfeeding rate (44.5%) reported by Mohamed et al. (2018) in Wajir County, and 45% reported by Talbert et al. (2020) in Kilifi County, both classified as ASAL areas in Kenya, were the same as the rates reported in this study. However, the exclusive breastfeeding rate reported in this present study was higher than those reported in similar studies. A rate of 36.7% (combined rate) was reported in Tanzania (Magoha et al., 2014), 28% in northern India (Mehta et al., 2020), 35.4% in Nigeria (Ezekiel et al., 2020), and 40% in Lebanon (Elaridi et al., 2017). The disparities among these rates could be due to varying degrees of constraints that lactating mothers face in exclusively breastfeeding their children. For instance, in this study, education status, occupational status, and age of breastfeeding children were all associated with the breastfeeding status of lactating mothers. These observations were also noted by KDHS (2014) and Jamaa et al. (2018).

All breastfeeding children in the study were introduced to breastmilk not more than 24 hours after delivery. This was higher than 58% reported by Jamaa et al. (2018) in Wajir County. The rate was, however, higher but comparable to the national rate (92.1%) (KDHS, 2014). The average age for introducing other foods alongside breastmilk was 3.34 months. This was similar to those reported in Kilifi County (3 months) by Talbert et al. (2020) but slightly higher than the 2.7 months reported by Mbagaya (2009) in the western region of Kenya. Complementary foods consumed in this study area (maize porridge, animal milk, and mashed ugali) were similar to those mentioned in other studies. In Kilifi County, maize porridge was the most frequently used complementary food (Talbert et al., 2020). In Makueni County, maize, sorghum, millet, and animal milk were mentioned by Kang'ethe et al. (2017). Animal milk was widely used in Wajir County (Jamaa et al., 2018). It is however surprising that over 60% of lactating mothers expressed the desire to continue breastfeeding for up to 2-years, surpassing the rates reported by KDHS (2014). Generally, a high non-exclusively breastfeeding rate characterized by frequent consumption of complementary foods was seen as an additional risk of aflatoxin exposure among breastfeeding children. These sentiments are similar to those of Kang'ethe et al. (2017). Mehta et al. (2021) and Ezekiel et al (2020) studies also concluded that non-exclusively breastfeeding children are exposed to higher levels of aflatoxin intake than those who are exclusively breastfed.

The average consumption quantity of breast milk in this study (559.6 g/day) was within the ranges of 525.3 to 793.1g/day reported in Tanzania (Magoha et al. 2014). The level was, however, below the reference consumption level of 750 g/day adopted in most aflatoxin M1 breast milk studies. A consumption quantity of 606.1 g/day was reported for exclusively breastfeeding children. They were lower than 841.2 g/day reported in western Kenya (Miller et al., 2019), and 750 g/day reported in northern India (Mehta et al., 2020). Despite non-exclusively breastfeeding children consuming other foods, it was surprising to report an insignificant difference between breast milk intake of exclusively and non-exclusively breastfeeding children. This shows that all breastfeeding children aged six months and below, regardless of breastfeeding status, are equally predisposed to aflatoxin M1 through breast milk in the study area. However, the statistical difference reported in breast milk intake between children age groups was expected. This intimates that age could be an underlying factor in determining the extent of exposure of breastfeeding children to aflatoxin M1 in breast milk.

5.3 Total aflatoxin and aflatoxin B1 in diets of lactating mothers in Kibwezi West

Prevalence of 85.4% of aflatoxin in the study was generally as high as those reported by Nabwire et al. (2020a) (100%), and Kang'ethe et al. (2017) (80.4%) in Makueni. This high prevalence confirms the suspicion of this study about the existence of prolonged and frequent occurrence of aflatoxin contamination in the area. In fact, the prevalence of this present study with that of Kilonzo et al. (2014) (45%, 20%, and 35% for maize kernels, *'muthokoi'*, and maize meal samples, respectively) in Makueni, points out that cooked maize dishes are also a source of dietary aflatoxin exposure in the area. Studies like the one of Obonyo and Salano (2018), and Nabwire et al. (2020a) reported a high prevalence of aflatoxin in raw maize. However, due to the handling and processing of maize before cooking, the prevalence in this study was expected to be at least moderate. This result could suggest that maize handling and processing practices in the study area are not effective enough in reducing contamination of aflatoxin in raw maize before cooking or milling.

Similarly, aflatoxin concentrations were expected to be lower than those reported for raw maize; however, this was not the case. The mean concentration of over 90% of food samples was shown to exceed 2 and 10 µg/kg set limits for total aflatoxin and aflatoxin B1, respectively. In fact, the mean of total aflatoxin (97.8 µg/kg) in this study was higher than 62.5 µg/kg of Nabwire et al. (2020a), and 41.5 µg/kg of Kang'ethe et al. (2017) in Makueni. They were, however, within the ranges of 6 to 480 µg/kg reported by Kilonzo et al (2014) in the same study area. These results reaffirm earlier statements about the high occurrence of aflatoxin contamination in the study area and are indicative of the existence of prolonged aflatoxin exposure through consumption of maize-based dishes. They also explain why the likelihood of consuming foods contaminated with aflatoxin between exclusively and non-exclusively lactating mothers reported in the study was comparable. Consequently, the results also support the findings that imply that once aflatoxin is inside the food matrix, it can withstand cooking temperatures without getting destroyed.

Levels of aflatoxin B1 however, were not comparatively high as those of total aflatoxin. The mean concentration level of aflatoxin B1 in this study was 9.0 µg/kg. However, that of Mahuku et al. (2019) was 39.0 ug/kg, while that of Nabwire et al. (2020a) was 59.3 µg/kg in Makueni. The mismatch between levels of total aflatoxin and aflatoxin B1 in this study is in agreement with the findings of Matumba et al. (2015). In this experiment, it was concluded that aflatoxin

ratios within the food matrix will always vary between those reported for total aflatoxin and individual types of aflatoxin. However, a high proportion of food samples exceeding the KEBS limit is alarming and should be a cause of food safety concerns in the study area.

5.3.1 Intake of total aflatoxin and aflatoxin B1 of lactating mothers in Kibwezi West

Mean dietary intake of total aflatoxin (7600 ng/kg b.w.t/day) among lactating mothers in this study was considerably higher than those reported by Kilonzo et al. (2014) (27.23, 291.66, and 59.31 ng/kg b.w.t/day for '*muthokoi*', maize kernel, and maize meal, respectively). They were also higher than those reported by Kang'ethe et al. (2017) (260 ng/kg b.w.t/day) in Makueni. Equally, the results were higher than 271 ng/kg b.w.t/day in Ghana (Kortei et al., 2019) and 1.29 ng/kg b.w.t/day in Turkey (Kabak, 2021). Mean intake of aflatoxin B1 (600 ng/kg b.w.t/day) on the other side, was comparatively higher than 451.8 ng/kg b.w.t/ day in Makueni and 148.4 ng/kg b.w.t/ day reported in western region of Kenya (Mahuku et al., 2019).

Exposure ranges for this study (0 to 1900 ng/kg b.w.t/ day) were wider than those estimated for Kenya adults (35-133 ng/kg b.w.t/ day) (Liu and Wu 2010) and surpassed the upper bound exposure levels of 3.25 ng/kg b.w.t/day estimated for adults from several studies (EFSA CONTAM Panel et al., 2020). The levels were equally higher than the 1.19 ng/kg b.w.t/ day reported in Ghana (Kabak, 2021). The high intake levels reported in this study could be because this present study also factored in the consumption frequency of foods within a day. However, going by these results, it is evident that dietary intake of both total aflatoxin and aflatoxin B1 is comparatively higher among lactating mothers in the study area. The high intake was greatly attributed to the high consumption of maize '*ugali'* followed by maize porridge, maize sorghum porridge, '*githeri',* and *'muthokoi'* in that order. This outcome, however, did not come as a surprise since maize ugali is the main staple food in Kenya, and is most frequently consumed among other maize-based foods. Kilonzo et al.'s (2014) findings in Makueni were also similar to the sentiments of this study. However, the absence of a significant association between dietary intake levels of aflatoxin and the breastfeeding status of lactating mothers reaffirms earlier sentiments that exclusively and non-exclusively breastfeeding mothers in the study area are at equal risk of being predisposed to dietary aflatoxin.

The margin of exposure (MOE) value below 10, 000 among lactating mothers was considerably low. The values were less than one (< 1) and extremely low compared to the results of other studies. For instance, Marijani et al. (2020) in Kenya reported a higher margin of exposure

value of 126.3 from consumption of '*omena'* (*Rastrineola argentea*). Kabak (2021) on the other hand, reported a margin exposure value of 336 in Ghana. The extremely low margin of exposure values reported in this study is indicative that lactating mothers in Kibwezi and its surrounding are considered to be at higher risk of carcinogenic exposure compared to other areas. As a result of this, risk characterization is highly recommended among lactating mothers and breastfeeding children in the study area.

5.4 Aflatoxin M1 in breastmilk of lactating mothers in Kibwezi West

To the best of my knowledge, research on the occurrence of aflatoxin M1 in the breast milk of lactating mothers in Kenya is scanty. It has only been conducted by Kang'ethe et al. (2017) on mothers with children below 5-years, and Maxwell et al. (1988) on pregnant mothers in Kenya. This study will be the third, but the first to determine the presence of aflatoxin M1 in the breast milk of mothers who are exclusively, and non-exclusively breastfeeding children aged six months and below in Kenya.

The overall prevalence of aflatoxin M1 in breast milk reported in this study (77.1%) was lower than 86.7% reported in Makueni but higher than 56.7% reported in Nandi (Kang'ethe et al., 2017). The rates, however, were higher than the 28% reported by Maxwell et al. (1988). These rates confirm that the prevalence of aflatoxin in breast milk is as high as those reported in food samples in the study area. Comparison with 41% in northern India (Mehta et al., 2021), 42% in Iran (Fakhri et al., 2019a), 90% in Columbia (Sánchez and Diaz, 2019), 93.8% in Lebanon (Elaridi et al., 2017), and 100% in Iran (Azarikia et al., 2018) show that occurrence of aflatoxin M1 in the breast milk of lactating mothers is a widespread problem and vary from region to region. For exclusively breastfeeding mothers, 80% was reported in this study against 18% in Nigeria (Ezekiel et al. 2020) and 22% in Iran (Mahdavi et al., 2010). These high rates especially for exclusively breastfeeding children are alarming in the study area.

Based on the concentration of aflatoxin M1 in breast milk, 35 ng/L reported in this study was considerably higher than 8.46 ng/L and 0.02 ng/L reported in Makueni and Nandi, respectively, using ELISA (Kang'ethe et al., 2019). However, ranges reported by Maxwell et al. (1989) (5-1379 ng/L) in 121 breast milk samples were higher than the overall ranges reported in this study (5-78 ng/L. This outcome points out that aflatoxin occurrence in the breast milk of lactating mothers is an under-evaluated risk in Kenya. While it is considered that breast milk is the safest food for children below six months of age, this might not be the case in the study

area. However, this problem also exists in other countries. The results of Mehta et al. (2021) (3.9-1200ng/L, median 13.7 ng/L) were higher than the overall ranges reported in this study (5-78 ng/L). Pooled mean for Iran (5.85 ng/L) (Fakhri et al. 2019), and 4.31 ng/L for Lebanon (Elaridi et al., 2017) was lower than the mean of this study. The mean of aflatoxin M1 in the breast milk of exclusively breastfeeding mothers in this study (38.0 ng/L) was almost 40 times greater than the 2.0 ng/L levels reported in Ogun estate, Nigeria (Ezekiel et al. 2020). It was almost six times greater than the 6.96 ng/L levels reported in rural areas of Iran (Mahdavi et al., 2010). However, mean levels reported in Tanzania (70 and 80 ng/L) during the rainy and dry season, respectively by Magoha et al. (2014), and 45 ng/L in Ecuador by Ortiz et al. (2018) were higher than the values reported in this study. The results of this study were however within the mean levels compiled by (Fakhri et al., 2019b) for Africa. These comparisons together with a high proportion (61.8%) of breast milk with aflatoxin M1 above 25 ng/L EU limits is a cause of food safety and health concern in the study area. Because of this, this study is in agreement with Kang'ethe et al. (2017) who concluded that infant children in Makueni are also exposed to aflatoxin M1 through breast milk. This study, therefore, confirms that breast milk in the study area is contaminated with aflatoxin. However, it adds that both exclusively and non-exclusively breastfeeding children aged six months and below in Makueni are equally exposed to aflatoxin M1 through breast milk.

5.4.1 Intake of aflatoxin M1 in breastmilk among breastfeeding children in Kibwezi West

Intake of aflatoxin M1 in this study was generally high among children regardless of their breastfeeding status. The overall mean intake (0.47 µg/kg b.w.t/day) was higher than 0.006 and 1×10^{-6} µg/kg b.w.t/day in Makueni and Nandi, respectively (Kang'ethe et al., 2017). They were also higher than those estimated using dairy milk in Nairobi (0.004 µg/kg b.w.t/day) (Alberg et al., 2018). The result of this study was comparable on an age group basis with those of Hernández et al. (2021). However, the levels were higher than 0.012 µg/kg b.w.t/day reported in Tanzania (Magoha et al., 2014), 0.069 µg/kg b.w.t/day reported in Lebanon (Elaridi et al., 2017), 0.003 µg/kg b.w.t/day reported in Serbia (Radonić et al., 2017), and 3.04×10^{-4} µg/kg b.w.t/day reported in India (Mehta et al., 2021). The results of this study were consistently higher thus pointing to the existence of high aflatoxin exposure among breastfeeding children aged six months and below in the study area. Consequently, a low margin of exposure value (<10000) showed that both exclusively and non-exclusively breastfeeding children in the study area were extremely exposed to high levels of aflatoxin

intake. These results confirm the findings of Coppa et al. (2019) who reported a margin of exposure of 0.27 for African countries. Because of this, this study acknowledges that the margin of exposure of breastfeeding children to aflatoxin is high in African countries. In Kenya, this study is the first of its kind to estimate the margin of exposure of breastfeeding children to aflatoxin M1 through breast milk. However, margin exposure reported in Serbia by (Milićević et al. 2021) was greater than 10,000 for children aged between 1 to 9 years. Similarly, those reported in Italy for toddlers were below 10, 000 but considerably higher than those reported in this study (Roila et al. 2021). Because of this, this study concludes that exclusively and non-exclusively breastfeeding children in the study area are remarkably at higher risk of carcinogenic exposure compared to other regions.

5.5 Aflatoxin M1 in the urine of breastfeeding children in Kibwezi West

The prevalence of aflatoxin M1 in the urine of breastfeeding children in this study was 100%. It was higher than 79 and 83% reported in Makueni and Nandi, respectively (Kang'ethe et al., 2017) for children below 5 years. It was also higher than 17% in Ethiopia (Ayelign et al., 2017) for infants 1-2 years, 98.8% in Ogun Nigeria (Ezekiel et al., 2018) for children below 2 years, 47% in Colombia for infants (Sanchez and Diaz, 2019), 53% in southern Ethiopia (Boshe et al., 2020) for children 6-23 months, and 43.5% in Bangladesh (Ali et al., 2020) for infants. The rates were also higher than the 4 and 12% rates reported for exclusive and non-exclusively breastfed children, respectively in Nigeria (Ezekiel et al., 2020), and the 22% rate reported for exclusively breastfed children in Iran (Mahdavi et al., 2010). The results are a reflection of a high dietary intake of aflatoxin by lactating mothers in Makueni, and mothers' dietary role in influencing aflatoxin M1 in breast milk in the study area.

Based on concentration, the overall mean of aflatoxin M1 in the urine of breastfeeding children in this study (390 ng/L) was lower than 1182.9 and 857.3 ng/L for children below 30 months in Makueni and Nandi, respectively (Kang'ethe et al., 2017). They were higher than 270ng/L (60-510 ng/L) in Nigeria (Ezekiel et al., 2018), and 214 (0-2582 ng/L) (Boshe et al., 2020) and 64 ng/L (Ayelign et al., 2017) in Ethiopia. The levels were also higher than 16 ng/L in Columbia (Sánchez & Diaz, 2019), 9.1, maximum of 55.6 ng/L in Bangladesh (Ali et., 2020), and 39 ng/L reported in Sweden (Mitropoulou et al., 2018). The mean for non-exclusive breastfed children in Nigeria (166 ng/L) (Ezekiel., 2020) was lower than the one reported in this study (420 ng/L). Likewise, that of exclusively breastfeeding children (23 ng/L) was lower than 350 ng/L reported in this study. Mean levels (96 ng/L) reported in Iran for exclusive

breastfed (Mahdavi et al. 2010) were also lower than the levels reported in this study. High levels of aflatoxin M1 in urine in this study were expected and confirmed that lactating mothers and breastfeeding children in Kibwezi are exposed to high levels of aflatoxin intake through maize-based foods. These results also reaffirm earlier sentiments of this study that aflatoxin occurrence is persistent in Makueni. However, significant variation of aflatoxin M1 noted between urine of non-exclusively breastfeeding children age groups could be a result of different complementary foods used before collecting urine samples.

5.6 Factors associated with the occurrence of total aflatoxin and aflatoxin B1 in food, and aflatoxin M1 in the breast milk of lactating mothers and urine of breastfeeding children in Kibwezi West

5.6.1 Factors associated with the occurrence of total aflatoxin and aflatoxin B1 in foods of lactating mothers

Though the predictor model showed that household size was not a significant influencer of total aflatoxin in the study, a positive significant correlation between them may suggest that larger households in the study area are at higher risk of consuming aflatoxin-contaminated maize. Since maize is the main staple food in Kenya and is consumed by almost everyone, its demand per person is hypothesized to be higher in larger households. This demand is matched by stocking for larger quantities of maize or frequently sourcing in smaller amounts from different sources. Either of these practices, combined with poor handling and storage reported among lactating mothers in the study, could increase the chances of maize being contaminated with aflatoxin in larger households. This hypothesis is in agreement with (Nabwire et al., 2020b) who found that children from smaller size families in Makueni had lower aflatoxin exposure as opposed to their counterparts.

Further, the model containing both women's dietary diversity and cleaning of maize before storage response was found to be a major predictor of dietary intake of total aflatoxin among lactating mothers. Even though the *p*-values for the two variables were not significant in the model, a significant negative correlation of dietary diversity of lactating mothers found in the study area concurs with the observation made by (Nabwire et al., 2020b) who showed that low dietary diversity in Makueni was associated with increased risk of aflatoxin intake. However, the positive correlation of cleaning maize with aflatoxin occurrence in this study was not expected as a study by (Lesuuda et al., 2021) mentioned that separating deformed grains was

associated with a low occurrence of aflatoxin. This correlation could therefore mean that the process is not effective enough to reduce a substantial amount of aflatoxin once in the maize food samples. The results could also be indicative of high levels of aflatoxin contamination in the study area as compared to others in Kenya. For other practices, no significant relationship was reported between concentration levels of total aflatoxin, and aflatoxin B1 in the analyzed foods with maize source, handling, processing, and storage practices in the study. These results were different from those reported by Nabwire et al. (2020a), and Daniel et al. (2011) among others who linked aflatoxin contamination with maize source, handling, processing, and storage practices in Makueni. These differences could be because the result of this study was based on mothers' responses, while the latter was based on experimental analysis.

Regression coefficients of concentration of total aflatoxin and aflatoxin B1 of individual analyzed foods to cumulative intake of aflatoxin reported in this study also point out that frequency of intake, food type, and quantities of foods consumed play an important role in determining the exposure levels of dietary aflatoxin intake among lactating mothers in the study. Therefore, it was not surprising to associate higher dietary intake of aflatoxin in the study area with consumption of maize ugali > maize porridge > maize sorghum porridge > '*githeri*' > '*muthokoi'* in that order.

Just as earlier results of this present study, education was also shown to reduce aflatoxin B1 intake by about 45% among exclusively lactating mothers. This result supports the findings of Leroy et al. (2015) but contrasts with the findings of Mehta et al. (2021). That notwithstanding, sentiments shared by Malusha et al. (2016), and Lesuuda et al. (2021) showed a negative correlation between knowledge, attitude, and practices with aflatoxin contamination on cereals, and underscore education as one of the strategies that can be implemented among lactating mothers to reduce dietary aflatoxin exposure in this present study.

Though the socio-economic status model did not predict aflatoxin B1 intake of lactating mothers in the study, its positive association with an increase in aflatoxin B1 intake was not expected. This is because the results of Leroy et al. (2015), Nabwire et al. (2020b), and a review by Omara et al. (2020) among others had associated poor households with a higher probability of aflatoxin exposure within the same study area of this present study. Similarly, this study showed that almost 95% of lactating mothers in the study were below the upper wealth index. Further investigation for a possible explanation was conducted. Results of this study though

nonsignificant, showed a positive association between socioeconomic status and an increase in the total number of meals consumed per day by lactating mothers, and an increase in women's dietary diversity in the study. But a predictor model showed that women dietary diversity negatively influenced aflatoxin B1 intake among non-exclusively lactating mothers. This outcome which showed that low dietary diversity was a risk factor for aflatoxin intake was also consistent with those reported by Leroy et al. (2015) and (Nabwire et al., 2020b) conducted in the same area of this study but inconsistent with those reported away from this study area by Mehta et al. (2021) in India. However, when focusing on foods that are only susceptible to aflatoxin contamination, the study by Andrew-Trevino et al. (2020) reported a positive correlation between socioeconomic status and consumption frequency of contaminated maize and groundnuts among Nepalese women**.** This study, therefore, suggests that the influence of socioeconomic status on aflatoxin levels in foods depends on the region of the study, available type and range of food diversity, and the prevalence of aflatoxin contamination in the area. When used on its own, it might not be a reliable pointer to aflatoxin intake in a study area. For instance, without basic knowledge of aflatoxin contamination and limited food choice, lactating mothers with higher socioeconomic status can still be susceptible to aflatoxin B1 exposure in the diet.

Even though Andrew-Trevino et al. (2020) reported a negative association between age and exposure to aflatoxin B1 in adduct of pregnant mothers, this study did not find any direct influence of age and consequently breastfeeding status of lactating mothers on total aflatoxin and aflatoxin B1 levels in the study area. This could probably be due to a range of sociodemographic and economic similarities drawn between exclusively and non-exclusively lactating mothers in the study.

5.6.2 Factors associated with the occurrence of aflatoxin M1 in the breast milk of lactating mothers

Total aflatoxin was the major predictor of aflatoxin M1 concentration levels in breastmilk of lactating mothers in the study. It accounted for 71% of aflatoxin M1 levels in breastmilk. Significant linearity was observed for both mothers who exclusively and non-exclusively breastfeed their children. Estimates of aflatoxin B1 intake in the study were also a major influencer of aflatoxin M1 levels in breast milk. However, this was reported for exclusively lactating mothers as opposed to non-exclusively lactating mothers. This one-sided influence could not be explained since a nonsignificant difference in aflatoxin B1 intake was reported

between exclusively and non-exclusively lactating mothers. However, the co-occurrence of aflatoxin B1 and M1 in breast milk, and the complex metabolization process of aflatoxin in the body could not be ruled out. That notwithstanding, these results are in total agreement with studies by Kang'ethe et al. (2017) among children below 5 years of age in Makueni, Kenya. Results in Nigeria were also consistent with the results of this study (Adejumo et al., 2013). The results were also similar to those of Azarikia et al. (2018) in Iran, Elaridi et al. (2017) in Lebanon, and (Mehta et al. 2021) in India.

Predicting model containing dietary diversity of mothers and aflatoxin weekly consumption score predicted about 32% of aflatoxin M1 intake among breastfeeding children in the study. This model emphasizes the importance of diverse diets in areas that are prevalent to aflatoxin contamination. It also points out that the type of food constituting a diverse diet plays an important role in reducing aflatoxin exposure among lactating mothers. Going by these results maize-based foods are still considered the greatest dietary contributor of aflatoxin M1 in breastmilk of lactating mothers in the study area (maize ugali > maize porridge > maize sorghum porridge > *'githeri'* > *'muthokoi'*).

Earlier results of this study showed a direct role of household size, education level, and socioeconomic status on levels of aflatoxin intake among lactating mothers. On the contrary, in addition to the age of mothers and breastfeeding children in the study, a direct influence on aflatoxin M1 in breastmilk was not observed. Consequently, no direct influence was observed on aflatoxin M1 intake among breastfeeding children. These observations are similar to those of Mehta et al. (2021) and Elaridi et al. (2017). However, they differ from those of Karayağiz and Özdemir (2020) who reported a positive significant association.

Among variables of breastfeeding practices in the study, time for initiating breast milk, number of children per lactating mother, and time for introducing complementary foods to children did not directly influence children's exposure to aflatoxin M1 through breast milk. This is worth noting since it is expected that introducing children to complementary foods also reduces suckling and thus intake of breast milk. Also, it is not possible to statistically determine the correlation between breast milk intake and aflatoxin intake of M1. However, it is clear in this study that children who are most frequently breastfed will be exposed to higher levels of aflatoxin M1 in breast milk. These remarks point to the need for having an elaborate plan in the study area to reduce aflatoxin exposure among lactating mothers. This is because breast

milk is the only food considered safe for children under the age of six months (Boquien, 2018). Similarly, a significant association was not observed between maize source, handling, and storage practices with the occurrence of aflatoxin M1 in breastmilk. However, this present study suggests the need of conducting a robust study that links the food supply chain with the occurrence of aflatoxin in breast milk in the study area. This is because studies by Nabwire et al. (2020a) and Daniel et al. (2011) have linked maize source, handling, and storage practices with aflatoxin contamination in Makueni and its surrounding.

5.6.3 Factors associated with aflatoxin M1 in the urine of breastfeeding children

This study confirmed that total aflatoxin in foods influenced aflatoxin M1 in the urine of exclusively and non-exclusively breastfeeding children in the study area. In fact, it explained about 15% of aflatoxin in the urine of all children whose samples were analyzed. Also, a model containing total aflatoxin, age of children, and socioeconomic status was able to explain 70% of aflatoxin M1 in the urine of exclusively breastfeeding children. This study thus concludes that maize ugali, indirectly, is the greatest contributor of aflatoxin M1 in the urine of breastfeeding children in the study area. This is followed by maize porridge, maize sorghum porridge, '*githeri*', and '*muthokoi'* in that order. Even though insignificant, a positive correlation between intake of total aflatoxin, aflatoxin B1, and aflatoxin M1 in breast milk with aflatoxin M1 in the urine of children is still supportive of Alegbe et al. (2018) findings. The findings showed that mothers' dietary intake patterns positively correlated with aflatoxin exposure. However, the absence of a direct link between aflatoxin B1 and aflatoxin M1 in urine in the study was due to the reasons stated by Ali et al. (2020) and the findings of Boshe et al. (2020). They both concluded that only a smaller percentage of aflatoxin B1 can be transferred to urine. Also, a significantly higher concentration level of aflatoxin M1the in the urine of non-exclusively breastfeeding children than that of exclusively breastfeeding children (p = 0.035) was noted. This difference pointed out that the use of complementary foods in the study area also influences aflatoxin M1 levels in the urine of breastfeeding children. This comparison is based on the findings of Ezekiel et al. (2020) and Magoha et al. (2014) who determined levels of aflatoxin among exclusively and non-exclusively breastfeeding children in Nigeria and Tanzania, respectively. They concluded that non-exclusively breastfeeding children are predisposed to higher levels of aflatoxin (breastmilk + complementary foods) than their counterparts who only consume breastmilk.

Children's age was not a significant predictor of aflatoxin M1 in the urine of exclusively breastfeeding children in the study. Despite this, a positive correlation intimated that an increase in age was associated with an increase in aflatoxin exposure. This was in parallel with the findings of this study that showed that non-exclusive breastfeeding rates increased with an increase in the age of children. This study, therefore, maintains that the age of children is an underlying factor in determining the extent of aflatoxin M1 exposure in the study area.

The negative correlation between socioeconomic status and aflatoxin M1 in the urine of exclusively breastfeeding children contradicted earlier findings of this study. The reports showed a positive correlation between socioeconomic status and aflatoxin B1 intake among exclusively lactating mothers. This contradiction reaffirms earlier sentiments of this study. It was suggested that socioeconomic status should not be a reliable pointer to aflatoxin exposure in an area where aflatoxin contamination is highly prevalent. Also, a direct influence of household size, age, and education level of lactating mothers was reported on aflatoxin intake in the study. On the contrary; this was not the case for aflatoxin M1 levels in the urine of breastfeeding children in the study. These findings were the same as those of Chan et al. (2018).

Similarly, a direct influence of women's dietary diversity, aflatoxin weekly consumption score, and the total number of meals consumed per day on mothers' aflatoxin intake was reported. Again, this was not the case for aflatoxin M1 in the urine of breastfeeding children in the study. These results were again similar to those of Chan et al. (2018) among children in Tanzania. Again, this study was not able to show any influence of sourcing, handling, storing, and processing of maize on aflatoxin M1 on the urine of breastfeeding children. This is despite studies by Nabwire et al. (2020a) and Daniel et al. (2011) linking various sources of maize and handling practices with aflatoxin contamination in the study area. A possible explanation for this was that this study did not determine the source of the analyzed food samples. Again, maize handling and storage practices in this study were only reported by mothers and were not based on any experimental results. However, there are no studies that have explored the relationship between maize handling and storage practices with the presence of aflatoxin in breast milk, and the urine of breastfeeding children less than six months of age.

5.7 Effect of aflatoxin exposure on weight-for-age of exclusively and non-exclusively breastfeeding children below 6 months in the study

The prevalence rate of underweight for exclusively and non-exclusively breastfeeding children was 13.3 and 11.6%, respectively. They were comparable to 13 and 18% reported in Tanzania among exclusive and non-exclusive breastfeeding children, respectively (Magoha et al. 2016). The overall prevalence rate (12.4%) for this study was almost similar to 10.2 and 11% reported for Makueni and Kenya, respectively (KDHS, 2014), but slightly lower than 14.6% reported by Kang'ethe et al (2017) also in Makueni. Despite comparable underweight rates, this present study did not show any direct influence of aflatoxin exposure on the weight-for-age outcome of breastfeeding children. This was contrary to the results of Kang'ethe et al. (2017), and (Nabwire, Thu, et al. 2020) who showed a negative association between aflatoxin exposure and weight-for-age among children aged below 5 years, and 6 and 12 years, respectively, in Makueni. However, one striking difference between the aforementioned studies and this present study was the difference in children's age. The aforementioned studies included older children whom this present study presumes to be exposed to more aflatoxin intake compared to breastfeeding children. However, Magoha et al. (2016) with children aged six months and below also reported a negative association between aflatoxin exposure and weight-for-age z-scores. Further analysis, however, revealed that the exclusive breastfeeding rate reported in this present study was higher than that of Magoha et al. (2016). Moreover, aflatoxin M1 levels in Magoha et al. (2016) were higher than the ones reported in this study. From these results, it may be concluded that children who are not being breastfed or are non-exclusively breastfed are highly exposed to aflatoxin intake than those who are exclusively breastfed. This is because most of the complementary foods being used in aflatoxin-prone areas have also been mentioned to be highly susceptible to aflatoxin contamination. These sentiments explain the reason why underweight prevalence rates (14.6%) reported by Kang'ethe et al. (2017) among children below 5 years, 17% reported by Ayelign et al. (2017) rate among infants in Ethiopia, and 17 and 21% reported by Chen et al. (2018) among children aged 24 and 36 months, respectively, in Tanzania were slightly higher than the ones reported in this study.

Further analysis revealed that age was the only predictor of weight-for-age z-scores of breastfeeding children in this study. This sentiment was in agreement with those of Hoffmann et al. (2018) who also concluded that the impact of aflatoxin exposure on growth parameters in children varies with their age. However, other variables including the age of lactating mothers, household size, education level, and socioeconomic status were shown not to have

any direct impact on the weight-for-age z-score of breastfeeding children in the study. Similarly, no direct influence of dietary consumption patterns and breastfeeding practices on weight-for-age z-scores was reported. However, while no direct effect of aflatoxin exposure on weight-for-age of exclusively and non-exclusively breastfeeding children was reported, this present study still emphasizes the need for exclusively breastfeeding children aged six months and below in the study area. This study further points out that the absence of direct impact of aflatoxin exposure on weight-for-age does not rule out imminent negative effects of chronic aflatoxin exposure in children's later life. Studies such as (Hoffmann et al., 2018), (Marchese et al. 2018), and (Kumar et al., 2021) have shown negative impacts of aflatoxin. It is therefore probable that breastfeeding children in this study area, due to high aflatoxin exposure, will be at risk of stunting, cancer, increased morbidities, and micronutrient deficiencies among other negative side effects. Because of this, this study recommends further follow-up and risk characterization. Clinical studies can help ascertain the impact of high aflatoxin exposure besides depending on weight-for-age z-scores in the study area.

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

The following conclusions were made in line with the objectives of the study:

Lactating mothers in the study area have similar socioeconomic and demographic characteristics; however, their influence on aflatoxin exposure varies and depends on the area of study.

Low dietary diversity, limited food consumption patterns, and breastfeeding practices influenced the exposure of lactating mothers and breastfeeding children, respectively, to aflatoxin intake. However, maize handling and storage practices among lactating mothers, which are not a predictor in this study, are associated with aflatoxin contamination in other studies.

High prevalence of total aflatoxin and aflatoxin B1 in maize-based foods, aflatoxin M1 in breastmilk, and urine of breastfeeding children establish mothers' dietary role of predisposing exclusively and non-exclusively breastfeeding children aged six months and below to aflatoxin intake in the study area.

However, there is no direct significant influence of aflatoxin exposure on weight-for-age z-scores of exclusively and non-exclusively breastfeeding children aged six months and below in the study.

6.2 Recommendations

The following recommendations were made according to the results of the respective objectives:

Sociodemographic and economic characteristics, and food consumption patterns of the lactating mothers

1. Income-generating activities should be introduced among poor lactating mothers to improve their access to food and consequently increase their food diversity.

2. Results underscore education as one of the strategies that can be implemented towards reducing aflatoxin contamination in diets and breast milk of lactating mothers, and exposure levels of breastfeeding children in the study area.

Levels of aflatoxin in breast milk, maize-based foods consumed by lactating mothers, and urine of breastfeeding children in Kibwezi West, Makueni County.

1. Despite reporting high aflatoxin M1 intake through breast milk, lactating mothers are still encouraged to adhere to WHO breastfeeding recommendations since this study shows that non-exclusively breastfeeding children are exposed to additional aflatoxin intake from complementary foods.

2. The high prevalence and presence of aflatoxin in foods, breastmilk, and urine of breastfeeding children is a public health concern and calls for the need of devising easy-to-use household food safety and monitoring measures in the study area.

3. The absence of a significant association between the response of lactating mothers to maize source, handling, processing, and storage with exposure to aflatoxin suggests the need of conducting a robust study that can trace the flow of aflatoxin from maize acquisition to breast milk of lactating mothers.

4. The results of this study provide an opportunity for Makueni County and others that are prevalent to aflatoxin contamination to initiate breast milk safety policy briefs. Integrating such policy briefs within existing food policies such as National Food Safety Policy 2013, Maternal, Infant and Young Child Nutrition 2013 among others that target exclusive breastfeeding of children under six months can reinforce risk communication and encourage management of aflatoxin among lactating mothers.

Nutrition status of breastfeeding children below six months based on weight-for-age z-scores in Kibwezi West, Makueni County.

1. The absence of direct influence of aflatoxin on weight-for-age z-scores of breastfeeding children despite high exposure, calls for the need to conduct clinical studies that can further elucidate the health impact of high aflatoxin exposure on exclusive and non-exclusive breastfeeding children in the study area.

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APPENDICES

Appendix 1: Informed Consent Form

Title: Aflatoxin exposure of lactating mother-child pairs and nutritional status of breastfeeding children 0-6 months in Makueni County, Kenya

Purpose

The purpose of the study is to generate information on levels of aflatoxin in food, breast milk, and urine of breastfeeding children, and their influence on the nutritional status of children as a basis for emphasizing the constant need for monitoring aflatoxin occurrence in at-risk regions.

Role

You are requested to co-operate in this study by answering questions in the questionnaire and providing any other information as pertains to the study. You are also requested to permit the investigator to collect food, breast milk, and urine samples for laboratory analysis.

Aim

The study aims to contribute to the improvement of food safety as well as maternal and infant and young child health and nutrition during the lactation period.

Risks

There are no foreseen risks associated with the study. Your participation is voluntary and therefore, you will not receive any form of compensation. This study is protected by the human ethics committee (KNH/ERC). If you have any questions regarding your rights as a participant, you are free to contact; Otieno Isaac Ogallo (+254720141182, otieno_isaac@yahoo.com) Or KNH/UON-ERC (Box19676/20723-00202, uonknh_erc@uonbi.ac.ke).

Volunteer Agreement

I have read the consent form describing the benefits, risks, and procedures for this study (*aflatoxin exposure of lactating mother-child pairs and nutritional status of breastfeeding children 0-6 months in Makueni County, Kenya)*

Name_____ **Signature** _____ **Date**_____

For official use only

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this study have been explained to the above individual

Date…… **Signature……**

Appendix 2: Study Questionnaire

Aflatoxin exposure of lactating mother-child pairs and nutritional status of breastfeeding children 0-6 months in Makueni County, Kenya				
Household Number				
IDENTIFICATION				
County		Sub-County		Constituency
Ward		Sub-ward		
Date of Interview		(dd/mm/year)		
Name of Interviewer				
SECTION A: DEMOGRAPHIC CHARACTERISTICS				
				Answer
1	Mothers' Age (Years)			
Ask the mother and choose one appropriate answer. Put the answer in the box				***Answe*r**
2	Marital Status		Married	
		2	Single	
		3	Separated	
		4	Widowed	
		5	Divorced	
				Answer
3	Level of Education	1	No formal education	
		2	Attempted primary education	
		3	Attempted primary education	
		4	Completed primary education	
		5	College/university education	
		6	Adult education	
				Answer
4	Occupation of the mother	1	Unemployed	
		2	Salaried employed	
		3	Self-employed	
		4	Housewife	
		5	Farmer	
		6	Casual labor	
		7	Other (Specify)	
Ask and write the total number in the answer box.				***Answer***
5	Total number of children			
6	Number of household members			

SECTION B: ECONOMIC CHARACTERISTICS				
NB: - Ask for a rough estimate. If the mother cannot estimate per month, probe per day and adjust on a monthly basis				
				KES
7	Household average monthly income			
8	Household Average Monthly expenditure			
9	Household Average Monthly Savings			
Assets				
NB: -Ask about assets owned in the household. Some can be observed without asking				
10	Do you own productive land e.g., agricultural?	Yes	No	***If yes, how many acreage***
11	Do you own any of this livestock?			***If yes, how many (Answer)***
11a	Goats	Yes	No	
11b	Poultry	Yes	No	
11c	sheep	Yes	No	
11d	Cows	Yes	No	
11e	Any other livestock (Specify)?			
	Total number of livestock			
	Do you own any of this…?			***If yes, how many (Answer)***
12	Own mobile phone	Yes	No	
13	Own media equipment (radio/television)	Yes	No	
14	Own means of transport (bicycle/motorbike/donkey/car)	Yes	No	

Section C: Food Consumption Pattern for Lactating Mothers (15-45 Years)

For each food item, indicate with a check mark the category that best describes the frequency with which you usually eat the particular food item and estimate the amounts using household measures.

*NB: *Scores for foods that are mostly susceptible to aflatoxins contamination*

Food	Consumption Frequency (per week) and estimated amount in grams								After every two weeks	Monthly	Never	Food Scores Daily=7 Once=1 Twice=2 Thrice=3 Four times=4 Five times=5 Six times=6 Per Fortnight=0 Per Month=0 Never=0
	Once/week	Twice/week	Thrice/week	Four/week	Five/week	Six/week	Daily	Number of intakes within a typical day				
Maize Products												
Maize meal (Ugali)												*
Sorghum flour ugali												*
Mixed flour ugali												*
Githeri												*
Muthokoi												*
Maize flour porridge												*
Sorghum flour porridge												*
Mixed flour porridge												*
Cassava flour porridge												*
Cereals, roots, tubers												
Sorghum												*
Rice												*
Irish potatoes												
Cassava												*
Sweet potatoes												
Finger millet												*
Raw bananas (matoke)												

Cont'd Section C: Food Consumption Pattern for Lactating Mothers (15-45 Years)

For each food item, indicate with a check mark the category that best describes the frequency with which you usually eat the particular food item and estimate the amounts using household measures.

*NB: *Scores for foods that are mostly susceptible to aflatoxins contamination*

Food	Consumption Frequency (per week) and estimated amount in grams								After every two	Monthly	Never	Food Scores Daily=7 Once=1 Twice=2 Thrice=3 Four times=4 Five times=5 Six times=6 Per Fortnight=0 Per Month=0 Never=0
	Once/week	Twice/week	Thrice/week	Four/week	Five/week	Six/week	Daily	Number of intakes within a typical day				
Fruits and vegetables												
Ripe bananas												
Pawpaw												
Mangoes												
Oranges												
Sukuma wiki												
Cabbages												
Carrots												
Cow peas leaves												
Amaranth												
Managu												
Protein Sources												
Beans												
Pigeon peas												
Green grams												
Peas												
Beef												*
Chicken												*
Egg												*
Fish												*
Milk												*
											Food score percent	(Total food score/504 × 100%)

Extra Questions	
1.How many meals do you have in a normal day?	
2.Please specify the meals (before morning, breakfast, etc.)	
3.Where do you obtain the cereals for cooking?	
4. How do you store foods that are waiting preparations?	
5. Where do you store the cereals	
4. What is the main cooking method used for preparing these foods?	

Section D: Breastfeeding Practices for Children below 6 months				
1. How many months is your breast-feeding child?				
2. What time did you initiate breast milk to the baby after birth				

(Probe whether immediately, after some hours, after a day etc.)			
3. How many times does your baby usually breast feed during the day?			
4. How many times does your baby usually breast feed during the night?			
5. Apart from breast milk are their other foods you give the baby? (Yes/No)			
6.If yes, can you mention them?			
7.What time did you start feeding the baby these foods? *(Probe whether immediately, after some hours, after a day etc.)*			
8.How often do you give these foods per week			
Name of the food	**Answer (1/2/3/4/5/6/7/Rarely/Never**		
9.For each food listed, how many times do you give the foods within a day	**Answer (1/2/3 etc.)**		
10.What is your child's weight (Kg)	1st reading		
	2nd reading		
	Average weight		
11. Classify the child a). Exclusively breastfeeding b). Non-exclusively breastfeeding			
10.What is your child's weight (Kg)	1st reading		
	2nd reading		
	Average weight		
11. Classify the child a). Exclusively breastfeeding b). Non-exclusively breastfeeding			

Section D: Women Dietary Diversity (15-45 Years)

**Establish whether the previous day and night was usual or normal for the woman. If unusual- feasts, funerals or most members absent, then another day should be selected*

**Establish food eaten both in and outside home*

Question Number	Food Group	Example	1 = Yes 0=No
1.	Cereals	Maize, wheat, rice, millet, sorghum and any other grains or foods made from these (e.g., bread, spaghetti, noodles, porridge, ugali, *muthokoi/ githeri*)	
2.	Roots and tubers	Irish potatoes, yams, cassava, or other foods made from these (e.g., chips/French fries,	
3.	Vitamin A rich vegetables and tubers	Pumpkin, carrots, squash, orange-fleshed sweet potato, other locally available vitamin A vegetables e.g., red sweet pepper,	
4.	Dark green-leafy vegetables	Dark green-leafy vegetables including wild forms and locally available vitamin A rich leaves such as amaranth, cassava leaves, kales, spinach etc.	
5.	Other vegetables	Other vegetables e.g., tomato, onion, egg plant, green bananas and any other locally available vegetable	
6.	Vitamin A rich fruits	Ripe mango, apricots, ripe pawpaw, ripe banana, avocado, 100% fruit juice and any other locally available vitamin A rich fruits	
7.	Other fruits	Including wild fruits, 100% fruit juice made from this.	
8.	Organ meat	Liver, kidney, heart and other organ meats and blood-based foods.	
9.	Flesh meats	Beef, pork, lamb, goat, rabbit, game, chicken, duck, other birds and insects.	
10.	Eggs	From chicken, duck, guinea fowl or any other eggs.	
11.	Fish and sea food	Fresh or dried fish	
12.	Legumes, nuts and seeds	Dried beans, dried peas, lentils, nuts, green grams, or food made from these e.g., peanut butter	
13.	Milk and milk products	Milk, cheese, mala, yogurt.	