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Study Report

Assessing the Relationship of Aflatoxin Exposure and Stunting in Children 6-59 Months of Age in 10 Districts of Nampula Province, Mozambique

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Assessing the Relationship of Aflatoxin Exposure and Stunting in Children 6-59 Months of Age in 10 Districts of Nampula Province, Mozambique

Report to USAID Mozambique submitted by
The Feed the Future Innovation Lab for Nutrition

Work conducted by
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Acronyms and Abbreviations

AFB1	Aflatoxin B1
AFM1	Aflatoxin M1
ANSA	Association for Food and Nutrition Security
APE	Agente Polivalente Elementar
BMGF	Bill and Melinda Gates Foundation
CAADP	Comprehensive African Agriculture Development Program
CHW	Community Health Workers
DHS	Demographic and Health Survey
EED	Environmental enteric dysfunction
GDP	Gross Domestic Product
GRM	Government of Mozambique
HCN	Nampula Central Hospital
HAZ	Height-for-age z-score, or length-for-age z-score
HIV	Human immunodeficiency virus
IFPRI	International Food Policy Research Institute
INE	Instituto Nacional de Estatística/National Institute of Statistics
INS	Instituto Nacional de Saúde/National Institute of Health
IRB	Institutional Review Board
IYCF-MDD	Infant and Young Child Feeding Minimum Dietary Diversity
Mg	Milligram
MISAU	Ministry of Health
MUAC	Mid-upper arm circumference
OR	Odds ratio
PAMRDC	Multi-Sectoral Action Plan for the Reduction of Chronic Malnutrition
pg	Picogram
PI	Principal Investigator
ppb	Parts per billion
PPS	Probability proportional to size
SAM	Severe acute malnutrition
SE	Standard error
SETSAN	Technical Secretariat for Nutrition and Food Security
USAID	United States Agency for International Development
WASH	Water, sanitation, and hygiene
WAZ	Weight-for-age z-score
WHZ	Weight-for-height z-score
ZOI	Zone of Influence

1. Executive Summary

The prevalence of stunting is high in Mozambique. This is particularly an issue in rural areas and northern provinces where United States Agency for International Development (USAID) activities linked to Feed the Future and Ending Preventable Child and Maternal Deaths initiatives are implemented. At the same time, little is known about the relationship of stunting and aflatoxin exposure in young Mozambican children. As noted by Turner (2013), "When one considers that worldwide 40% of the 11 million deaths in children aged less than five years old occur in sub-Saharan Africa, and that approximately half of the deaths linked to infectious diseases in sub-Saharan African children point to undernutrition and slowed growth as an underlying cause, the urgent need for further research into the effect of these food contaminants on public health becomes self-evident (Turner, 2013)." In order to better understand the extent of the relationship, a study was conducted in 10 districts of the Nampula province including Angoche, Larde, Malema, Meconta, Mecuburi, Mogovolas, Moma, Monapo, Murrupula, and Rapale.

The aim of this study was to enumerate the serum aflatoxin B1 (AFB1) in children 6-59 months of age, and estimate its association with linear growth in these children. To accomplish this aim, the team conducted a cross-sectional study that focused on understanding the extent and level of aflatoxin exposure as measured through presence in a serum sample from children. We also studied the association between serum aflatoxin and chronic malnutrition (stunting), accounting for other risk factors. The specific objectives of this study include:

1. Assess the mean serum aflatoxin in children 6-23 months and 24-59 months of age
2. Examine differences in serum aflatoxin (mean) by age group
3. Enumerate the association between serum aflatoxin and linear growth adjusting for confounders

Data from the household head, mother or female caregiver, and child were collected by enumerators on key measures of interest, including child and maternal health status, diets, food security, household variables, socio-economic status, agriculture, and food processing practices. Anthropometric measurements were conducted by nutritionists from Universidade Lúrio (UniLúrio). Venous blood samples from children were drawn by phlebotomists from Nampula Central Hospital (HCN) and were analyzed for aflatoxins using validated assays at the University of Georgia (UGA). Data on the child's exposure to aflatoxins was linked to the child's growth status and reported at a level of disaggregation by sex and age group.

The training of the enumerators (41), anthropometrists (6), and phlebotomists (3) took place from the 22nd to the 26th of October 2018 in Nampula City. The training included information on the project, objectives and methodology, ethical aspects, biosecurity, anthropometry, venous blood draw, and training of questions contained in the study instruments. Training was conducted by staff from Tufts University, ANSA, UniLúrio, National Institute of Health (INS), and HCN. Data collection took place from November to December 2018.

Sample size calculations were based on the need to assess the association between children's aflatoxin exposure and height-for-age z-score (HAZ) (Objective 3). Gong et al. (2002 and 2003) have shown a significant correlation between individual AFB1-albumin adduct and HAZ ($p<0.001$) with 480 children 9-60 months (Gong et al., 2003, 2002). In order to see the same magnitude of association in Mozambique, and accounting for attrition of 25% and a design effect of 1.5, 1,800 children were required, with 900 in each age group (6-23 months and 24-59 months). The survey used a stratified, two-stage cluster sampling design with DHS-defined enumeration areas as the primary sampling units, and household as the secondary sampling unit (Feedback. FtF, 2014). We utilized the enumeration areas/clusters of the most recent National Institute of Statistics (INE) census, which was made available by INE.

The study achieved a sample size of 1001 households, short of the required number. The study found a high detection rate with over 90% of the children having detectable levels of aflatoxins. The arithmetic mean ± SE aflatoxin concentration was 7.3 ± 1.2 pg/mg albumin while the geometric mean was 1.4 pg/mg albumin (95% CI: 1.3-1.5 pg/mg albumin). The median was 1.9 pg/mg albumin, and levels ranged from 0.4 pg/mg albumin (not

detected) to 401.7 pg/mg albumin. We found that aflatoxin levels vary by age group with levels being higher in the older children. The mean ± SE AFB1 level of children 6-23 months of age was 0.24 ± 0.04 pg/mg albumin, while mean ± SE of children 24-59 months of age was 0.37 ± 0.05 (p=0.026).

With respect to nutritional status, almost half of the children were stunted, with children 24-59 months of age being 2.1 times more likely to be stunted than children 6-23 months of age (p=0.002), and the mean ± SE HAZ of the younger children was significantly higher than that of the older children (-1.42 ± 0.10 for children 6-23 months and -2.07 ± 0.07 for children 24-59 months). We found high levels of malaria and anemia, with malaria being highly prevalent in children aged 24-59 months and anemia being highly prevalent in children aged 6-23 months. Most of the children did not meet requirements for minimum dietary diversity and less than 30% of female caregivers consumed the minimum five food groups as measured by the Minimum Dietary Diversity for Women (MDD-W). A majority of the households experienced some level of household food insecurity measured using the Household Food Insecurity Access Scale (HFIAS) and reliance on agriculture was predominant with 75% of households involved in agricultural production.

We examined the association of aflatoxin levels and stunting or HAZ using logistic regression and ordinary least square regression models respectively. A significant association was found between stunting and aflatoxin exposure adjusting for child's weight-for-height z-score (WHZ), age, age squared, sex, and detectable AFB1. One unit increase in aflatoxin was associated with a 60% increase in likelihood of being stunted (OR=1.60; p=0.028). The association was unchanged when adjusting for child's anemia status (OR=1.60; p=0.028), which also had a significant association with stunting (OR=1.80; p=0.010). A similar relationship between stunting and AFB1 was found when multivariate analyses only included children with detectable AFB1 levels (OR=1.56; p=0.038). Similar results were found when assessing the association between aflatoxin exposure and HAZ.

As part of secondary analysis, we examined the dietary determinants of aflatoxin exposure as well as agricultural determinants. We found that groundnut consumption was significantly associated with higher levels of aflatoxin, particularly in older children (24-59 months of age). While consumption of maize was associated with aflatoxin levels in younger children, this relationship was not significant when adjusting for WHZ, age of the child, meeting minimum dietary diversity (IYCF-MDD), and detectable aflatoxin. We also found a strong and significant association between aflatoxin and cassava consumption. This latter finding is critical. Studies have found cassava flour to be contaminated with the Aspergillus spp, this coupled with our finding suggests the need for the development and implementation of aflatoxin contamination mitigation during processing of cassava into flour (Cambaza, Koseki, and Kawamura 2018; "Mould and Aflatoxin Contamination of Dried Cassava Chips in Eastern Uganda: Association with Traditional Processing and Storage Practices" n.d.).

With respect to agricultural determinants, we found that maize drying location was significantly associated with aflatoxin: children living in households who dried maize after bringing it from the field had significantly lower levels of aflatoxin compared to those that lived in households that dried their maize in the field only. Models were adjusted for child and household factors, as well as production practices such as crop rotation and intercropping, as these are known to affect quality of the grain. Improved drying practices for maize, including drying grain with fans, on platforms or plastic sheets, and hanging under a roof, were also important, finding that children living in households who used improved practices had lower levels of aflatoxin than households that used unimproved drying practices (unimproved practices include drying only in the field, spreading grain directly on the dirt or floor, and drying on the roof). Similarly, households with improved groundnut drying practices had children with lower levels of aflatoxin.

Our findings are particularly important within the context of Mozambique given the high rates of stunting as well as the high rates of contamination of agricultural commodities with aflatoxin. The 2013 Lancet series on maternal and child nutrition highlighted the importance of focusing research and programming activities on the prevention of stunting during the first 1,000 days of a child's life (Black et al., 2013). The period of pregnancy, birth, and infant

growth up to two years of age is critical to human physical and cognitive development, as well as to the attainment of optimal linear growth and productive life from two years onwards. A series of 10 evidence-based targeted, or nutrition-specific, interventions were proposed in the Lancet series, which could reduce the prevalence of stunting by at least 20% if scaled up to address 90% of need in countries carrying the highest current burdens of undernutrition (Bhutta, 2013). Importantly, the 2013 Lancet series on maternal and child nutrition also underlined that additional complementary actions are needed in the domain of so-called 'nutrition-sensitive' actions, to ensure that stunting can be reduced by *more than* 20%. This brings a renewed focus to investments required in sectors other than health, including agriculture and its associated value chains, not simply to make more food available to consumers but to make that food safer and of higher quality. One of the principal foci of the food safety/quality agenda today is mycotoxin contamination of foods consumed by pregnant women and young children, and its potential links to stunting. Our findings support the need to assess the potential of nutrition sensitive actions such as aflatoxin mitigation through good agricultural and post-harvest storage practices in supporting further reductions in stunting in Mozambican children.

2. Introduction

Tackling the nutritional status of children under five years in Mozambique is particularly crucial, as stunting is still a significant issue in Mozambique. According to the 2011 DHS, the national prevalence of stunting is 43% in children under five years of age, showing little change in stunting prevalence since 1997, with rates of 49% in 1997, 50% in 2001, 47% in 2003, and 44% in 2008 (Health. Mo, Statistics. NIo, & International. I, 2011). Examining the DHS data by age group, we find that stunting is as high as 30% in Mozambican infants under six months of age with an increase to over 50% between 6 and 36 months of age.

Mycotoxins are toxic chemicals produced by fungi, some of which are known to contaminate a wide variety of crops before, during, and after harvest. They are extremely stable and are able to survive in foodstuffs after long periods of storage or after cooking (IFPRI/BMGF, n.d.). Mycotoxins have been found in breast milk, dairy products, and poultry, as well as animal feed. Aflatoxins, the most common type of mycotoxins, are known to cause human liver cancer and to compromise protein metabolism and immunity. There is evidence that demonstrates the association between this form of biological contamination and malnutrition; however, there is very little data within the realm of causality. It is also known that aflatoxin contamination is likely to impair nutrient absorption in the gut, with reductions in the absorptive capacity of several key nutrients including iron and protein (IFPRI/BMGF, n.d.).

3. Aflatoxins in Mozambique

Aflatoxins, a common type of mycotoxin, are ubiquitous in the value chains of key staple crops, such as maize and groundnuts, in many developing countries where agriculture is a significant contributor to Gross Domestic Product (GDP). Agriculture constitutes 24% of the GDP in Mozambique, with 80% of the population dependent on it as a source of income (Probst et al., 2014). Some of the most commonly cultivated crops in Mozambique are easily contaminated by aflatoxins and are widely consumed by the population, including maize, cassava, and groundnuts. Additionally, Mozambique participates in significant formal and informal trade of maize and beans with Malawi, Tanzania, Zambia, and Zimbabwe, which are likely to be contaminated as well.

A review of studies on crop and soil levels of aflatoxins in maize samples across 18 Sub-Saharan African countries (including 42 samples from across Mozambique) has shown *Aspergillus flavus* predominantly present in the samples (Probst et al., 2014). There are clear time points within the agricultural production cycle and the food system where contamination could occur by way of interactions between host plant, fungal population, environment, and farming practices. Starting from pre-harvest through harvest and post-harvest, several different factors could affect levels of the toxin that will ultimately become part of the diet. An assessment of crop and soil levels has been conducted in specific areas of Nampula Province and the mold *Aspergillus flavus* has been documented. Though there is potential variation in crop and soil levels, it is noted that once the mold is found in the soil, it is hard to remove.

During the 2004 aflatoxicosis outbreak in Eastern Kenya, 40 patients who had been hospitalized with jaundice and 80 controls were recruited for a case-control study. Findings from this study showed much higher levels of aflatoxin in both maize and blood samples from the cases than the controls. The geometric mean of contamination in maize present in the case households was much higher than the geometric mean of maize in the control households (354.5 parts per billion (ppb) compared to 44.1 ppb) (Barrett, 2005). Another case-control study examined serum aflatoxin levels in 181 sugarcane workshop employees with occupational airway aflatoxin exposure and 203 controls (Lai et al., 2014). This study found a much higher percentage of cases with detectable serum aflatoxin B1 (AFB1) albumin adducts than controls (56% and 6%, respectively). Within the context of Mozambique, up to the implementation of this study, such data do not exist. A study testing 122 food samples from local markets of Nampula province found high levels of aflatoxin and other mycotoxins, especially in maize and groundnuts (Warth et al., 2012). In 2018 one study reported that out of 57 commercial markets and supermarkets in Maputo, the average concentration of AFB1

in raw peanuts was 2.71 μg/kg (0.00 to 72.93) and the prevalence above the limit of the European Union legislation (8 μg/kg) was 3.5% (Hlashwayo, n.d.).

4. Agriculture and Nutrition Policy and Initiatives in Mozambique

In Mozambique, several policy initiatives target agricultural production and increasing incomes through agriculture. The Action Plan for Reducing Poverty (2011-2014) aimed to reduce food insecurity from 55% to 42% by focusing on increasing agricultural production, promoting employment, as well as human and social development. The Strategic Plan for Agricultural Development (2010-2020), which is fully aligned with the Comprehensive African Agriculture Development Program (CAADP), aimed to increase agricultural production, food security, and incomes of agricultural producers in a competitive and sustainable manner, while promoting social and gender equality. Efforts are also in place to link agricultural growth initiatives to nutrition (USAID, 2011). In addition, the Food and Nutritional Security Strategy 2011-2015 is implemented in parallel to Mozambique's Multi-Sectoral Action Plan for the Reduction of Chronic Malnutrition (PAMRDC) and the Action Plan for Food and Nutrition Security (USAID, 2011). Several donor initiatives, including Feed the Future, are aligned with the Government of Mozambique's (GRM) five-year National Investment Plan for the Agricultural Sector under CAADP and PAMRDC. Feed the Future has two primary objectives in Mozambique. The first is to increase the productivity and competitiveness of Mozambique's agricultural sector, and secondly to improve the nutritional status of pregnant and lactating women and children under five, with a particular emphasis on pregnant women and children under two (known as the "1,000 days" population), in 23 districts across four provinces of Mozambique. Both agriculture and nutrition interventions are being implemented in 10 Feed the Future-designated priority districts in Nampula province (USAID, 2014).

The Feed the Future Initiative expects to have national-level impact through the scaling up of innovative private sector-led models to increase productivity among competitive smallholder farmers, emerging farmers, and agro enterprises. It anticipates increasing technology transfer and smallholder access to improved agricultural inputs, technical assistance, finance, and training. Within the context of nutrition, it aims to develop (where relevant) and/or implement existing best practices for community nutrition interventions that integrate water, sanitation, and hygiene (WASH) approaches. At the policy level, Feed the Future aims to strengthen policy-making capacity, advocate for enabling policies for agricultural productivity, and for development and dissemination of higher-yielding, disease-resistant crop varieties.

Nampula has major health deficits and is the most populated province in the country (USAID, 2011). According to the 2011 DHS, Nampula has the highest rate of stunting (55%) in the country, and approximately 52% of households in Nampula were concentrated in the lowest two nation-wide wealth quintiles. In Mozambique, there is a significant urban/rural divide, with 45% of children in rural areas classified as stunted, while 35% are classified as stunted in urban areas (Health. Mo, Statistics. NIo, & International. I, 2011).

5. Aflatoxins, Linear Growth, and other Environmental Risk Factors

There are only a handful studies that have examined the association of aflatoxins in the blood and linear growth. Aflatoxin levels in mothers' blood during pregnancy are significantly correlated with impaired birth outcomes (Andrews-Trevino et al., 2019; Lauer et al., 2019). This important result derived from birth cohorts, not cross-sectional studies. The negative birth effects were seen on the infants' birth weight, head circumference, being born small-for-gestational age (SGA), and stunting at birth in Uganda, where aflatoxin levels in maternal blood samples were extremely high (Lauer et al., 2019). In Nepal, the effects were mainly a higher risk being born SGA (Andrews-Trevino et al., 2019). A longitudinal study in Gambia also found a significant relationship between maternal aflatoxin exposure during pregnancy and growth faltering during infancy (Turner et al., 2007).

Presence of AFB1 in cooked food samples and high rates of hepatocellular cancer linked to hepatitis B virus have been documented in Inhambane Province, Mozambique in the 1980s (Van Rensburg et al., 1985). More recently, a study conducted in Nampula Province in 2012 used a multi-toxin method covering 247 fungal and bacterial metabolites, and found 63 metabolites in 122 samples of maize, groundnuts, sorghum, millet, rice, wheat, soy, dried fruits, other processed foods and animal feeds (Warth et al., 2012). AFB1 was more frequently found in maize than groundnuts (46% incidence, median=69.9 μg/kg versus 14% incidence, median=3.4 μg/kg). Specific to aflatoxins, International Institute of Tropical Agriculture researchers report that between 2007 and 2009, there were several incidents of rejection of peanuts and peanut kernels by the European Union for exceeding aflatoxin legal limits. Furthermore, a survey supported by USAID/Mozambique in 2013 found aflatoxin hotspots in the northern and central parts of Mozambique (Augusto et al., 2014).

No studies examining the association between aflatoxins, stunting and linear growth specifically in Mozambique were found. Furthermore, there are no data that allow us to estimate the prevalence of aflatoxin exposure in the population of Mozambique, which would provide an estimate of the severity of the situation. For instance, the World Health Organization considers a stunting rate greater than or equal to 40% as very high severity of malnutrition. Given a lack of data, it is impossible to determine a cutoff for aflatoxin level in humans, and particularly in children under two years of age, who are the most vulnerable with respect to stunting.

Emerging evidence around aflatoxins, with both animal and human data, is summarized in a review by Khlangwiset et al. (Khlangwiset et al., 2011). Several studies have shown an inverse relationship between HAZ or weight-for-age z-scores (WAZ) and serum aflatoxin. One study found that children born to mothers with above median maternal aflatoxin-albumin levels were significantly shorter ($p=0.031$) at 52 weeks of age than children born to mothers with below median levels (Turner et al., 2007). Of significance, higher maternal aflatoxin levels were related to significantly lower WAZ and lower HAZ. The analysis noted that a decrease in maternal aflatoxin level of 100pc/mg was linked to an impact at one year of age in the infant of -0.87 WAZ, -800 grams in weight, -1.0 HAZ and -2.0 centimeters in height. Infant blood aflatoxin levels at 16 weeks of age were inversely predictive of HAZ at one year of age.

In contrast to this data from infants, a study of 472 older Gambian children aged six to nine years who had been followed since birth found no relationship between blood aflatoxin levels and nutritional status scores. These children belonged to a cohort of 2047 live births (the authors re-recruited 472 at six to nine years of age and followed them for a 60 day period. The data on the anthropometric indices were collected once during the 60-day period, thus, the analysis is associative and there is no indication that any of these re-recruited children died).

Results showed an association with immune parameters, which indicates a link to increased risk of morbidity in these children due to infectious disease. This suggests that the influence of aflatoxins on growth is most significant during the rapid growth period in utero and in early childhood (Turner et al., 2003). This is biologically plausible, given the far less rapid rate of growth in middle childhood compared to growth rate during the first 1,000 days of life.

Stunting, or poor linear growth, could be affected by factors other than aflatoxin exposure. These include poor hygiene and sanitation practices that could lead to an increased risk of acute infections, or an increased risk of inflammation and environmental enteric dysfunction (EED). Two studies conducted in the Gambia have linked EED to growth faltering. In one study, Lunn et al. (1991) found that infants as young as three to six months of age had increased intestinal permeability, and concluded that EED could potentially explain up to 43% of observed variation in length and 39% of variation in weight gain in Gambian infants two to fifteen months old (Lunn et al., 1991). Another study by Campbell et al. (2003) in the Gambia found that lactulose excretion was inversely correlated with growth, and concluded that intestinal permeability could account for 22% of growth failure (Campbell et al., 2004; Denno et al., 2014). While our study did not examine EED in detail, through comprehensive questionnaires, data were be collected on factors that are likely to contribute to EED, such as poor quality of

drinking water, sanitation, and hygiene practices. These WASH practices will be used as a proxy for EED measures. This is especially important given that 39% of Mozambicans consume non-potable drinking water, with 12% using surface water, 40% practicing open defecation, and 35% using unimproved sanitation facilities (Health. Mo, Statistics. NIo, & International. I, 2011). In addition, poor feeding and caring practices that lead to poor diet quality and low nutrient value of the diet are practiced. These are likely to be important contributors, as only 43% of children under six months in Mozambique are exclusively breastfed, only 13% of 6-23 month old children have a minimum acceptable diet, and only 30% meet the minimum dietary diversity requirement (Health. Mo, Statistics. NIo, & International. I, 2011).

Additionally, high levels of morbidity have been significantly associated with severe stunting. Malaria can confound the relationship of aflatoxin with linear growth, and the prevalence of malaria in Nampula province in children aged 6-59 months is 42% (Health. Mo, Statistics. NIo, & International. I, 2011). Malaria in stunted children is likely to increase inflammation and impair immunity, factors that are likely to confound the relationship of aflatoxins with stunting (Verhoef et al., 2002). An intervention study from western Kenya has shown that malaria prevalence was lower, and heights and weights of children in villages that received bed nets were significantly better than those that did not receive bed nets (ter Kuile et al., 2003).

Another potential confounding illness is HIV, which affected approximately 1.5 million people in Mozambique in 2015. According to the Joint United Nations Programme on HIV/AIDS, 10.5% of adults aged 15 to 49 years in Mozambique were living with HIV in 2015 ("Mozambique | UNAIDS," n.d.). Just as being malnourished can impair the immune system leaving one more vulnerable to HIV transmission, HIV has compromising effects on the immune system that can increase ones vulnerability to malnutrition(Saloojee et al., 2007). In addition to the direct effect HIV has on an individual's nutritional status, results from a study conducted over 18 sub-Saharan African countries (not including Mozambique) showed that children under five were more likely to be stunted if the child's mother had HIV (Magadi, 2011).

6. Project Rationale

In 2012, a research prioritization meeting organized by International Food Policy Research Institute (IFPRI) and Bill and Melinda Gates Foundation (BMGF) on food-borne toxins concluded, "While there is solid association of stunting with exposure to mycotoxins (Turner et al., 2007), the causality has not been proven at a public health scale and the percentage of stunting at a population level that is attributable to mycotoxins in general or to specific mycotoxins is not known particularly in different African countries (Gong et al., 2003, 2002; IFPRI/BMGF, n.d.)." In other words, the extent of the problem, although widely suspected, has been poorly documented and the biological mechanisms thought to be involved remain poorly understood. According to Khlangwiset et al. (2011), "aflatoxin [a type of mycotoxin] exposure and its association with growth impairment in children may contribute to a significant public health burden, especially in less developed countries (Khlangwiset et al., 2011)." Furthermore, Leroy (2013) argues that "while only a small number of observational studies have been carried out, the majority have found strong associations between aflatoxin exposure and stunted fetal, infant, and child growth, thus providing evidence for the first criterion for causality (Leroy, 2013)." No doubt the case for aflatoxin mitigation to target stunting is slowly being made, however there is still lack of data to understand the extent of the problem at the national and sub-national level in most countries in Sub-Saharan Africa and South Asia, the regions of the world with the highest burden of malnutrition in the form of stunting (Black et al., 2013). Moreover, there is very little knowledge of if and how the presence of aflatoxin in the body interacts with other risk factors of stunting, thereby exacerbating the problem. For example, there is evidence from a small (n=400) longitudinal cohort study in Northern Uganda that has shown that human immunodeficiency virus (HIV) positive women who were found to have detectable aflatoxin in the blood during pregnancy had infants with significantly lower height-for-age z-scores (HAZ) at one year of age, compared to HIV negative women with detectable aflatoxin, as well as HIV positive or negative women with no aflatoxin in their bodies (Natamba et al., 2016). Furthermore, while there is literature in

other parts of Sub-Saharan Africa that links aflatoxin levels to child growth, these studies are specific to those agro-ecological contexts, and are not representative of those countries, let alone Mozambique or for that matter, Nampula province. There is sufficient emerging evidence in food and feed data from Warth et al. (2012) to justify the study in Nampula province, Mozambique.

As noted by participants of the IFPRI/BMGF (2012) meeting, "only 35% of stunting of children can be attributed to known factors (IFPRI/BMGF, n.d.)" This leaves room for research to uncover other suspected contributors to this critical, worldwide nutrition problem, and determine the scale of the problem and test innovative interventions to address these suspected contributors. A key question from a public health perspective is "in areas of high prevalence of stunting, does one find a high prevalence of aflatoxin in the human body (particularly that of children under five years of age) and if so, are they interrelated?" Answering this question at a public health level is essential for policy makers and stakeholders to advocate for future investment in aflatoxin mitigation strategies at scale as another measure to tackle stunting.

6.1. Study Aim

The aim of this study is to enumerate the serum aflatoxin in children 6-59 months of age, and estimate its association with linear growth in these children. To accomplish this aim, the team conducted a cross-sectional survey with a sample size of 1,800 children (900 children 6-23 months and 900 children 24-59 months). We utilized a sampling frame and strategy such that the findings are representative of children 6-59 months, children 6-23 months, and children 24-59 months of age in 10 Feed the Future ZOI districts of Nampula province, Mozambique. The study focused on understanding the extent and level of aflatoxin exposure as measured through presence in a serum sample from children. We have also studied the association between serum aflatoxin and chronic malnutrition (stunting), accounting for other risk factors.

6.2. Study Objectives

The objective of this study is to enumerate the mean serum aflatoxin levels, and to examine the association of serum aflatoxin and linear growth within a representative sample of Mozambican children 6-59 months of age from 10 Feed the Future ZOI districts in Nampula province.

Specific objectives include:

1. Assess the mean serum aflatoxin in children 6-23 months and 24-59 months of age
2. Examine differences in serum aflatoxin (mean) by age group
3. Enumerate the association between serum aflatoxin and linear growth adjusting for confounders

To achieve these specific objectives, the Nutrition Innovation Lab worked closely with USAID/Mozambique and stakeholders in the Government of Mozambique. Specifically, Government of Mozambique stakeholders included the Ministry of Agriculture and Food Security, the Technical Secretariat for Nutrition and Food Security (SETSAN), the INE, the INS, and the Department of Nutrition at the National Directorate of Public Health - Ministry of Health (MISAU). Additionally, the Nutrition Innovation Lab partnered with a local university (UniLúrio) and non-governmental organization (NGO; Association for Food and Nutrition Security; ANSA). The Nutrition Innovation Lab worked closely with provincial officials from the Nampula Provincial Directorate of Health and the Nampula Provincial Directorate of Agriculture, as well as the Central Hospital in Nampula (HCN). The Nutrition Innovation Lab worked with UniLúrio, ANSA, INE, and INS to train local staff on human nutrition research methodology and biological sample collection and establishing a cold chain system for transportation and storage of biological samples.

7. Research Methods

7.1. Study Design

Using a cross-sectional design, this study included 1,800 children 6-59 months of age. Data from the household head, mother or female caregiver, and child was collected by local enumerators and nutritionists on key measures of interest, including child and maternal health status, diets, food security, household variables, socio-economic status, agriculture, and food processing practices. Further information on the type of data collected is provided in the data collection section of the protocol.

Using ethically reviewed gold standards to approach and recruit children, venous blood samples from children were drawn by phlebotomists from HCN. Blood samples were processed by a laboratory technician from HCN and stored at HCN until they were shipped to UGA for analysis. Data on the child's exposure to aflatoxins was linked to the child's growth status and reported at a level of disaggregation by sex and age group.

Several molecular biomarkers have been developed that allow for the assessment of aflatoxin exposure using biochemical epidemiology and go beyond the determination of levels in foods and feeds. Biomarkers such as serum aflatoxin are routinely used in epidemiological studies in combination with questionnaires to monitor exposure, as they have the advantage of being directly relevant to human risk (Leong et al., 2012). They bridge the gap between hazard characterization and exposure assessment, particularly in cases where other components in the food matrix or in the environment (e.g. other environmental factors, diseases) could affect the bioavailability and thus the systemic dose of the substance (in this case, aflatoxin). These include AFB1 metabolites in the serum, as well as aflatoxin M1 (AFM1) and AFB1-N7-guanine that are measured in urine (Leong et al., 2012; Wang et al., 2001). We estimated the AFB1-albumin adduct as a measure of aflatoxin instead of urinary AFM1; the serum AFB1-albumin adduct has been reported to better reflect longer term intake and presence of AFB1 in the serum due to a longer half-life of albumin in humans compared to the urinary metabolites (Groopman et al., 1994). Additionally, the serum measure is found to be more stable and has less fluctuations than the urinary metabolites over the same period of time, as observed by Groopman et al. 1993 and Wild et al. 1992 (Groopman et al., 1994; Wild et al., 1992). Thus, it is a more accurate measure of aflatoxin exposure.

7.2. Ethical Review and Protection of Human Subjects

Prior to data collection, the protocol and all study tools were reviewed and approved by the Tufts University Health Sciences Institutional Review Board (IRB#12838), the Institutional Health Bioethics Committee at UniLúrio (Ref. 09/Abril/CIBSUL/18) , and the Mozambican National Bioethics Committee (Ref: 388/CNBS/18). Informed consent procedures were followed as established in the IRB approved protocol, including providing an overview of the study to the parent/guardian of the child and requesting a signed, hard copy of the consent form. The informed consent form consisted of a brief description of the study, risks and benefits to participation, privacy protection, and study personnel contact information. The participants were informed that the child or child's parent/guardian could refuse to participate in the study at any time, and their relationship with UniLúrio, ANSA, or Tufts would not be influenced in any manner. Electronic survey data collection via tablets began upon receipt of informed consent from the parent/guardian of the child. Specific inclusion and exclusion criteria, listed in Table 2, outline clinical exclusion criteria for children, which triggered immediate referral to local health services for appropriate treatment.

Households and children enrolled in the study were given a study ID number, and only de-identified data are available for analysis. Names of study participants were not collected. Only those participants with a signed informed consent form on file are included in the analyses, and all informed consent forms are stored at Tufts University in Boston.

7.3. Sample Size Calculation

Sample size calculations were based on the need to assess the association between children's aflatoxin exposure and HAZ (Objective 3). Gong et al. (2002 and 2003) have shown a significant correlation between individual AFB1-albumin adduct and HAZ ($p<0.001$) with 480 children 9-60 months (Gong et al., 2003, 2002). In order to see the same magnitude of association in Mozambique, and accounting for attrition of 25% and a design effect of 1.5, 1,800 children were required, with 900 in each age group (6-23 months and 24-59 months).

A secondary analysis of interest is to examine the difference in mean serum aflatoxin between children 6-23 months and children 24-59 months (Objective 2). Gong et al. (2002 and 2003) observed a 23.1 pg/mg AFB1-albumin adduct difference between children aged 6-23 months and 24-59 months (Gong et al., 2003, 2002). The sample size of 1,800 calculated to satisfy Objective 2 would have 80% power to detect a 17.5 pg/mg difference in mean aflatoxin level between age groups, with a design effect of 3.38 (from the Feed the Future Mozambique Baseline Report).

A tertiary analysis of interest is to examine the mean serum aflatoxin levels among children 6-23 months and 24-59 months (Objective 1). With the proposed sample size of 900 children in each age group, a standard error of 2.31 could be achieved for the 6-23 month age group, and a standard error of 3.79 for the 24-59 month age group.

The 25% attrition[1] rate accounted for the following criteria:

1. Low quality or incomplete sample collection. Occasionally, the first blood collection is unsuccessful (e.g. difficulty identifying vein, low blood volume, child makes abrupt move and dislodges needle) and can result in insufficient or low quality (e.g. lysed) sample specimens that are unsuitable for analysis.
2. Participant non-response or data collection refusal. This occurs when the parent/consent-giver refuses to participate and/or give a blood sample from the child.
3. Principal Investigator (PI) withdrawal. Involuntary inability of the child to participate, as assessed by the PI. This occurs when the child has a potentially life threatening illness (e.g. severe acute malnutrition (SAM) or severe anemia).
    a. SAM is characterized by low weight-for-height z-score (WHZ; <-3), low mid-upper arm circumference (MUAC; <11.5cm), or bilateral pitting edema (excessive swelling cause by the accumulation of fluid in the body tissues). Children with signs of SAM will be excluded from the study and referred to the designated health clinic.
    b. Severe anemia is defined by low hemoglobin level (<7g/dL). Children with severe anemia will be excluded from the study and referred to the designated health clinic. Anemia will be measured using a HemoCue® Hb 301 system.

7.4. Sampling Strategy and Site Selection

The survey used a stratified, two-stage cluster sampling design with DHS-defined enumeration areas as the primary sampling units, and household as the secondary sampling unit (Feedback. FtF, 2014). As per the 2011 DHS, the enumeration areas are the smallest area unit and consist of 100 to 150 households in urban areas and 80 to 120 households in rural areas (Health. Mo, Statistics. NIo, & International. I, 2011). We utilized the enumeration areas/clusters of the new INE census, which was made available by INE.

[1] The attrition rate of 25% is an overage calculation for the sample size to prevent a loss of sample size that would affect our statistical analysis. This addition accounts for two ways sample size can be affected in a cross-sectional survey: lack of response/non-response or refusal to participate, and an involuntary inability to participate as assessed by the PI, also called the PI withdrawal. Lack of response/non-response or refusal to participate occurs when the caregiver refuses to consent to participate in the survey and/or provide a blood sample. PI withdrawal occurs when the blood sample is low quality or incomplete, or if the child has SAM (indicated by low WHZ, MUAC, or edema) or severe anemia. If the child has SAM or severe anemia, a serum sample may not be taken and the child must be referred to the health authorities for treatment.

The first stage of sampling was to enumerate the INE clusters into rural and urban clusters (Figure 1). The number of clusters from rural and urban strata was determined proportional to the total number of clusters in each stratum (INE-Mozambique, 2010). Utilizing the probability proportional to size (PPS), clusters were randomly selected from the urban and rural strata in each district. The second sampling stage involved randomly selecting 35 households in each cluster. The number of households selected in each cluster was determined from the proportion of households in Mozambique that have at least one child 6-23 months and/or at least one child 24-59 months from the 2011 DHS data.

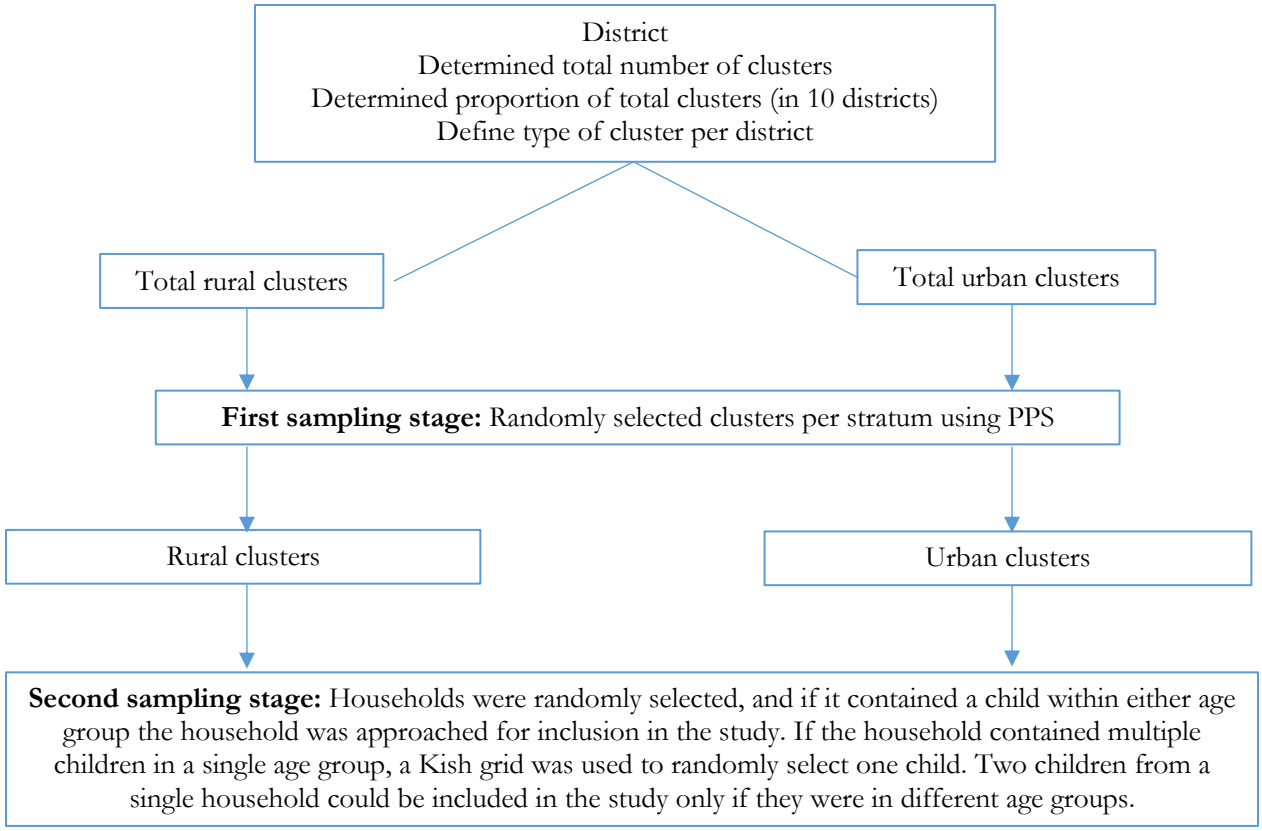

Figure 1. Sampling strategy

The selection of Nampula Province was based on several criteria, including nutritional characteristics, sufficient population to sample from, and accessibility with respect to blood sampling and cold chain management. Table 1 provides demographic details of the province. Ten districts within Nampula Province were selected due to location in the Feed the Future ZOI. The 10 districts that were included in the study are Angoche, Larde, Malema, Meconta, Mecuburi, Mogovolas, Moma, Monapo, Murrupula, and Rapale.

Table 1. Site selection characteristics

	Nampula
Number of selected Districts (Total in province= 19)	10
Moderate stunting in under five (%)	55.3
Severe stunting in under five (%)	30.0
Total population	4,084,700
Female population	2,068,900
Population density (per sq. km)	50.1

Source: DHS Mozambique, 2011

7.5. Subject Characteristics

Households with children 6-59 months of age living in the study area were invited to join the study. Eligible children with informed consent provided by a parent or legal guardian were enrolled in the study; inclusion and exclusion criteria is provided in Table 2.

As noted under exclusion criteria in Table 2, any SAM or severe anemia identified in the child was immediately referred to the health center. Children diagnosed with malaria were also referred to the health center for treatment.

8. Study Plan

8.1. Development and Translation of Tools

The survey tools were developed based on inputs from all collaborators, building on those already approved by the ethical review boards in prior conducted research. The questionnaire modules were originally designed in English and have been translated to Portuguese and Emakhuwa, the most common local language in Nampula Province. The translated versions were utilized on the handheld tablet device. Standard procedures for translation and programming were followed. Paper forms of the questionnaires were also available as back-ups in case there were technical difficulties (e.g. uncharged, mechanical failure) with the tablets. No hard copies of the questionnaire were used to collect survey data.

8.2. Piloting/Pretesting of the Tool

The questionnaires in both paper and electronic format were pre-tested in a non-study district, Ilha de Moçambique, in Nampula Province in August 2018. This activity was used to refine the instrument/tools and help the investigative team understand how respondents interpreted the questions.

8.3. Training and Piloting of Electronically Formatted Tools

The study questionnaires were programmed in electronic form by the Tufts research team, to enable administration of the tool using hand-held devices. After completion of the programming, the tablet-based tools were piloted to test their functionality and revisions to the tablet-based tool were completed prior to data collection. The enumerators were trained specifically on the subject matter of the research, the ethics of human research, and their role in the study by staff from Tufts University, ANSA, UniLúrio, INE, and INS.

The training was first conducted on all of the investigators and co-investigators, as well as the staff and supervisors within the study team, followed by a training of the enumerators and the members of the medical team. Enumerators were trained on using the tools loaded on handheld Android devices in Nampula, as well as on collecting anthropometric data and administering questionnaires. The enumerators and anthropometrists hired for this study were recent graduates of UniLúrio, and the anthropometrists were trained on conducting anthropometry measurements according to the requirements of this study. The phlebotomists and laboratory technician hired for the study were medical professionals from HCN; however, they were trained on conducting capillary pricks, venous blood draws, and laboratory practices for processing the blood samples according to the requirements of this study.

8.4. Data Collection Team

Data collection was conducted by three groups of enumerators and supervisors, reference diagram in Figure 2. Each group was composed of 12 enumerators, three of whom also acted as small team leaders, and one supervisor for the entire group (big team leaders). Each group of enumerators and supervisors traveled to separate clusters to administer the questionnaire. Household and agriculture modules of the questionnaire were conducted in the household, while anthropometry, capillary prick, and venous blood draw were conducted at a designated health center. The data collection team had at least three contacts with each cluster. On the day prior to administering the

questionnaire, the big team leader visited the cluster to make contact with local leaders and encourage households to participate in the study, make themselves available the following day, and to answer any remaining questions. During the interview, enumerators instructed the mother/caregiver and child(ren) to meet at a central location the following day to be transported by bus to the designated health center with the other participants from that cluster for the anthropometry, capillary prick, and venous blood draw modules. After the first two districts, the strategy was modified to bring the mother/caregiver and child(ren) to the health center on the same day as the questionnaire. At the health center, trained Mozambican anthropometrists conducted all anthropometry measurements and trained Mozambican phlebotomists/nurses hired for the study conducted the capillary prick and blood draw. Small team leaders and enumerators held daily meetings with the big team leader to discuss any problems encountered in the field. The study coordinator and investigators made regular visits to field sites and had frequent contact with field supervisors by phone and email to discuss weekly progress and plans. The study coordinator and investigators from ANSA, UniLúrio, INS, and Tufts University held necessary meetings on study progress.

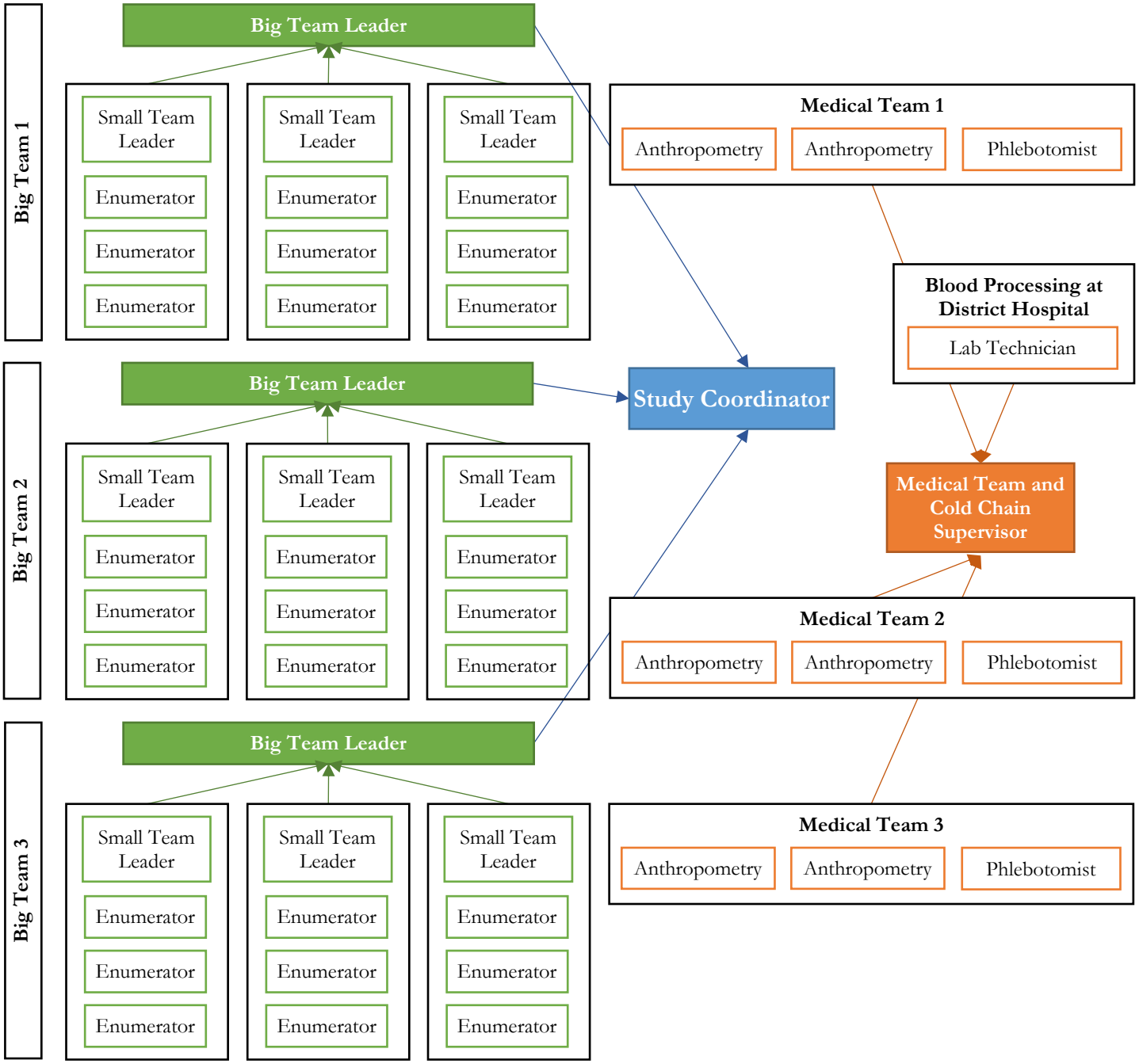

Figure 2. Data collection team structure

8.5. Province/District/Community Entry and Mobilization

On October 22, 2018, a half-day long launch event was held to commemorate the beginning of the study and to introduce local leaders to the study objectives and study team. Government representatives from the 10 study districts attended the event, as well as study partners and the data collection team. Presentations were given on an overview of aflatoxin and the state of malnutrition in Nampula, a description of the study design, and the plan for data collection. Ample time was provided for the local leaders to ask questions in order to gain a better understanding of the study.

This community-based study depended heavily on the active support and participation of all levels of the government, especially the local communities. Entry and mobilization required considerable attention to ensure mothers/caregivers felt comfortable participating in all aspects of the study, including the venous blood draw from their child(ren). Study personnel initiated engagement with provincial and district level officials and other government outreach workers (e.g. agricultural extension workers) through individual one-on-one meetings and small, targeted meetings to describe the study, its potential benefits and risks, and the types of support needed from various government actors. This mobilization team moved ahead of the data collection team so that communities were aware of the study and prepared to engage with the data collection team. With their support, study personnel approached local leaders, health facility staff, Agentes Polivalentes Elementares (APEs), and Community Health Workers (CHWs).[2] Through a series of individual and small group meetings, the mobilization team explained the study and requested the participation of the local agents and the communities.

8.6. Inclusion and Exclusion Criteria

Households with at least one child 6-59 months old was eligible to participate in the study. Households were requested to enroll the selected child(ren) in the study after confirming informed consent and meeting the inclusion and exclusion criteria as outlined in Table 2. A maximum of two children in each household were allowed to participate in the study: if the household contained multiple children in a single age group, one child was randomly selected. Two children from a single household were included in the study only if they were in different age groups.

Table 2. Example of sample inclusion/exclusion criteria and definitions

Type	Criteria and definitions
Inclusion into study	**Child**
	6-59 months old
	Household
	Head of household/caregiver provides informed consent for the himself/herself, the mother or caregiver of the child, and the child
Exclusion and immediate referral	**Child**
	Severely malnourished: WHZ<-3, MUAC<11.5cm, or bilateral pitting edema
	Severe anemia: Hb<7g/dL
Drop out	**Household or child**
	Refusal: participant indicates lack of interest and refuses to participate in the survey
	Withdrawal: participant completes a portion of the interview
	Loss to follow-up: participant completes the interview but does not attend the appointment at the health clinic for anthropometry measurements, finger prick, or blood draw

[2] CHWs are trained through the APE program, launched by the MISAU in 1978. APEs are responsible for disseminating information regarding general health promotion, including maternal care, nutrition, and childhood diseases (Advancing Partners & Communities, 2013).

8.7. Identification and Recruitment

Households were randomly selected from the 2017 INE census, which includes GPS coordinates of the household. With the assistance of INE, GPS locations of selected households were programmed onto a tablet-based application. Enumerators used the application and APEs/local guides to locate selected households. Mothers or female caregivers of children 6-59 months of selected households were approached for participation at their homes (accompanied by APEs/local guides). Information was provided to them and time was given in order for them to consult with their husband and/or other family members (e.g. mothers-in-law). Given the cultural context where women may require permission from their households to participate, the study team ensured that all steps are taken to ensure that the mother made an informed decision in collaboration with her household. The interviews took place at the household, while the anthropometry, finger prick, and blood draw occurred at a designated health facility.

During recruitment, the selected child's (or children's) MUAC was used as a screening criterion, and any child with a MUAC measurement less than 11.5cm was excluded from the study. Children that were identified with SAM were invited to go to the health clinic with the study participants to receive the appropriate treatment. Weight, height, MUAC, and hemoglobin were measured at the health clinic prior to the blood draw, allowing for children who were found to have SAM or severe anemia to be excluded from the blood draw and referred for treatment. The MUAC measurement taken in the household was for screening purposes only and was not entered as study data. Only the measurements taken in the health clinic were recorded as study data.

8.8. Drop Out of Subjects

Recruited subjects were able to opt out at any time and/or refuse to answer any specific questions/modules that they did not want to answer. Reference Table 2 for the definitions of types of drop out. All data collected were analyzed with "intent to treat", thus if subjects refuse to answer any part of the survey, their data is still included in the study. A complete withdraw or drop out was based on a participant/subject's request to be removed from the study.

9. Data Collection, Management, Calculation, and Analysis

Data collection was conducted over six weeks, from November 1 to December 15, 2018, following the launch event and week of training. Training included information on the project, objectives and methodology, ethical aspects, biosecurity, anthropometry, venous blood draw, and training of questions contained in the study instruments. Three sets of trainings were conducted simultaneously, with separate training for the survey enumerators, anthropometrists, and phlebotomists. Training was conducted by staff from Tufts University, ANSA, UniLúrio, INS, INE, and HCN.

9.1. Interviews

All interviews were conducted at one time. The questionnaires were administered to respondents designated as eligible for each module. The modules were separated by household head and mother/female caregiver, and interviews were conducted simultaneously if possible. However, in some households the mother/caregiver was the head of the household and therefore was the respondent for both household and caregiver's modules. Two members of the study team visited each household, one to conduct the head of household interview and the other to conduct the mother/female caregiver interview. This allowed the interviews to be completed within 1 to 2 hours. Conducting modules simultaneously with two household members reduced the respondent burden and optimized the data collection process. See Table 3 for a description of the modules conducted, eligible respondent, and estimated survey duration.

Table 3. Survey modules, eligible respondent, and estimated duration

Type of module	Eligible respondent	Estimated duration	Topics covered
Household questionnaire			
Household module	Head of the household, if available	1-2 hours	• Socio-economic indicators • Current agricultural production conditions • Knowledge, attitude, and practices (KAP) related to aflatoxin
Caregiver's module	Mother or primary caregiver of child	2 hours	• Household characteristics • Caregiver and child food consumption • Household food security • Gender and decision making • Pregnancy and birth history • Child morbidity • Household WASH practices • Knowledge, attitude, and practices (KAP) related to nutrition • Breastfeeding and complementary feeding practices • Knowledge, attitude, and practices (KAP) related to aflatoxin
Anthropometry measurements and blood draw			
Biomarker module	Child	30 minutes	• Anemia test (HemoCue®) • Malaria test • Aflatoxin test
Anthropometry module	Child and mother of child	30 minutes	• Height/length, weight, mid-upper arm circumference, knee-heel length, and head circumference of child • Height, weight, and mid-upper arm circumference of mother

The study team administered specific questionnaires related to household-level socio-economic and demographic characteristics, food security, and food supply, including agricultural and post-harvest processing and practices. At the individual level, questionnaires regarding the intake and diet diversity of the child and caregiver, breastfeeding, duration and type of complementary feeding, as well as morbidity status of the child were administered.

9.2. Biomarker and Anthropometry Measurements

During the initial interviews in the first two districts, mothers/caregivers and children were instructed to meet at a central location on the following day in order to be taken to the designated health clinic for biomarker and anthropometry measurements. Due to higher than expected loss to follow-up, the study team modified the strategy and brought mothers/caregivers and children to the health center directly following completion of the household questionnaire. Anthropometry of the mother and child were collected, including the weight, height/length, and MUAC of both individuals, as well as the knee-heel length and head circumference of the child. Anthropometry measurements of the child's caregiver were not taken if the caregiver was not the child's biological mother.

All children had their hemoglobin measurements taken using the HemoCue® analyzer during the appointment. The HemoCue® uses a standard protocol: the nurse/enumerator cleaned the area to be lanced (finger or heel depending

on the age of the child) with an alcohol swab, pierced with a sterile lancet and used the microcuvette to collect the blood and read using a HemoCue® 301 analyzer. Any participant found to have severe anemia was referred to the appropriate personnel in the health clinic for treatment.

All children were also tested for malaria. Using blood from the same capillary prick used for the HemoCue® analysis, a rapid diagnostic test was conducted. The malaria test was used, in part, to distinguish the cause of a child's fever, as malaria and other respiratory infections can cause fever. Any participant found to have malaria was referred to the appropriate personnel in the health clinic for treatment.

Since the anthropometry measurements, hemoglobin, and malaria tests were conducted in the health clinic, a referral system was established between the study and the health clinic. By this referral system, children found to have severe acute malnutrition, severe anemia, or malaria received a referral slip that the health clinic accepted, for which children were given the appropriate treatment at the health clinic.

Anthropometry measurements and the finger prick were conducted prior to the blood draw, therefore any child found to have severe acute malnutrition or severe anemia were not included in the blood draw. A 1-3 mL blood sample was collected from the child in pre-coded and de-identified vials. A minimum of 1 mL and maximum of 3 mL sample was collected from the child in accordance with recommendations by the Tufts University Human Subjects research ethics committee, which states that the amount drawn may not exceed 3 mL per kg in an eight-week period, and collection may not occur more frequently than two times per week.

9.3. Conducting Blood Draws

The blood draws on children were taken in local health facilities by well-trained phlebotomists from HCN, using new materials for each attempt. To minimize any risk of adverse events that might take place, proper techniques, procedure, and equipment were used. Specific to children, a maximum of two attempts on different areas were made to draw blood. For children 6-23 months, blood draw attempts were made on the dorsal hand vein. If the first hand failed, an attempt on the other hand was made. For children 24-59 months, blood draw attempts were made on the antecubital fossa vein. If the first side failed, a second attempt on the other side was made. All blood samples collected were sufficient in quantity and were made on the first attempt.

9.4. Handling, Transportation, Processing, and Storage of Blood Samples

Blood samples were handled in a manner that conforms to clinical standards. Coded and de-identified blood samples were stored in a portable refrigerator immediately after the blood draw was taken. Once all blood samples were taken in the cluster, they were transported to a district hospital where the technician centrifuged all blood samples collected by the three teams. A sufficient amount of serum was transferred to a pre-labeled cryovial for analysis, with the remaining serum transferred to a second pre-labeled cryovial for backup. Serum samples were stored in a portable refrigerator until the end of the day, when the technician transferred all serum samples to a -20°C freezer. After all sample collection in the district was complete, the technician transferred all serum samples to HCN, where he placed them into the -80°C freezer. Samples were kept in a robust cooler during transport.

9.5. Transportation of Serum Samples for AFBI Analysis

Once all samples were collected, samples designated for analysis were shipped on dry ice to the University of Georgia (UGA) for AFB1 analysis. Sample transfer to the United States was conducted by Biocair, Inc., and followed all appropriate protocols, including filing of paperwork in Mozambique and the US. A materials transfer agreement was signed between HCN and UniLúrio as the providers of the materials with UGA as the recipient of the materials. No samples were lost during transport and the integrity of all samples was maintained. The backup samples are stored at HCN.

9.6. Analysis of Serum Samples

Serum samples were analyzed for aflatoxin by skilled laboratory technicians in the Peanut and Mycotoxin Innovation Lab at UGA. The AFB1-albumin adduct levels were analyzed using validated methods accepted by the Centers for Disease Control and Prevention. A high performance liquid chromatography method using fluorescence detection was used, resulting in the quantification of AFB1-lysine adducts in the child's blood, which reflects aflatoxin exposure in the past 2 to 3 months. Results are presented as picogram of AFB1-lysine adducts/mg albumin, allowing the results to be comparable to recent human aflatoxin studies.

9.7. Data Entry and Management

Survey data were collected electronically using the Qualtrics platform on Android tablets. Data were stored on the tablet and transferred daily to a secure central database by wireless technology. All supervisors, enumerators, anthropometrists, and phlebotomists were trained on Qualtrics, the use of the Android tablet and in uploading data. As a data check, the research manager downloaded data from the database and reviewed for errors on a daily basis. The electronic questionnaires were programmed as to prevent any errors (pre-coded), as well as utilizing checks that did not allow enumerators to move from one screen to another without inputting responses. Any issues raised from data collection was be brought to the immediate attention of the investigators and followed up with the supervisors. Any missing data required the data collector to return to the household to re-obtain the data. The Nutrition Innovation Lab staff conducted random checks on data quality. Assessments on anthropometric techniques as well as interview techniques were conducted, and refresher training was provided by the local study team as necessary. Aflatoxin data were obtained post analyses and merged with the electronic data generated on all other measurements.

Data management was conducted by INS and an independent contractor, and included downloading the raw data, conducting a daily review of raw data. Any identified issues were brought to the attention of the study coordinators and field supervisors and were also sent to individual supervisors for resolution in the field. Additionally, data cleaning was conducted by the Tufts team following the completion of data collection. This included reviewing and organizing raw data, creating derived variables (inclusive of calculating indices and other derivations, aggregating, and disaggregating raw variables), checking variable names, checking labels, and generating a codebook.

9.8. Variable Generation

The primary outcome measures were HAZ and stunting. Length or height measurements were converted to HAZ, according to WHO child growth standards using the "igrowup" Stata macro. Stunted children were defined as those having an HAZ<-2. Child's weight was also converted to WAZ using the "igrowup" macro and was used to create a binary variable for underweight status. Children with WAZ<-2 were defined as underweight (*unicef-drp/igrowup_update*, 2020).

Due to the typical skewed distribution of AFB1-lysine adduct data, AFB1 levels were transformed using the natural log prior to all analyses. Children with AFB1-lysine adduct level below the lower limit of detection of 0.4 pg/mg albumin were assigned a value of 0.2 and a dummy variable was also created indicating if the child had a detectable AFB1 level. A complex relationship between AFB1, age, weight, and HAZ was observed during analysis, finding that the AFB1-HAZ relationship changed based on the child's age and weight. Figure 3 describes these different relationships in the whole study sample (6-59 months), showing that study data predict a negative relationship between HAZ and AFB1 in children with low weight, no relationship in children with average weight, and a positive relationship in children with high weight. Furthermore, different relationships are observed when the predictions are conducted at specific ages, as shown in Figure 4. To account for this, a weight-adjusted AFB1 variable was constructed by dividing the AFB1-lysine level by body weight in kg and then transforming using the natural log.

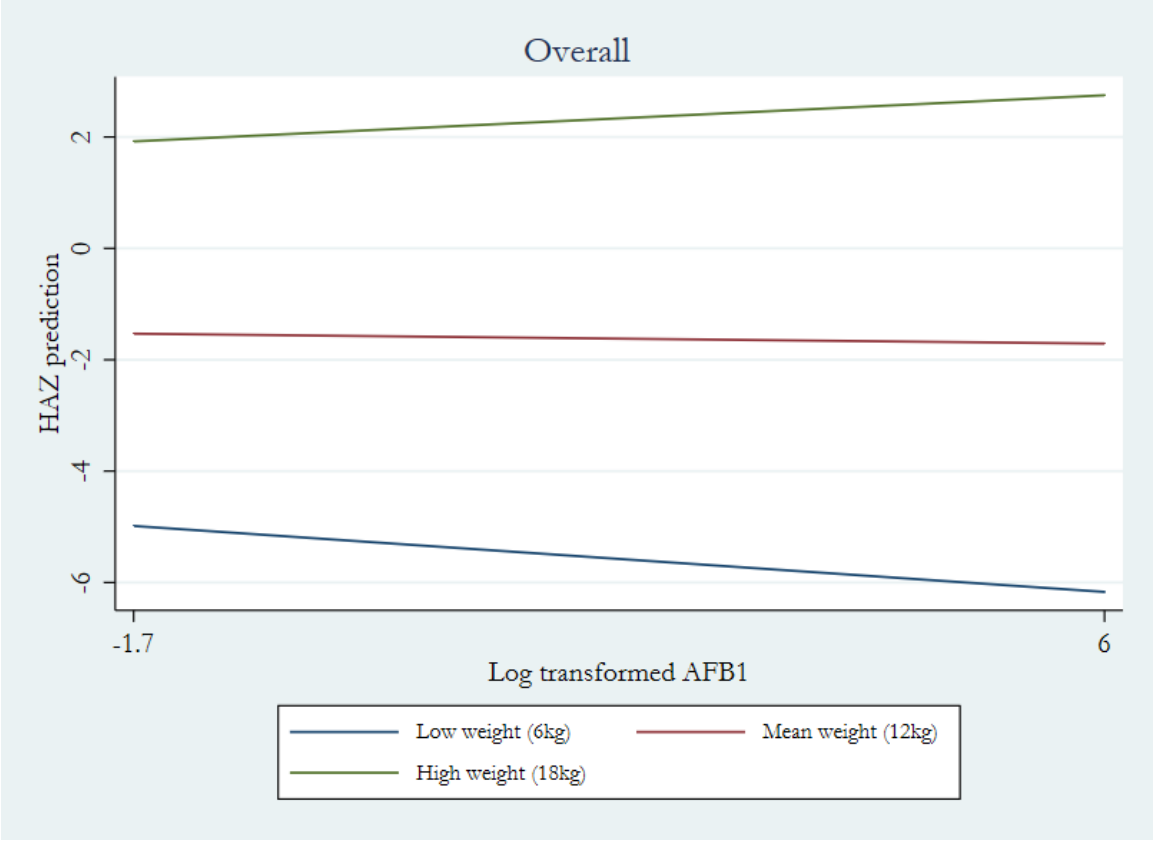

Figure 3. Predicted relationship between HAZ and AFB1 at varying child weights

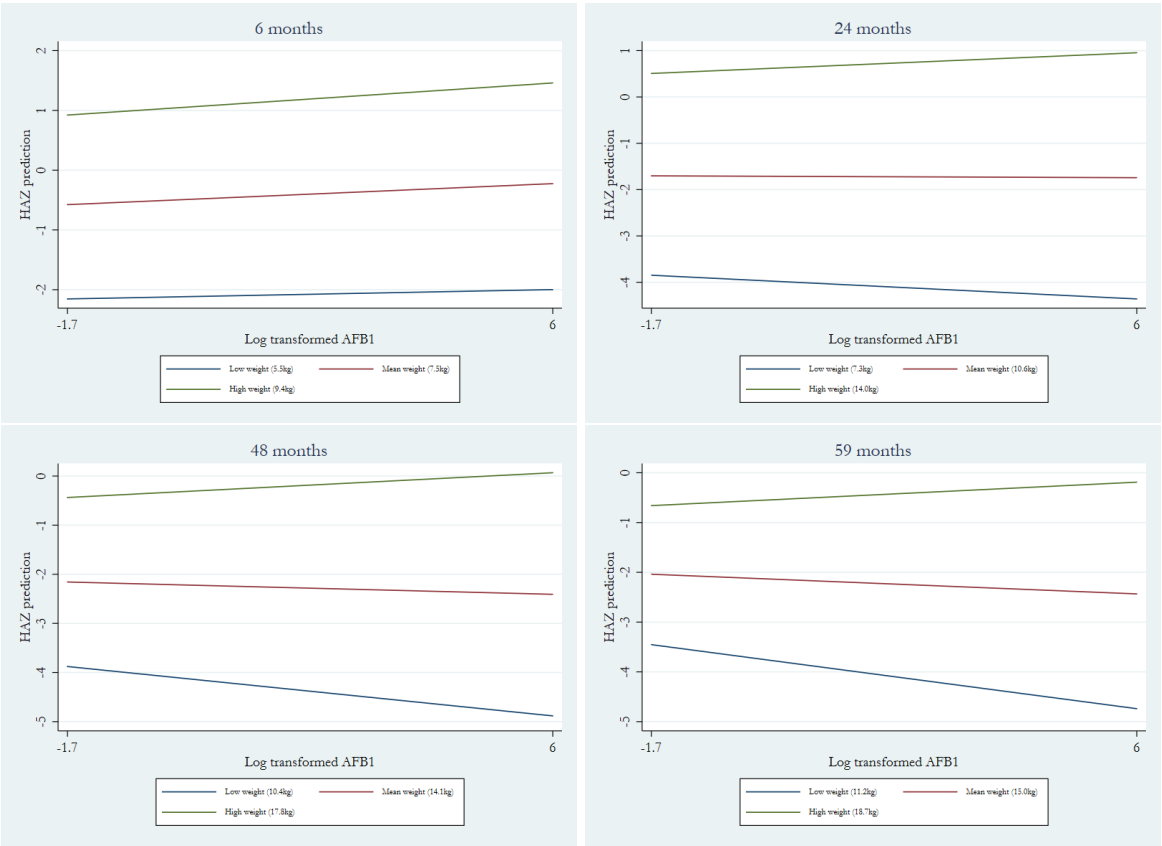

Figure 4. Predicted relationship between HAZ and AFB1 at varying child weights and ages

Anemia status was determined from the hemoglobin concentration in the capillary prick, measured by the HemoCue® Hb 301 system. In alignment with WHO standards for anemia status in children 6-59 months of age, mild anemia was defined as Hb 10.0-10.9g/dL, moderate anemia as Hb 7.0-9.9, and severe anemia as Hb less than 7.0g/dL ("haemoglobin.pdf," n.d.). The Malaria P.f/Pan Antigen rapid diagnostic test from Cypress Diagnostics was used to assess children's malaria status. This test detects *Plasmodium falciparum* antigen and a lactate dehydrogenase antigen that is common to all four species of malaria. This immunochromatographic test produced positive or negative results based on indicator lines on the device, which were used for the binary malaria variable ("359," n.d.).

Data from the 24-hour diet recall were used to create the child's and female caregiver's dietary diversity indicator. According to the UNICEF IYCF-MDD indicator, children who consumed 4 or more of the 7 food groups were considered to have met the minimum dietary diversity requirement (World Health Organization (WHO), 2008). The 7 food groups used in this indicator were: grains, roots, and tubers; legumes and nuts; dairy products; meat, fish, poultry, and organ meats; eggs; vitamin A-rich fruits and vegetables; and other fruits and vegetables. According to the FAO MDD-W indicator, female caregivers who consumed 5 or more of the 10 food groups were considered to have met the minimum dietary diversity requirement ("Minimum Dietary Diversity for Women- A Guide to Measurement," n.d.). The 10 food groups used in the MDD-W indicate were: grains, white roots and tubers, and plantains; beans, peas, and lentils; nuts and seeds; dairy; meat, fish, and poultry; eggs; dark green leafy vegetables; other vitamin A-rich fruits and vegetables; other vegetables; and other fruits.

Consumption of foods prone to aflatoxin contamination was also examined, including maize, groundnuts, and cassava consumption in the past 7 days and 24 hours.

Household-level indicators focused on food security and agriculture production and practices. The validated Household Food Insecurity Access Scale questionnaire was used to measure and categorize the level of each household's food insecurity (Coates et al., 2007). Household's engagement with production of maize and groundnuts in the past 12 months was gathered. Among households producing maize or groundnuts, post-harvest production practices were evaluated, including drying method and location. Drying location focused on drying the grain only in the field compared to drying it in the field and after bringing it in from the field or only after bringing it in from the field. The two groups of households who dried grain after bringing in from the field (in addition to drying in the field or only drying out of the field) were combined, creating a binary indicator for grain drying location. Options for drying method were categorized into improved and unimproved groups. Improved methods included drying grain with fans, on platforms or plastic sheets, hung under the roof or hung in the kitchen. Unimproved methods put grain at a higher risk for moisture, mold, and aflatoxin growth due to greater exposure to weather and higher labor requirements to prevent such exposure ("i2433e10.pdf," n.d.). These methods included only drying the grain in the field, spreading directly on the dirt, spreading directly on a cement or brick floor, or spreading on the roof.

9.9. Statistical Methods

Descriptive statistics, including mean ± standard errors (SE), frequency distributions, and proportions were used to describe demographic, health, diet, and nutritional characteristics. The arithmetic mean (basic average) and geometric mean (calculated by multiplying all values and taking the nth root) of AFB1-lysine results were calculated; the geometric mean was calculated due to the non-normal distribution of AFB1 data. Bivariate analyses were used to assess AFB1 levels across variables of interest, and multivariate analyses were used to assess the relationship between HAZ and AFB1, stunting and AFB1, AFB1 and consumption of aflatoxin contamination-prone foods, as well as AFB1 and post-harvest practices for aflatoxin contamination-prone crops. All descriptive statistics, bivariate analyses, and multivariate analyses were adjusted for survey design, including clustering and sample weights for the different age groups of children. Results are representative of children 6-59 months of age in the 10 Feed the Future Zone of Influence districts of Nampula province.

The relationship between HAZ and AFB1 was evaluated using unadjusted and adjusted OLS regression models, and the relationship between stunting and AFB1 was evaluated using unadjusted and adjusted logistic regression models. Models were adjusted for factors that are known to affect HAZ or stunting, including child's age and sex. As mentioned previously, the relationship between HAZ and AFB1 was found to differ based on the child's age and weight, therefore weight adjusted AFB1 was used in the models. WHZ was also included in the model to account for this adjustment of AFB1 by weight. The dummy variable indicating children with AFB1-lysine level below the lower limit of detection was included to account for the constant value assigned to those children. Finally, binary anemia status was included in the model to account for its effect on malnutrition and linear growth. Models including malaria status (in place of anemia) were also run, producing similar results to the models with anemia. Because of the similarities between the models, only models including anemia are reported in Section 10.

OLS regressions were used to examine associations of AFB1 and dietary consumption in the past 24 hours. Models focused on the consumption of maize, groundnuts, and cassava, as these foods are commonly contaminated with AFB1. Unadjusted and adjusted models were run including all children, children 6-23 months of age, and children 24-59 months of age, due to dietary difference in the different age groups. ABF1 standardized by body weight was used as the dependent variable, with a binary consumption variable as the main outcome of interest. Models were adjusted for WHZ, child's age, AFB1 detectable level dummy, and a binary indicator for meeting the IYCF-MDD to adjust for overall diet quality.

OLS regressions were used to assess agricultural determinants of aflatoxin exposure, including drying location, drying technique, and storage practices for maize and groundnuts. Storage practices examined include location (e.g. granary, house, etc.), method (i.e. in sacks/container directly on the floor, hanging, in sacks raised from the floor), and the state of the grain (i.e. in husk or shell, on cob but not in husk (maize only), as loose grain). No significant relationship was found between any storage practice and aflatoxin exposure for maize or groundnuts. The relationship of AFB1 and two key drying aspects were assessed for maize and groundnuts: drying location (drying the grain only in the field or drying after bringing in from the field) and overall drying practice (unimproved vs. improved drying techniques). Unadjusted and adjusted models were conducted, adjusting for child's age and household head's education level in all adjusted models. Production practices of crop rotation and intercropping were included in adjusted models, as they are known to affect the quality of the grain.

All analyses were conducted using Stata version 15.1 software.

10. Results

10.1. Enrollment Results

In total, 1,001 households participated in the study, with 487 children 6-23 months of age and 767 children 24-59 months of age enrolled, See Table 4. After attrition and exclusion from the blood draw, the complete study sample size was 749 households, of which 310 were children 6-23 months of age and 584 were children 24-59 months of age.

Table 4. Household and Child Enrollment and Participation

	Households	Children 6-23 months	Children 24-59 months
Approached	2,626		
Refused	198		
Not home	135		
No child 6-59 months of age	1,167		
Excluded from enrollment (MUAC<11.5cm)	13	8	5
Enrolled	**1,001**	**487**	**767**
Loss to follow-up (participated in household survey but did not come to health center)	148	69	109
Excluded from blood draw (WHZ<-3)	3	3	0
Excluded from blood draw (Hb<7.0 g/dL)	83	62	24
Refused blood draw	51	32	25
Complete data (household survey, anthropometry, and blood draw)	**749**	**310**	**584**

10.2. Aflatoxin Levels

Nearly 90% of children had detectable AFB1 level. The arithmetic mean ± SE aflatoxin concentration was 7.3 ± 1.2 pg/mg albumin while the geometric mean was 1.4 pg/mg albumin (95% CI: 1.3-1.5 pg/mg albumin). The median was 1.9 pg/mg albumin, and levels ranged from 0.4 pg/mg albumin (not detected) to 401.7 pg/mg albumin. Due to the skewed nature of AFB1 data (see Figure 5), log-transformed concentrations will be used and any reference to AFB1 levels from here on is log-transformed AFB1.

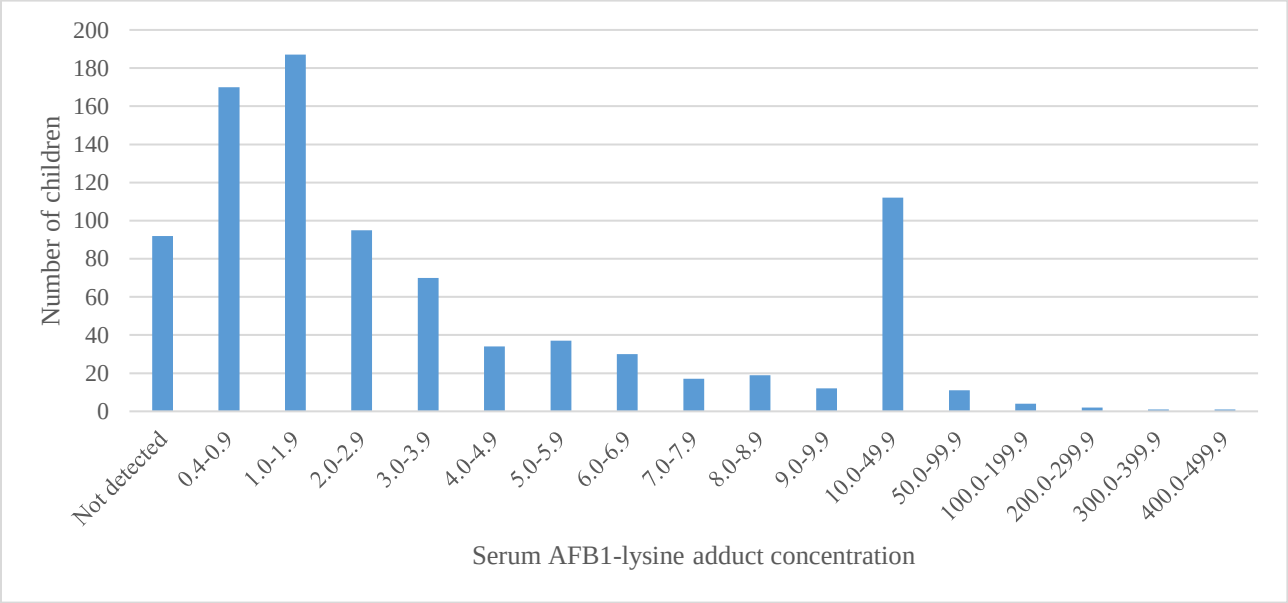

Figure 5. Distribution of serum AFB1-lysine adduct concentrations

AFB1 levels varied significantly by age group, see Figure 6. The mean ± SE AFB1 level of children 6-23 months of age was 0.24 ± 0.04 pg/mg albumin, while mean ± SE of children 24-59 months of age was 0.37 ± 0.05 (p=0.026).

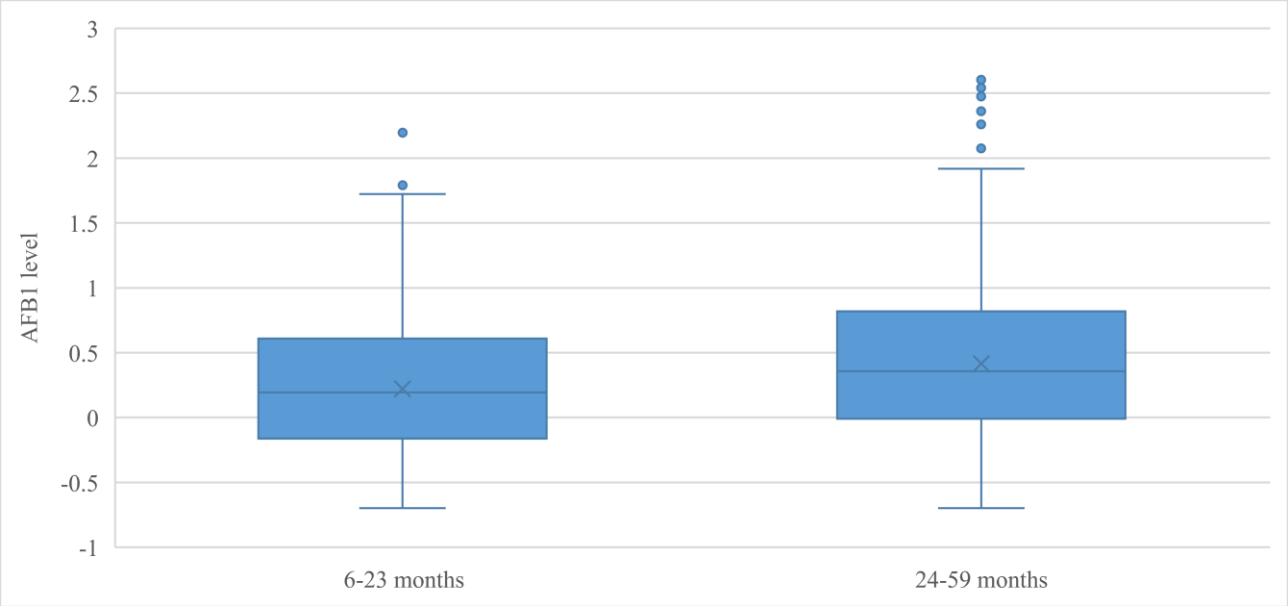

Figure 6. AFB1 levels by age group

10.3. HAZ and Stunting

Almost half of the children were stunted (45% of children; HAZ<-2), with a mean ± SE HAZ of -1.9 ± 0.06. The median HAZ was -1.9 and values ranged from -5.8 to 3.7. Children 24-59 months of age were 2.1 times more likely to be stunted than children 6-23 months of age (p=0.002), and the mean ± SE HAZ of the younger children was significantly higher than that of the older children (-1.42 ± 0.10 for children 6-23 months and -2.07 ± 0.07 for children 24-59 months). Figure 7 shows the distribution of HAZ for each age group, showing the shift in curves between children 6-23 months and children 24-59 months.

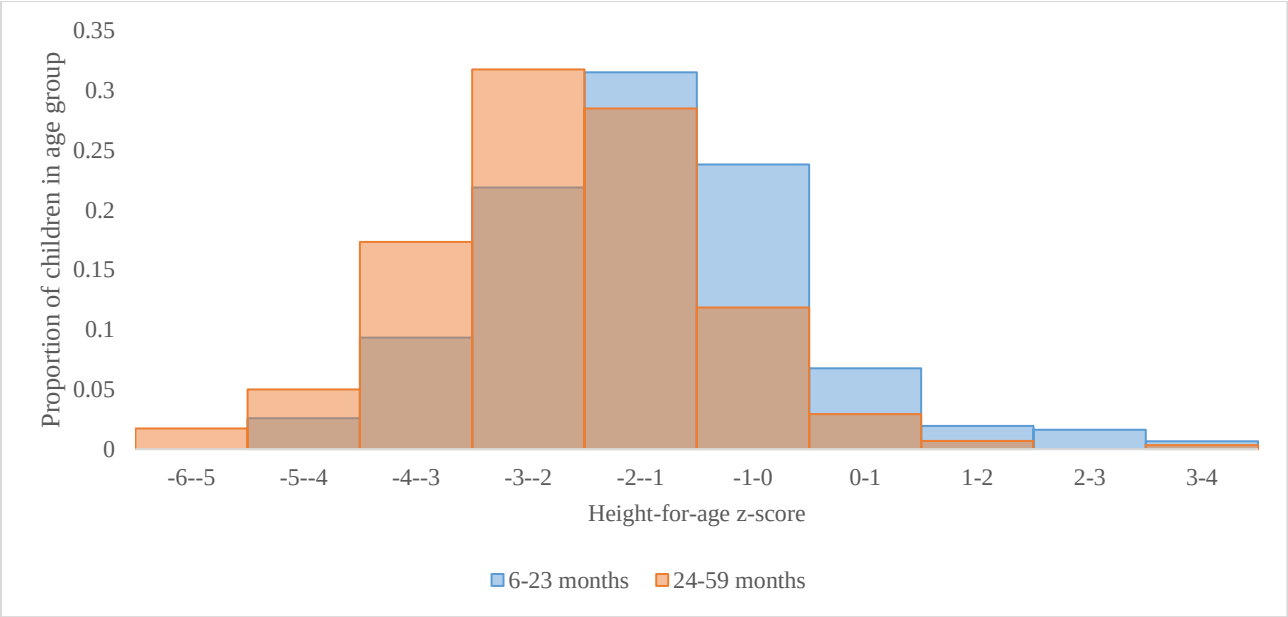

Figure 7. Distribution of HAZ by age group

10.4.Child and Household Descriptive Characteristics

Many child-level and household-level characteristics were examined to provide an overall picture of the study population and to determine if they might be a confounder in the association between aflatoxin and HAZ or stunting. Child-level anthropometric indicators of stunting and underweight, biological indicators of anemia and malaria, along with dietary diversity and consumption of aflatoxin-prone food groups were examined. Household characteristics of food security and agriculture production and practices, as well as caregiver characteristics of education and dietary diversity were examined.

The study found similar levels of stunting compared to recent DHS reports, see Figure 8, with lower rates among children in the younger age group (6-23 months of age).

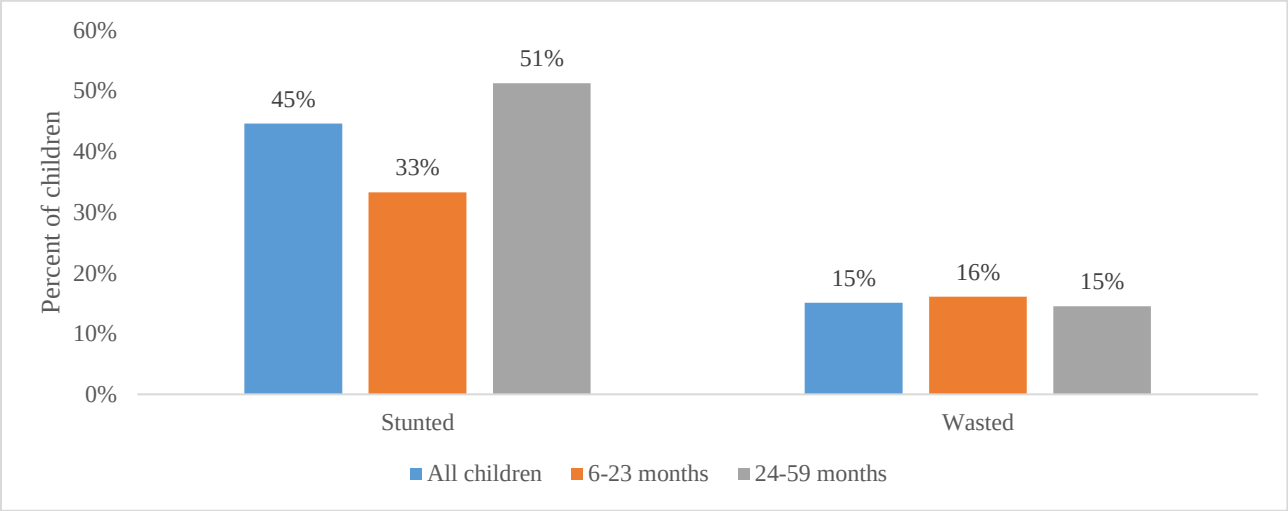

Figure 8. Anthropometric indicators by age group

High levels of malaria and anemia were found, especially malaria in the 24-59 month old children and anemia in the 6-23 month old children, see Figure 9.

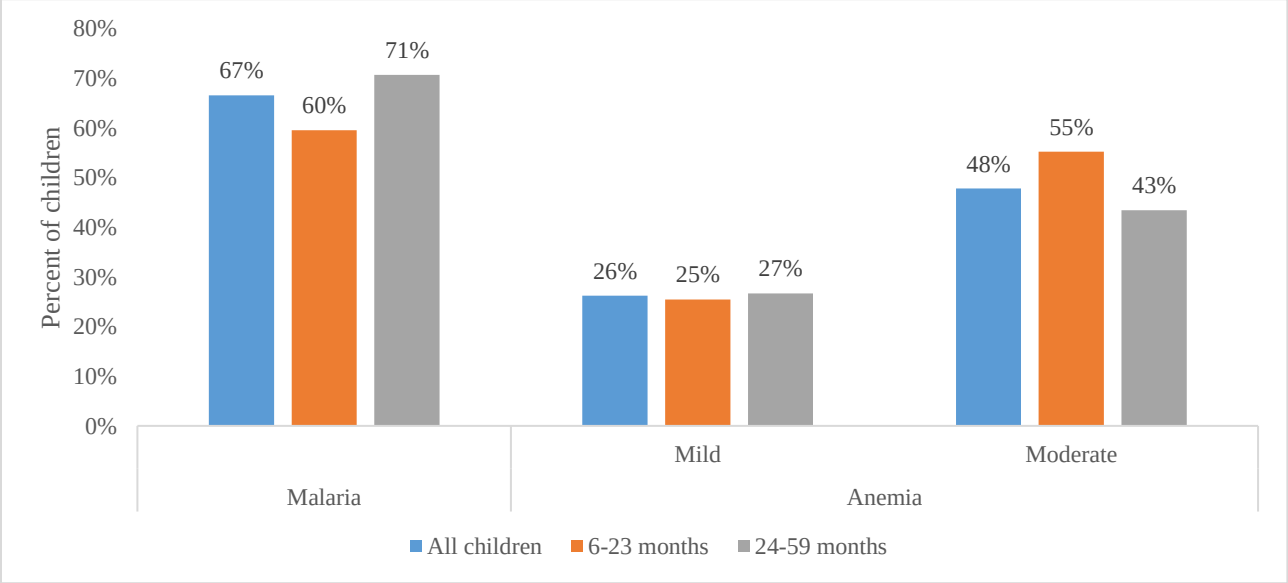

Figure 9. Biological indicators by age group

Less than half of children met the Infant and Young Child Feeding Minimum Dietary Diversity (IYCF-MDD) threshold of consuming four or more food groups from the following seven groups: grains, roots, and tubers;

legumes and nuts; dairy products (milk, yogurt, and cheese); meat, fish, poultry, and organ meats; eggs; vitamin A-rich fruits and vegetables; and other fruits and vegetables, in the past 24 hours. Less than 30% of female caregivers met the Minimum Dietary Diversity for Women (MDD-W) threshold of consuming five or more food groups from the following ten groups: grains, roots, and tubers; pulses; nuts and seeds; dairy; meat, poultry, and fish; eggs; dark green leafy vegetables; other vitamin A-rich fruits and vegetables; other vegetables; and other fruits, in the past 24 hours.

Unsurprisingly, most participants consumed cassava in the past 24 hours, especially children 24-59 months of age and female caregivers, see Figure 10. About 35% of children and caregivers consumed maize, and only a quarter of consumed groundnuts in the past 24 hours.

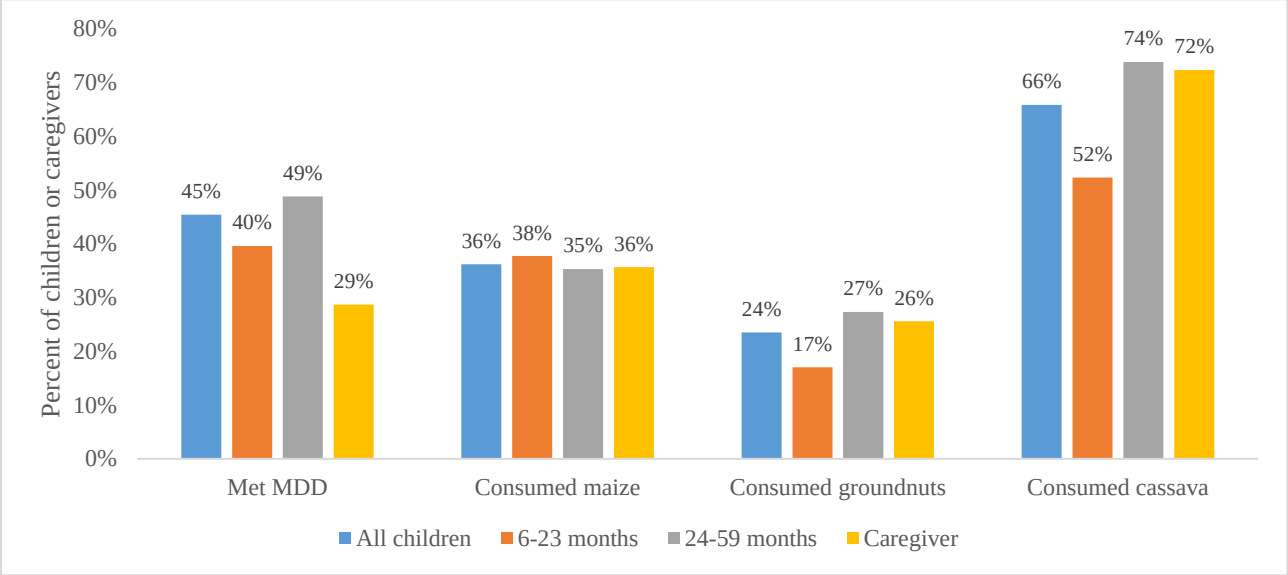

Figure 10. Dietary diversity and consumption of aflatoxin-prone foods

Female caregivers were less educated than heads of household, with two-thirds of female caregivers having no education or did not complete primary 1, see Figure 11.

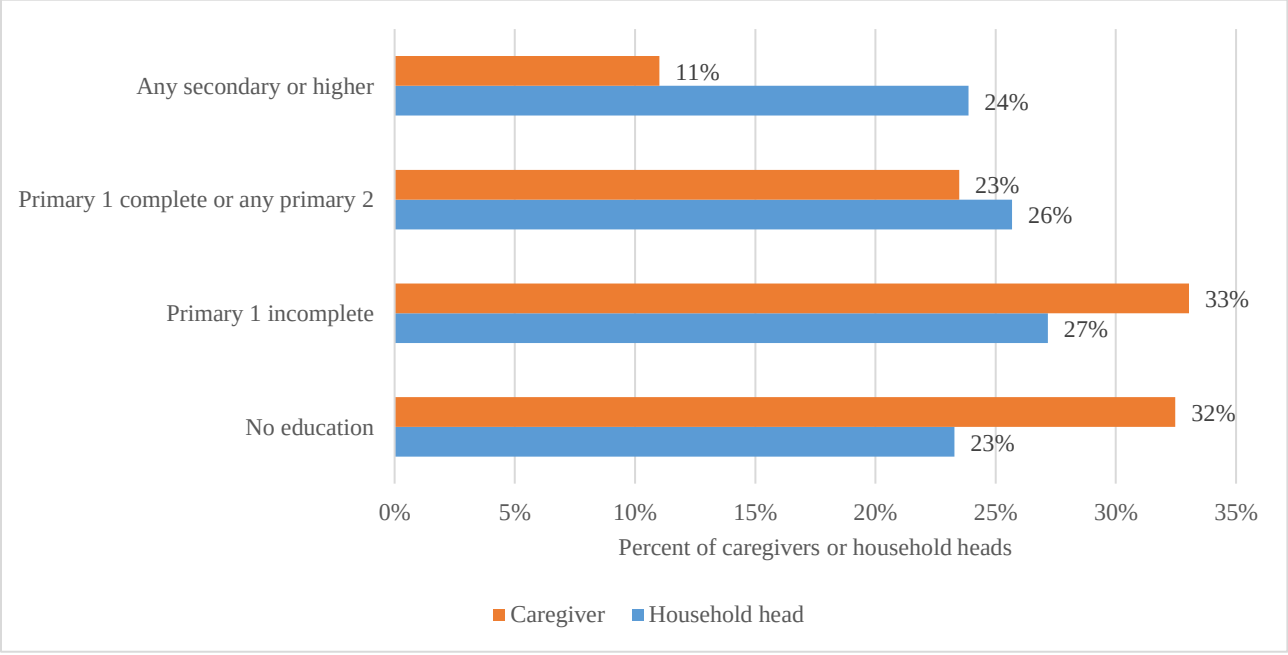

Figure 11. Household head and caregiver's education level

The majority of households in the study were experiencing some degree of food insecurity, as measured by the HFIAS, see Figure 12. Less than a quarter of the households were food secure.

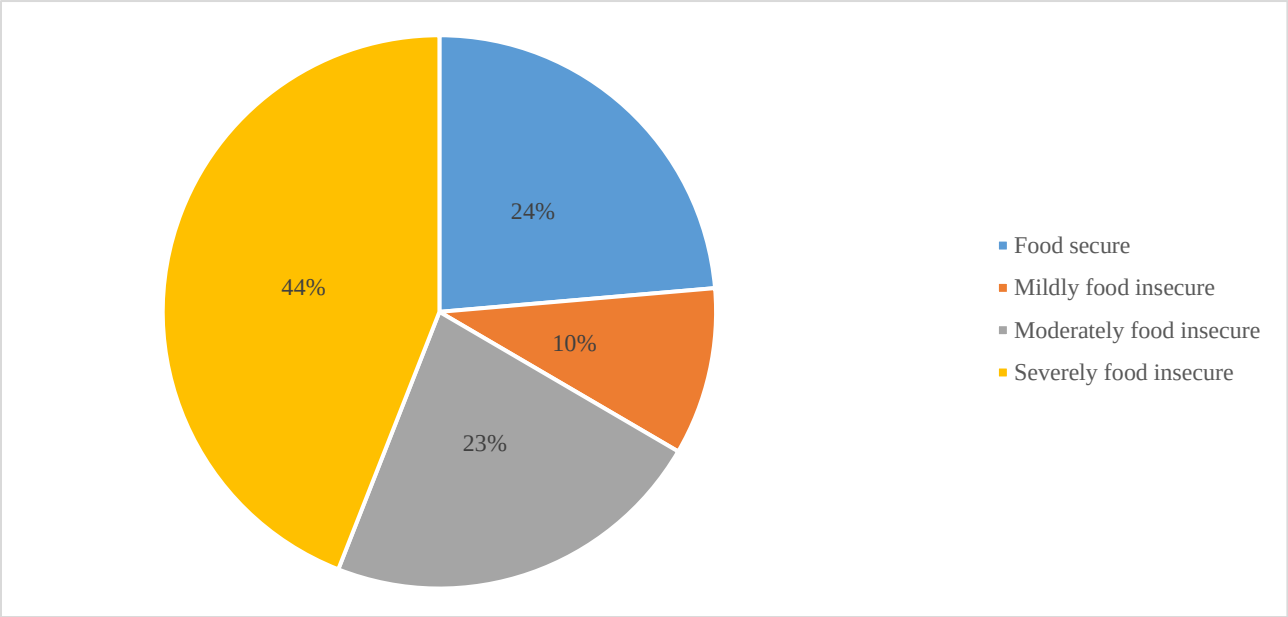

Figure 12. Household food security

As expected, the majority of the households in the study participate in some kind of agriculture, see Figure 13. Over half reported growing maize in the past year, and over 60% reported growing groundnuts.

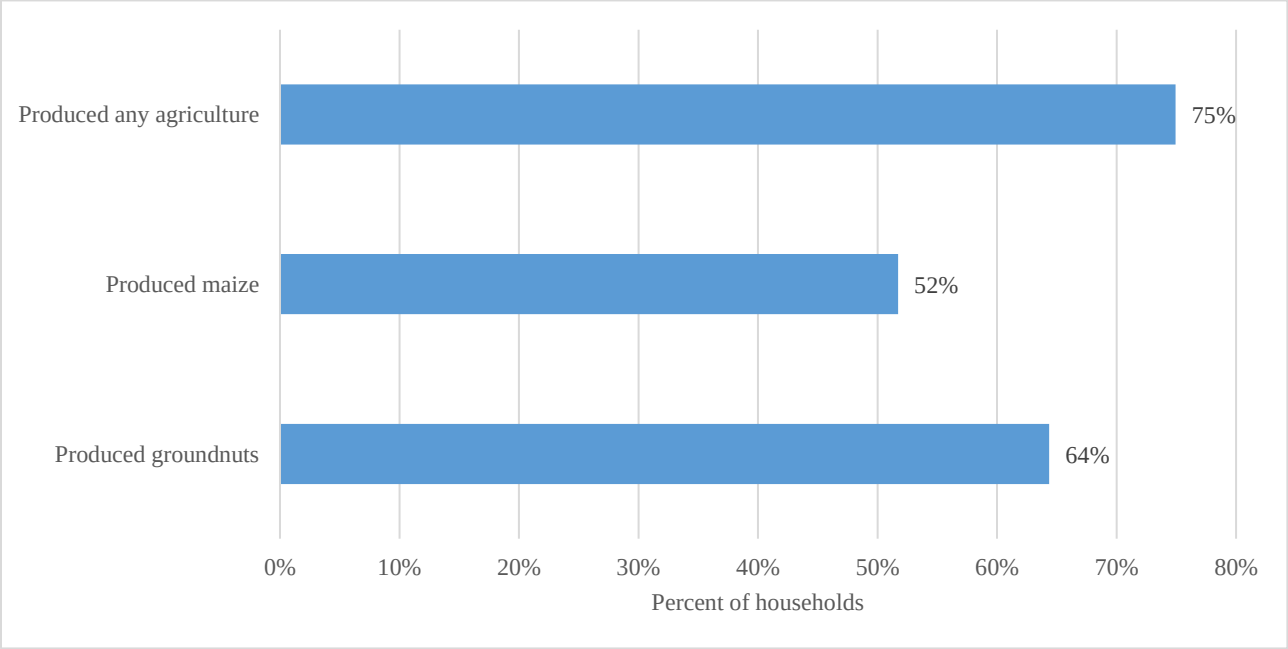

Figure 13. Household agriculture production

10.5. Aflatoxin Levels by Child and Household Characteristics

Child's age and weight were significantly positively associated with AFB1 level in bivariate analyses. See Table 5 for AFB1 levels by child health and diet characteristics, and Table 6 for AFB1 levels by household food security and agricultural production and practices. Due to the skewed nature of AFB1 data, natural log transformed AFB1 levels

are presented in Tables 5 and 6. Furthermore, due to the complex relationship between AFB1, weight, age, and HAZ as described in Section 10, AFB1 levels divided by child's weight are also presented in Tables 5 and 6.

Table 5. AFB1 levels by child health and individual diet characteristics

		Log-transformed AFB1		Log-transformed AFB1 by child's weight	
	n %	Mean	SE	Mean	SE
Age category					
6-23 months	30.4%	0.24*	0.04	-0.70	0.04
24-59 months	69.6%	0.37	0.05	-0.74	0.05
Sex					
Male	48.6%	0.37	0.04	-0.70	0.04
Female	51.4%	0.30	0.05	-0.75	0.05
Stunting					
Not stunted (HAZ≥-2)	54.8%	0.30	0.04	-0.77	0.04
Stunted (HAZ<-2)	45.2%	0.38	0.05	-0.68	0.05
Underweight					
Not underweight (WAZ≥-2)	85.7%	0.33	0.04	-0.75	0.04
Underweight (WAZ<-2)	14.3%	0.35	0.08	-0.63	0.08
Anemia					
Not anemic (Hb≥7 g/dL)	25.1%	0.32	0.07	-0.76	0.07
Anemic (Hb<7 g/dL)	74.9%	0.34	0.04	-0.72	0.04
Malaria					
Child tested negative for malaria	33.0%	0.26	0.05	-0.79	0.05
Child tested positive for malaria	67.0%	0.36	0.04	-0.71	0.04
Child's dietary diversity					
Met IYCF-MDD	46.4%	0.29	0.05	-0.79	0.05
Did not meet IYCF-MDD	53.6%	0.37	0.04	-0.68	0.05
Maize consumption					
Child consumed maize in past 7 days	58.7%	0.28*	0.04	-0.78*	0.04
Child did not consume maize in past 7 days	41.3%	0.40	0.05	-0.66	0.05
Child consumed maize in past 24 hours	36.1%	0.27	0.05	-0.79	0.05
Child did not consume maize in past 24 hours	63.9%	0.37	0.04	-0.70	0.04
Groundnut consumption					
Child consumed groundnuts in past 7 days	54.0%	0.34	0.04	-0.73	0.04
Child did not consume groundnuts in past 7 days	46.0%	0.32	0.04	-0.73	0.04
Child consumed groundnuts in past 24 hours	24.4%	0.36	0.06	-0.71	0.06
Child did not consume groundnuts in past 24 hours	75.6%	0.32	0.04	-0.74	0.04
Cassava consumption					
Child consumed cassava in past 7 days	83.0%	0.36	0.04	-0.71	0.04
Child did not consume cassava in past 7 days	17.0%	0.20	0.08	-0.82	0.08
Child consumed cassava in past 24 hours	66.4%	0.39**	0.04	-0.68*	0.04
Child did not consume cassava in past 24 hours	33.6%	0.22	0.05	-0.82	0.05
Female caregiver's dietary diversity					
Met MDD-W	28.9%	0.27	0.06	-0.80	0.06
Did not meet MDD-W	71.1%	0.36	0.04	-0.70	0.04

Stars indicate significant difference in mean log-transformed AFB1 or log-transformed AFB1 standardized by weight between bivariate groups. * $p<0.05$; ** $p<0.01$.

Table 6. AFB1 levels by household food security and agriculture practices

		Log-transformed AFB1		Log-transformed AFB1 by child's weight	
	n %	Mean	SE	Mean	SE
Household Food Insecurity Access					
Household is food secure	22.0%	0.39	0.07	-0.68	0.07
Household is mildly food insecure	10.2%	0.21	0.08	-0.87	0.09
Household is moderately food insecure	22.7%	0.28	0.05	-0.79	0.05
Household is severely food insecure	45.1%	0.36	0.05	-0.69	0.05
Maize production					
Household produced maize	51.2%	0.33	0.05	-0.72	0.05
Household did not produce maize	48.8%	0.33	0.04	-0.73	0.04
Maize drying location					
Household dried maize outside the field	36.2%	0.20	0.08	-0.85*	0.08
Household dried maize only in the field	63.8%	0.41	0.05	-0.65	0.05
Maize drying method					
Household dried maize with improved practices[3]	13.8%	0.22	0.10	-0.85	0.10
Household did not dry maize with improved practices	86.2%	0.35	0.06	-0.70	0.06
Groundnut production					
Household produced groundnuts	64.1%	0.37	0.04	-0.70	0.04
Household did not produce groundnuts	35.9%	0.28	0.05	-0.78	0.05
Groundnut drying location					
Household dried groundnuts outside the field	64.4%	0.34	0.05	-0.73	0.05
Household dried groundnuts only in the field	35.6%	0.41	0.05	-0.65	0.06
Groundnut drying method					
Household dried groundnuts with improved practices	17.2%	0.22*	0.07	-0.85*	0.07
Household did not dry groundnuts with improved practices	82.8%	0.39	0.04	-0.67	0.04

Stars indicate significant difference in mean log-transformed AFB1 or log-transformed AFB1 standardized by weight between bivariate groups. * $p<0.05$; ** $p<0.01$.

10.6. Association between Aflatoxin Exposure and Stunting or HAZ

The multivariate logistic regression found a significant and positive association between stunting and aflatoxin exposure by child's weight when adjusting for child's WHZ, age, age squared, sex, and detectable AFB1, see Table 7. An increase in one unit of aflatoxin exposure adjusted for child's weight is associated with a 60% increase in likelihood of being stunted (OR=1.60; p=0.028). The association was unchanged when adjusting for child's anemia status (OR=1.60; p=0.028), which also had a significant and negative association with stunting (OR=1.80; p=0.010). A similar relationship between stunting and AFB1 by weight was found when multivariate analyses only included children with detectable AFB1 levels (OR=1.56; p=0.038). An analysis of stunting and aflatoxin exposure not adjusted by child's body weight was also conducted, with no significant relationships found.

[3] Improved drying practices include drying the maize or groundnuts with fans, on platforms, on plastic sheets, hung under the roof, or hung in the kitchen. Unimproved practices include drying only in the field, spreading directly on the dirt, spreading directly on cement or brick floor, or spreading on the roof.

Table 7. Logistic association of stunting and log-transformed AFB1 with biological variables[4]

	Log-transformed AFB1		Log-transformed AFB1 by weight	
	Unadjusted model OR (95% CI)	**Adjusted model** OR (95% CI)	**Unadjusted model** OR (95% CI)	**Adjusted model** OR (95% CI)
Log-transformed AFB1	1.231 (0.934, 1.623)	1.287 (0.841, 1.969)	1.295 (0.974, 1.721)	1.595* (1.053, 2.418)
WHZ		1.179 (0.985, 1.412)		1.198 (0.994, 1.445)
Detectable AFB1 dummy		0.599 (0.287, 1.252)		0.470* (0.225, 0.980)
Child is anemic		1.802** (1.178, 2.757)		1.798** (1.161, 2.786)
Child's age (month)		1.106** (1.043, 1.173)		1.110*** (1.048, 1.175)
Child's age squared (month^2)		0.999** (0.999, 0.999)		0.999** (0.998, 1.000)
Female child		0.732 (0.489, 1.093)		0.730 (0.486, 1.096)
Constant	0.769* (0.611, 0.967)	0.151** (0.042, 0.551)	0.994 (0.797, 1.240)	0.267 (0.059, 1.216)
n	894	894	894	894

* p<0.05; ** p<0.01; *** p<0.001

Linear regression analyses also found a significant and negative association between HAZ and aflatoxin exposure by child's weight (β=-0.24; p=0.028) when adjusting for child's WHZ, age, age squared, sex, and detectable AFB1, see Table 8. When only children with detectable AFB1 are included in the model, the relationship between HAZ and aflatoxin exposure remains (β=-0.23; p=0.029). Similar to before, the analysis of HAZ and aflatoxin exposure not adjusted by child's body weight was conducted, finding no significant relationships.

Table 8. Linear association of HAZ and log-transformed AFB1 with biological variables

	Log-transformed AFB1		Log-transformed AFB1 by weight	
	Unadjusted model β-coefficients (SE)	**Adjusted model** β-coefficients (SE)	**Unadjusted model** β-coefficients (SE)	**Adjusted model** β-coefficients (SE)
Log-transformed AFB1	-0.105 (0.083)	-0.067 (0.113)	-0.134 (0.081)	-0.234* (0.104)
WHZ		-0.032 (0.055)		-0.038 (0.055)
Detectable AFB1 dummy		0.196 (0.182)		0.383* (0.176)
Child is anemic		-0.463*** (0.122)		-0.459*** (0.121)
Child's age (month)		-0.086*** (0.020)		-0.086*** (0.020)
Child's age squared (month^2)		0.001** (0.000)		0.001** (0.000)
Female child		0.149 (0.104)		0.147 (0.102)
Constant	-1.837*** (0.068)	-0.129 (0.341)	-1.970 (0.088)	-0.474 (0.382)
n	894	894	894	894
R-squared	0.002	0.117	0.004	0.125

* p<0.05; ** p<0.01; *** p<0.001

[4] All regression models were adjusted for survey design including clustering and sample weights for the different age groups. Results are representative of children 6-59 months of age in the 10 Feed the Future ZOI districts in Nampula province.

10.7. Dietary Determinants of Aflatoxin Exposure

As maize, groundnuts, and cassava are known to be easily contaminated by aflatoxin, the relationship of consuming these foods in the past 24 hours and aflatoxin exposure was assessed. Consumption of maize among children 6 to 23 months of age was significantly associated with log-transformed aflatoxin level (β=-0.18; p=0.018), although this relationship became insignificant when adjusting for WHZ, child's age, meeting IYCF-MDD, and detectable AFB1, see Table 9. Interestingly, the relationship was negative indicating that children who consumed maize had lower log AFB1.

Groundnut consumption was significantly associated with higher log AFB1 levels among all children (β=0.11; p=0.024), when adjusting for WHZ, child's age, meeting IYCF-MDD, and detectable AFB1. The relationship was not significant among children 6 to 23 months but became stronger among children 24 to 59 months of age (β=0.13; p=0.014), see Table 10. Cassava consumption (Table 11) also showed a significant association with higher log AFB1 levels among all children in both unadjusted and adjusted models (β=0.18; p=0.003 and β=0.12; p=0.021, respectively). A similar relationship was seen in the unadjusted model among children 24 to 59 months of age, but became insignificant when adjusting for WHZ, child's age, meeting IYCF-MDD, and detectable AFB1.

Table 9. Linear association of log-transformed AFB1 and maize consumption

Variable	All children		Children 6-23 months		Children 24-59 months	
	Unadjusted β-coefficients (SE)	**Adjusted** β-coefficients (SE)	**Unadjusted** β-coefficients (SE)	**Adjusted** β-coefficients (SE)	**Unadjusted** β-coefficients (SE)	**Adjusted** β-coefficients (SE)
Child consumed maize in past 24 hours	-0.098 (0.056)	-0.050 (0.055)	-0.183* (0.075)	-0.117 (0.080)	-0.051 (0.071)	-0.027 (0.063)
WHZ		0.015 (0.023)		-0.029 (0.030)		0.027 (0.031)
Child's age (months)		0.003 (0.002)		0.007 (0.007)		-0.001 (0.002)
Child met the IYCF-MDD		-0.078 (0.050)		0.082 (0.082)		-0.148* (0.061)
AFB1 result is detectable		1.126*** (0.032)		1.018*** (0.038)		1.173*** (0.040)
Constant	0.368*** (0.040)	-0.722*** (0.056)	0.316*** (0.054)	-0.769*** (0.107)	0.390*** (0.45)	-0.572*** (0.111)
N	890	890	311	311	579	579
R squared	0.006	0.303	0.024	0.334	0.002	0.302

* p<0.05; ** p<0.01; *** p<0.001

Table 10. Linear association of log-transformed AFB1 and groundnut consumption

Variable	All children		Children 6-23 months		Children 24-59 months	
	Unadjusted β-coefficients (SE)	**Adjusted** β-coefficients (SE)	**Unadjusted** β-coefficients (SE)	**Adjusted** β-coefficients (SE)	**Unadjusted** β-coefficients (SE)	**Adjusted** β-coefficients (SE)
Child consumed groundnuts in past 24 hours	0.038 (0.052)	0.105* (0.045)	0.076 (0.085)	-0.001 (0.091)	0.010 (0.063)	0.135* (0.053)
WHZ		0.013 (0.023)		-0.033 (0.029)		0.025 (0.031)
Child's age (months)		0.003 (0.002)		0.008 (0.008)		-0.001 (0.003)
Child met the IYCF-MDD		-0.103* (0.050)		0.069 (0.091)		-0.175** (0.059)
AFB1 result is detectable		1.139*** (0.030)		1.033*** (0.041)		1.197*** (0.041)
Constant	0.324*** (0.036)	-0.768*** (0.052)	0.230*** (0.047)	-0.840*** (0.099)	0.370*** (0.045)	-0.636*** (0.108)
N	890	890	311	311	579	579
R squared	0.001	0.306	0.003	0.324	0.000	0.310

* p<0.05; ** p<0.01; *** p<0.001

Table 11. Linear association of log-transformed AFB1 and cassava consumption

Variable	All children		Children 6-23 months		Children 24-59 months	
	Unadjusted β-coefficients (SE)	**Adjusted** β-coefficients (SE)	**Unadjusted** β-coefficients (SE)	**Adjusted** β-coefficients (SE)	**Unadjusted** β-coefficients (SE)	**Adjusted** β-coefficients (SE)
Child consumed cassava in past 24 hours	0.176** (0.056)	0.123* (0.052)	0.137 (0.083)	0.085 (0.077)	0.163* (0.074)	0.120 (0.066)
WHZ		0.013 (0.023)		-0.033 (0.030)		0.026 (0.031)
Child's age (months)		0.002 (0.002)		0.006 (0.008)		-0.001 (0.002)
Child met the IYCF-MDD		-0.090 (0.047)		0.062 (0.075)		-0.155** (0.058)
AFB1 result is detectable		1.119*** (0.033)		1.030*** (0.040)		1.161*** (0.042)
Constant	0.217*** (0.051)	-0.789*** (0.061)	0.178** (0.057)	-0.848*** (0.099)	0.251** (0.073)	-0.670*** (0.112)
N	890	890	311	311	579	579
R squared	0.018	0.309	0.014	0.329	0.013	0.308

* p<0.05; ** p<0.01; *** p<0.001

10.8. Agricultural Determinants of Aflatoxin Exposure

Linear regressions were used to assess agricultural determinants of aflatoxin exposure, including drying location, drying technique, and storage practices for maize and groundnuts. No significant relationship was found between any storage practice and aflatoxin exposure for maize or groundnuts.

Maize drying location was found to be significantly associated with aflatoxin level, where children living in households who dried maize after bringing it in from the field had lower log AFB1 levels than children living in households that only dried their maize in the field (β=-0.21; p=0.009), see Table 12. This relationship became stronger after adjusting for child's age, household head's education level, and production practices of crop rotation and intercropping.

Table 12. Linear association of log transformed AFB1 and maize drying location

Variable	Unadjusted β-coefficients (SE)		Adjusted 1 β-coefficients (SE)		Adjusted 2 β-coefficients (SE)	
Household dried after bringing in from the field	-0.205**	(0.075)	-0.217*	(0.098)	-0.227*	(0.096)
Child's age (month)			0.007**	(0.002)	0.007**	(0.002)
Household used crop rotation			0.030	(0.086)		
Household intercropped					0.069	(0.069)
Household head's education level (none reference)						
Primary 1 incomplete			0.074	(0.098)	0.086	(0.094)
Primary 1 complete or any primary 2			0.009	(0.101)	0.015	(0.099)
Any secondary or higher			-0.109	(0.095)	-0.105	(0.096)
Constant	0.407***	(0.054)	0.167	(0.129)	0.130	(0.141)
N	483		369		369	
r2	0.023		0.061		0.063	

* p<0.05; ** p<0.01; *** p<0.001

Maize drying practice was also found to be significantly associated with aflatoxin level (Table 13). Children living in households who used improved maize drying practices had lower log AFB1 levels than children living in households that only used unimproved drying practices (β=-0.26; p=0.015 and β=-0.26; p=0.017, respectively), when adjusting for child's age, household head's education level, and crop rotation or inter-cropping.

Table 13. Linear association of log transformed AFB1 and maize drying practices

Variable	Unadjusted β-coefficients (SE)		Adjusted 1 β-coefficients (SE)		Adjusted 2 β-coefficients (SE)	
Household used improved maize drying techniques	-0.129	(0.106)	-0.260*	(0.103)	-0.263*	(0.106)
Child's age (month)			0.007**	(0.002)	0.007**	(0.002)
Household used crop rotation			0.034	(0.086)		
Household intercropped					0.043	(0.072)
Household head's education level (none reference)						
Primary 1 incomplete			0.071	(0.101)	0.081	(0.099)
Primary 1 complete or any primary 2			0.009	(0.098)	0.015	(0.096)
Any secondary or higher			-0.079	(0.093)	-0.073	(0.094)
Constant	0.351***	(0.057)	0.124	(0.118)	0.101	(0.133)
N	483		369		369	
r2	0.005		0.054		0.054	

* p<0.05; ** p<0.01; *** p<0.001

Similarly, children living in households who used improved groundnut drying practices had lower log AFB1 levels than children in households who only used unimproved drying practices (β=-0.17; p=0.030), see Table 14. The relationship became slightly stronger when adjusting for child's age, household head's education level, and crop rotation or inter-cropping (β=-0.19; p=0.023 and β=-0.19; p=0.020, respectively).

Table 14. Linear association of log transformed AFB1 and groundnut drying practices

Variable	Unadjusted β-coefficients (SE)		Adjusted 1 β-coefficients (SE)		Adjusted 2 β-coefficients (SE)	
Household used improved groundnut drying techniques	-0.169*	(0.076)	-0.189*	(0.081)	-0.192*	(0.081)
Child's age (month)			0.005*	(0.002)	0.005*	(0.002)
Household used crop rotation			0.046	(0.084)		
Household intercropped					-0.004	(0.068)
Household head's education level (none reference)						
Primary 1 incomplete			0.030	(0.076)	0.036	(0.077)
Primary 1 complete or any primary 2			-0.064	(0.097)	-0.058	(0.095)
Any secondary or higher			-0.077	(0.091)	-0.072	(0.093)
Constant	0.393***	(0.041)	0.224	(0.112)	0.235	(0.128)
N	590		500		500	
r2	0.010		0.037		0.036	

* p<0.05; ** p<0.01; *** p<0.001

11. Discussion

Aflatoxin exposure can affect the nutritional status of young children which in turn has long term implications related to their growth and development. The objectives of this study were to enumerate the serum aflatoxin in children 6-59 months of age, examine differences in exposure by age and estimate the association of aflatoxin levels in these children and their risk of being stunted. As secondary objectives, we also wanted to examine the dietary and agricultural determinants of aflatoxin exposure thus providing insights on avenues for program and policy intervention. To accomplish these objectives, we conducted a cross-sectional survey with a sample size of 1,001 households utilizing a sampling frame and strategy such that the findings are representative of children 6-59 months, children 6-23 months, and children 24-59 months of age in 10 Feed the Future ZOI districts of Nampula province, Mozambique. The 10 districts included in the study are Angoche, Larde, Malema, Meconta, Mecuburi, Mogovolas, Moma, Monapo, Murrupula, and Rapale.

The study found a high detection rate of aflatoxin exposure with over 90% of the children exhibiting detectable levels of aflatoxin. The serum aflatoxin levels varied by age group with levels being higher in older children than in younger children. We also found higher aflatoxin levels in children with higher weights.

Stunting was high across both age groups with 33% of children 6-23 months and 51% of children 24-59 months being classified as stunted. Older children (24-59 months) were twice as likely to be stunted as younger children. Rates of malaria and anemia were high with 71% of children 24-59 months and 60% of children 6-23 months diagnosed with malaria and 55% of children 6-23 months and 43% of children 24-59 months classified as moderately anemic. Only 40% of children 6-23 months and 49% of children 24-59 months met their minimum dietary diversity. With respect to consumption of aflatoxin prone foods in the past 24 hours, 74% of children 24-59 months, 52% of children 6-23 months and 72% of female caregivers reported consuming cassava. Ground nut consumption ranged from 17% in infants aged 6-23 months to 26% in caregivers while maize consumption ranged from 35-38% in both groups of children and their caregivers. A critical point to note here is that most households experienced some degree of food insecurity.

A complex relationship between AFB1, age, weight, and HAZ was observed. We observed a positive HAZ-AFB1 relationship in children at higher weights, no relationship in children at average weight, and a negative relationship in children at lower weights. These relationships also changed when analyzed at specific ages. In multivariable analyses adjusting for confounders, we found a significant and positive association between being stunted and aflatoxin levels adjusted for body weight with a one unit increase in aflatoxin levels being associated with a 60% increase in likelihood of being stunted. Similarly, a significant and negative association was also found between HAZ and aflatoxin levels.

Assessing the dietary determinants of aflatoxin, we found groundnut consumption was significantly associated with aflatoxin levels in all children but specifically in children aged 24-59 months. We also found a significant association between cassava consumption and aflatoxin levels in all children and specifically in children aged 24-59 months. The lack of association in younger children could be a reflection of lower levels of consumption of these food items in that age group. Studies have found cassava flour to be contaminated with the Aspergillus spp, this coupled with our finding suggests the need for the development and implementation of aflatoxin contamination mitigation during processing of cassava into flour.

Finally, we examined the agricultural determinants of aflatoxin levels and found in adjusted analyses that the location of drying maize was significantly associated with aflatoxin levels with children in households that dried their maize only in the field had significantly higher levels than children in households that dried maize after it was brought in from the field. Children living in households with improved maize drying practices or ground nut drying practices also had lower levels of aflatoxin than those living in households with unimproved practices for drying of either crop. While individual cassava consumption was significantly associated with aflatoxin levels, we were unable

to assess the association of cassava production, storage and handling practices due to the lack of data, which is a critical limitation of our analyses. Despite this, our findings indicate the importance of addressing aflatoxin in the food system for optimal growth and development of Mozambican children.

12. Program and Policy Implications

We found that children in Nampula province had substantial exposure to aflatoxin which in turn was associated with poorer anthropometric outcomes. Furthermore, aflatoxin levels were predicted by their diet, with consumption of cassava and groundnuts being significant issues. We also note the agricultural dimension within our findings wherein, improved drying practices of maize and groundnuts being critical factors related to aflatoxin levels in all children irrespective of age. Our findings highlight the importance of addressing both nutrition specific and nutrition sensitive factors that affect early life health and nutrition particularly within the context of Mozambique given the high rates of stunting as well as the high rates of contamination of agricultural commodities with aflatoxin.

The 2013 Lancet series on maternal and child nutrition highlighted the importance of focusing research and programming activities on the prevention of stunting during the first 1,000 days of a child's life (Black et al., 2013). While the series identified 10 evidence-based targeted, or nutrition-specific, interventions (Bhutta, 2013), it also underlined that additional complementary actions are needed in the domain of 'nutrition-sensitive' actions, to ensure that stunting can be reduced by *more than* 20%. This brings a renewed focus to investments required in sectors other than health, including agriculture and its associated value chains, not simply to make more food available to consumers but to make that food safer and of higher quality. One of the principal foci of the food safety/quality agenda today is mycotoxin contamination of foods consumed by pregnant women and young children, and its potential links to stunting.

Within the policy and program context of Mozambique, our findings highlight the need to focus and continue efforts in alleviating malnutrition through nutrition and health specific actions particularly given the high rates of malnutrition, poor dietary diversity, high levels of food insecurity, anemia and malaria. While our study did not assess underlying causes of malnutrition such as livelihoods, resilience and income poverty, the continued emphasis on these aspects of development continue to remain critical. In addition, there is need for an increased emphasis on nutrition sensitive actions through the support of research and programming on agricultural and post-harvest drying and storage technologies that support aflatoxin mitigation across the food system. There is need to couple the nutrition specific and nutrition sensitive actions outlined above in a way that rural households are being exposed to both sets of interventions at the same time. There is need to ensure that households are exposed for a sufficient period of time to interventions (be they nutrition specific or nutrition sensitive) in order to effect change. Our study also highlights the need to assess the cassava production, processing and storage viz-a-viz aflatoxin contamination. It also indicates the need for the development of mitigation and regulatory measures that not only target standard aflatoxin prone crops such as maize and groundnuts and their products but also cassava and its products, e.g., cassava flour.

In conclusion, our findings are important as they help identify nutrition sensitive actions such as aflatoxin mitigation through good agricultural and post-harvest storage practices that would support further reductions in stunting in Mozambican children.

13. References

"359." n.d. Cypress Diagnostics. Accessed March 17, 2021. https://diagnostics.be/product/359.

Advancing Partners & Communities. 2013. "Country Profile: Mozambique Community Health Programs." Arlington, VA: Advancing Partners & Communities.

Andrews-Trevino, Johanna Y., Patrick Webb, Gerald Shively, Beatrice L. Rogers, Kedar Baral, Dale Davis, Krishna Paudel, et al. 2019. "Relatively Low Maternal Aflatoxin Exposure Is Associated with Small-for-Gestational-Age but Not with Other Birth Outcomes in a Prospective Birth Cohort Study of Nepalese Infants." *The Journal of Nutrition* 149 (10): 1818–25. https://doi.org/10.1093/jn/nxz122.

Augusto, J, R Bandyopadhyay, J Akello, and J Atehnkeng. 2014. "Aflatoxin Outlook in Mozambique." IITA.

Barrett, Julia R. 2005. "Liver Cancer and Aflatoxin: New Information from the Kenyan Outbreak." *Environmental Health Perspectives* 113 (12): A837–38.

Bhutta, Zulfiqar A. 2013. "Early Nutrition and Adult Outcomes: Pieces of the Puzzle." *The Lancet* 382 (9891): 486–87. https://doi.org/10.1016/S0140-6736(13)60716-3.

Black, Robert E., Cesar G. Victora, Susan P. Walker, Zulfiqar A. Bhutta, Parul Christian, Mercedes De Onis, Majid Ezzati, et al. 2013. "Maternal and Child Undernutrition and Overweight in Low-Income and Middle-Income Countries." *The Lancet* 382 (9890): 427–51. https://doi.org/10.1016/S0140-6736(13)60937-X.

Cambaza, Edgar, Shigenobu Koseki, and Shuso Kawamura. 2018. "Aflatoxins in Mozambique: Impact and Potential for Intervention." *Agriculture* 8 (7): 100. https://doi.org/10.3390/agriculture8070100.

Campbell, David I., Graham Mcphail, Peter G. Lunn, Marinos Elia, and Donald J. Jeffries. 2004. "Intestinal Inflammation Measured by Fecal Neopterin in Gambian Children With Enteropathy: Association With Growth Failure, *Giardia Lamblia,* and Intestinal Permeability." *Journal of Pediatric Gastroenterology and Nutrition* 39 (2): 153–57.

Coates, Jennifer, Anne Swindale, and Paula Bilinsky. 2007. "Household Food Insecurity Access Scale (HFIAS) for Measurement of Food Access: Indicator Guide: Version 3: (576842013-001)." American Psychological Association. https://doi.org/10.1037/e576842013-001.

Denno, Donna M., Kelley VanBuskirk, Zakia C. Nelson, Christine A. Musser, Hay Burgess, Deborah C, and Phillip I. Tarr. 2014. "Use of the Lactulose to Mannitol Ratio to Evaluate Childhood Environmental Enteric Dysfunction: A Systematic Review." *Clinical Infectious Diseases* 59 (suppl_4): S213–19. https://doi.org/10.1093/cid/ciu541.

Feedback. FtF. 2014. "Feed the Future Mozambique Zone of Influence Baseline Report."

Gong, Y. Y., K. Cardwell, A. Hounsa, S. Egal, P. C. Turner, A. J. Hall, and C. P. Wild. 2002. "Dietary Aflatoxin Exposure and Impaired Growth in Young Children from Benin and Togo: Cross Sectional Study." *BMJ* 325 (7354): 20–21.

Gong, Y. Y., S. Egal, A. Hounsa, P. C. Turner, A. J. Hall, K. F. Cardwell, and C. P. Wild. 2003. "Determinants of Aflatoxin Exposure in Young Children from Benin and Togo, West Africa: The Critical Role of Weaning." *International Journal of Epidemiology* 32 (4): 556–62. https://doi.org/10.1093/ije/dyg109.

Groopman, John D., Gerald N. Wogan, Bill D. Roebuck, and Thomas W. Kensler. 1994. "Molecular Biomarkers for Aflatoxins and Their Application to Human Cancer Prevention." *Cancer Research* 54 (7 Supplement): 1907s–11.

"Haemoglobin.Pdf." n.d. Accessed March 17, 2021. https://www.who.int/vmnis/indicators/haemoglobin.pdf.

Health. Mo, Statistics. NIo, & International. I. 2011. "Mozambique. in Demographic and Health Surveys." MISAU, INE, ICFI, Calverton, Maryland, USA.

Hlashwayo, Delfina Fernandes. n.d. "Aflatoxin B1 Contamination in Raw Peanuts Sold in Maputo City, Mozambique and Associated Factors," 10.

"I2433e10.Pdf." n.d. Accessed March 17, 2021. http://www.fao.org/3/i2433e/i2433e10.pdf.

IFPRI/BMGF. n.d. "Aflatoxin: Impact on Stunting in Children and Interventions to Reduce Exposure. Co-Organized by International Food Policy Research Institute and the Enteric and Diarrheal Diseases, Agriculture and Nutrition." Accessed June 27, 2017. http://docplayer.net/35651373-Aflatoxin-impact-on-stunting-in-children-and-interventions-to-reduce-exposure.html.

INE-Mozambique. 2010. "Projecções Anuais Da População Total, Urbana e Rural, Dos Distritos Da Província de Nampula: 2007 – 2040." Instituto Nacional de Estatística.

Khlangwiset, Pornsri, Gordon S. Shephard, and Felicia Wu. 2011. "Aflatoxins and Growth Impairment: A Review." *Critical Reviews in Toxicology* 41 (9): 740–55. https://doi.org/10.3109/10408444.2011.575766.

Kuile, Feiko O. ter, Dianne J. Terlouw, Simon K. Kariuki, Penelope A. Phillips-Howard, Lisa B. Mirel, William A. Hawley, Jennifer F. Friedman, et al. 2003. "Impact of Permethrin-Treated Bed Nets on Malaria, Anemia, and Growth in Infants in an Area of Intense Perennial Malaria Transmission in Western Kenya." *The American Journal of Tropical Medicine and Hygiene* 68 (4 Suppl): 68–77.

Lai, Hao, Xianwei Mo, Yang Yang, Ke He, Jun Xiao, Chao Liu, Jiansi Chen, and Yuan Lin. 2014. "Association between Aflatoxin B1 Occupational Airway Exposure and Risk of Hepatocellular Carcinoma: A Case-Control Study." *Tumour Biology* 35 (10): 9577–84. https://doi.org/10.1007/s13277-014-2231-3.

Lauer, Jacqueline M., Christopher P. Duggan, Lynne M. Ausman, Jeffrey K. Griffiths, Patrick Webb, Jia-Sheng Wang, Kathy S. Xue, Edgar Agaba, Nathan Nshakira, and Shibani Ghosh. 2019. "Maternal Aflatoxin Exposure during Pregnancy and Adverse Birth Outcomes in Uganda." *Maternal & Child Nutrition* 15 (2): e12701. https://doi.org/10.1111/mcn.12701.

Leong, Yin-Hui, Aishah A. Latiff, Nurul Izzah Ahmad, and Ahmad Rosma. 2012. "Exposure Measurement of Aflatoxins and Aflatoxin Metabolites in Human Body Fluids. A Short Review." *Mycotoxin Research* 28 (2): 79–87. https://doi.org/10.1007/s12550-012-0129-8.

Leroy, Jef L. 2013. "Child Stunting and Aflatoxins." http://ebrary.ifpri.org/cdm/ref/collection/p15738coll2/id/127874.

Lunn, P. G, C. A Northrop-Clewes, and R. M Downes. 1991. "Intestinal Permeability, Mucosal Injury, and Growth Faltering in Gambian Infants." *The Lancet*, Originally published as Volume 2, Issue 8772, 338 (8772): 907–10. https://doi.org/10.1016/0140-6736(91)91772-M.

Magadi, Monica A. 2011. "Household and Community HIV/AIDS Status and Child Malnutrition in Sub-Saharan Africa: Evidence from the Demographic and Health Surveys." *Social Science & Medicine (1982)* 73 (3): 436–46. https://doi.org/10.1016/j.socscimed.2011.05.042.

"Minimum Dietary Diversity for Women- A Guide to Measurement." n.d., 82.

"Mould and Aflatoxin Contamination of Dried Cassava Chips in Eastern Uganda: Association with Traditional Processing and Storage Practices." n.d. Accessed March 20, 2021. https://doi.org/10.3923/jbs.2010.718.729.

"Mozambique | UNAIDS." n.d. Accessed June 29, 2017. http://www.unaids.org/en/regionscountries/countries/mozambique.

Natamba, Barnabas K., Jia-Sheng Wang, Sera L. Young, Shibani Ghosh, and Jeffrey K. Griffiths. 2016. "HIV-Infected Pregnant and Lactating Women Have Higher Serum Aflatoxin Levels than HIV–Uninfected Women and Aflatoxin Levels Are Higher during Early Postpartum than during Pregnancy among HIV-Infected Women." *The FASEB Journal* 30 (1 Supplement): 668.5-668.5.

Probst, C., R. Bandyopadhyay, and P. J. Cotty. 2014. "Diversity of Aflatoxin-Producing Fungi and Their Impact on Food Safety in Sub-Saharan Africa." *International Journal of Food Microbiology* 174 (March): 113–22. https://doi.org/10.1016/j.ijfoodmicro.2013.12.010.

Saloojee, Haroon, Tim De Maayer, Michel L. Garenne, and Kathleen Kahn. 2007. "What's New? Investigating Risk Factors for Severe Childhood Malnutrition in a High HIV Prevalence South African Setting." *Scandinavian Journal of Public Health. Supplement* 69 (August): 96–106. https://doi.org/10.1080/14034950701356435.

Turner, Paul C., Andrew C. Collinson, Yin Bun Cheung, YunYun Gong, Andrew J. Hall, Andrew M. Prentice, and Christopher P. Wild. 2007. "Aflatoxin Exposure in Utero Causes Growth Faltering in Gambian Infants." *International Journal of Epidemiology* 36 (5): 1119–25. https://doi.org/10.1093/ije/dym122.

Turner, Paul C, Sophie E Moore, Andrew J Hall, Andrew M Prentice, and Christopher P Wild. 2003. "Modification of Immune Function through Exposure to Dietary Aflatoxin in Gambian Children." *Environmental Health Perspectives* 111 (2): 217–20.

Turner, Paul Craig. 2013. "The Molecular Epidemiology of Chronic Aflatoxin Driven Impaired Child Growth." *Scientifica* 2013. https://doi.org/10.1155/2013/152879.

Unicef-Drp/Igrowup_update. (2019) 2020. Stata. UNICEF Data & Analytics. https://github.com/unicef-drp/igrowup_update.

USAID. 2011. "Mozambique: FY 2011–2015 Multi-Year Strategy." USAID, Mozambique.
———. 2014. "Request for Proposals (RFP) No. SOL-656-14-000004 –Strengthening Agribusinesses and Fostering Rural Alimentation (SAFRA) Project in Mozambique." USAID.
Van Rensburg, S. J., P. Cook-Mozaffari, D. J. Van Schalkwyk, J. J. Van der Watt, T. J. Vincent, and I. F. Purchase. 1985. "Hepatocellular Carcinoma and Dietary Aflatoxin in Mozambique and Transkei." *British Journal of Cancer* 51 (5): 713–26.
Verhoef, Hans, Clive E. West, Jacobien Veenemans, Yves Beguin, and Frans J. Kok. 2002. "Stunting May Determine the Severity of Malaria-Associated Anemia in African Children." *Pediatrics* 110 (4): e48–e48. https://doi.org/10.1542/peds.110.4.e48.
Wang, Jia-Sheng, Salahaddin Abubaker, Xia He, Guiju Sun, Paul T. Strickland, and John D. Groopman. 2001. "Development of Aflatoxin B1-Lysine Adduct Monoclonal Antibody for Human Exposure Studies." *Applied and Environmental Microbiology* 67 (6): 2712–17. https://doi.org/10.1128/AEM.67.6.2712-2717.2001.
Warth, Benedikt, Alexandra Parich, Joseph Atehnkeng, Ranajit Bandyopadhyay, Rainer Schuhmacher, Michael Sulyok, and Rudolf Krska. 2012. "Quantitation of Mycotoxins in Food and Feed from Burkina Faso and Mozambique Using a Modern LC-MS/MS Multitoxin Method." *Journal of Agricultural and Food Chemistry* 60 (36): 9352–63. https://doi.org/10.1021/jf302003n.
Wild, C. P., G. J. Hudson, G. Sabbioni, B. Chapot, A. J. Hall, G. N. Wogan, H. Whittle, R. Montesano, and J. D. Groopman. 1992. "Dietary Intake of Aflatoxins and the Level of Albumin-Bound Aflatoxin in Peripheral Blood in The Gambia, West Africa." *Cancer Epidemiology and Prevention Biomarkers* 1 (3): 229–34.
World Health Organization (WHO). 2008. *Indicators for Assessing Infant and Young Child Feeding Practices: Conclusions of a Consensus Meeting Held 6-8 November 2007 in Washington D.C., USA*. Washington, D.C.: World Health Organization (WHO).

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