SOURCES AND EFFECT OF MATERNAL AFLATOXIN EXPOSURE THROUGH DIET ON GROWTH AND MORBIDITY OF INFANTS 0 - 3 MONTHS IN KISUMU COUNTY, KENYA

BY

MARY IBAYO OBADE

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DECLARATION

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CANDIDATE

SIGN _____DATE_____

MARY IBAYO OBADE

PG/PHD/085/2010

This Thesis has been submitted for examination with our approval as the University supervisors.

SIGN_____DATE_____

DR. PAULINE ANDANG'O
Maseno University,
School of Public Health and Community Development
Department of Nutrition and Health

SIGN_____DATE_____

PROF. CHARLES O. OBONYO
Kenya Medical Research Institute
Kisumu, Kenya

SIGN_____DATE_____

DR. FRANCESCA LUSWETI
Kenya Agricultural and Livestock Research Organization (KALRO)
Kitale, Kenya

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DEDICATION

This work is dedicated to my dear and loving husband, **Mr. Henry Philip Obade**, my children, Bernard Obade, Evelyn Obade, Fred Obade, Mildred Obade and Kelvin Obade.

ABSTRACT

Aflatoxins, naturally occurring carcinogenic toxins produced by species of fungi, *Aspergillus flavus* and *Aspergillus parasiticus*, are associated with poor growth outcomes, especially stunting and underweight as well as morbidity in young children. Although evidence supports mother to infant exposure during pregnancy and breastfeeding, evidence of its effect on growth is limited to the period after introduction of complementary foods. It is therefore unclear whether early maternal exposure to aflatoxin affects infant growth and morbidity right from birth. Prevalence of aflatoxin levels of 40% has been observed in Nyanza region, and 22.7% of children under 5 years are stunted. The purpose of this study was to determine effect of maternal aflatoxin exposure on growth and morbidity of infants 0-3 months old in Kisumu County, Kenya. Specific objectives were to: determine aflatoxin contamination levels in selected common foods (maize, sorghum, cassava, groundnuts, rice, omena and milk); assess aflatoxin exposure in pregnant women; assess the effect of maternal aflatoxin exposure on infant growth indicators (length, weight, WLZ, WAZ, and LAZ); assess the effect of maternal aflatoxin exposure on infant morbidity. In a cross sectional survey, 297 solid food samples selected by a combination of cluster and systematic sampling; and 80 milk samples selected using the European model from market outlets were analyzed for aflatoxin contamination in June-August, 2013. Out of 730 potential participants, 553 pregnant women were screened for aflatoxin exposure. Of these, 137 exposed and 137 non-exposed women, matched for age and household income, participated in an 8-month cohort study. The women were followed up to delivery and their infants up to 3 months after delivery. Infant length and weight data was collected monthly and morbidity data fortnightly for 3 months. Length-for-age (LAZ), weight-for-length (WLZ)) and weight-for-age (WAZ) were generated. Aflatoxin levels were analyzed using Enzyme Linked Immunosorbent Assay (ELISA) in parts per billion (ppb). Women consuming foods with aflatoxin levels above 10 ppb were considered exposed. Effects of aflatoxin on infant growth outcomes were assessed using multivariate linear and logistic regression. Effect of maternal aflatoxin exposure on infant length, weight, LAZ, WLZ, WAZ and morbidity was determined using Cox regression with constant time at risk. Aflatoxin B_1 and M_1 levels in market foods ranged between 0 ppb to 34.5 ppb and 0.012 ppb to 0.127 ppb respectively. Sorghum had the highest aflatoxin median levels (median=14.2; IQR= (8.5-19). Women exposed to aflatoxin levels above 10 ppb were 24.8%. Weight (95% CI:-0.85,-0.53), length (95% CI: -4.08, -3.36), LAZ (95% CI: -1.93, -1.16) and WAZ (95% CI:-1.03, -0.54) were lower in infants of exposed women at 3 months of age, but there was no difference in WLZ (95% CI:-0.03, 0.74). Risk for stunting was higher in infants of exposed women (RR=4.08; 95% CI: 1.35, 12.29). There was no difference in the risk for underweight (RR=6.61; 95% CI: 0.80-54.33) and wasting (RR=0.37; 95% CI: 0.40, 3.39, P=0.38). Risk of malaria (RR= 2.04; 95% CI: 1.05, 3.99) and diarrhea (RR=4.13; 95% CI: 1.16, 14.76) was higher in infants of exposed women. Infants of women exposed to aflatoxin are at risk of stunting, malaria and diarrhea. These results underpin the need to reduce aflatoxin exposure in infants and young children who are very vulnerable.

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LIST OF ABBREVIATIONS AND ACRONYMS

AFB_1	Aflatoxin B_1
AFM_I	Aflatoxin M_1
AOAC	Association of Official Analytical Chemists
BMGF	Bill and Melinda Gates Foundation
CGoK	County Government of Kisumu
CDC	Centre for Disease Control
DoA	Department of Agriculture
EAAPP	Eastern Africa Agricultural Productivity Project
EC	European Commission
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
FDA	United States Food and Drugs Administration
GM	Geometric Mean
GDP	Gross Domestic Product
GPS	Global Positioning System
HPLC	High performance liquid chromatography
IARC	International Agency for Research on Cancer
EDD	Enteric and Diarrheal Diseases
IFPRI	International Food Policy Research Institute
KEBS	Kenya Bureau of Standards
KALRO	Kenya Agricultural and Livestock Research Organization
KCIDP	Kisumu County Integrated Development Plan

LAZ	Length for Age Z-scores
LOD	Limitation of Detection
MMWR	Morbidity and mortality weekly report.
MoA	Ministry of Agriculture
MoFD	Ministry of Fisheries Development
MoLD	Ministry of Livestock Development
MoP&ND	Ministry of Planning and National Development
MoPHS	Ministry of Public Health and Sanitation
NCCS	National Cancer Control Strategy
NFNSP	National Food and Nutrition Security Policy
NHANES	National Health and Nutrition Examination Survey
ng	Nano grams
PACA	Partnership for Aflatoxin Control in Africa
ppb	Parts Per Billion
RNA	Ribonucleic Acid
SD	Standard Deviation
SPSS	Statistical Package for Social Sciences
SDGs	Sustainable Development Goals
WLZ	Weight for length Z-scores
WAZ	Weight for age Z-scores

DEFINITION OF TERMS

Aflatoxins: Toxic compounds produced by *Aspergillus flavus* and *Aspergillus parasiticus* species of fungi.

Aflatoxicol: The reduction metabolite of AFB_1

Aflatoxicosis: A disease of the liver characterized by failure or acute poisoning associated with consuming extremely high levels of aflatoxin in food.

Carcinogenic: The tendency to cause cancer.

Hepatotoxicity: Hepatic poisoning or the toxicity to the liver

Mycotoxins: Secondary metabolites produced by fungi/moulds in food crops that are highly toxic to animals and humans.

Nanogram (ng): One billionth (10^{-9}) of a gram

Microgram (µg): One millionth of (10^{-6}) a gram

Parts per billion (ppb): One part in a billion

Z-score: Z-score refers to the number of standard deviations below or above the reference median value. In this study it is applied to nutrition status indicators.

OPERATIONAL DEFINITIONS

Diet: The sum of foods consumed by the group of pregnant women in Kisumu County measured as the average food consumption by the study participants in one day.

Limit of detection (LOD): The mean plus two standard deviations of multiple determinants of an aflatoxin free commodity extract.

Growth: Increase in length and weight of infant

Length: Measurement of linear growth made with the infant lying down.

Weight-for-age: Body mass relative to chronological age defined by 2 standard deviations above or below WHO growth standards (z-scores).

Weight for length: Body mass relative to length defined by 2 standard deviations above or below WHO growth standards (z-scores).

Length for age: A measure of linear growth relative to age defined by 2 standard deviations above or below WHO growth standards (z-scores).

Infant increase in length. The difference in length measurements between birth and 3 months of age.

Exposure to aflatoxin: Consumption of food with aflatoxin levels above 10 parts per billion (ppb).

Levels of exposure: Aflatoxin levels below or above 10 ppb in consumed foods.

Exposed woman: A woman consuming food containing aflatoxin levels above 10 ppb.

Non-exposed woman: A woman consuming food containing aflatoxin levels ≤ 10 ppb.

Infant exposure to aflatoxin: Exposure of infant to aflatoxin based on maternal aflatoxin exposure as determined by results of analyzed aliquot food samples.

Stunting: A child's length-for-age being 2 standard deviations below the WHO growth reference (LAZ ≤ - 2SD)

Underweight: A child's weight-for- age being 2 standard deviations below the WHO growth reference (WAZ ≤ - 2SD)

Wasting: A child's weight-for-height being 2 standard deviations below the WHO growth reference (WAZ ≤ - 2SD)

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CHAPTER ONE: INTRODUCTION

1.1 Background of Study

Aflatoxins are food contaminants that cause liver and other cancers in human and other animals (International Agency for Research on Cancer [IARC], 2002). The toxins are also associated with poor growth outcomes, especially stunting in young children (Gong et al., 2004, Turner et al., 2007). It is indicated that about 25% of the world's food could be contaminated with aflatoxins (FAO, 2004) and about 4.5 billion people globally exposed to aflatoxins through contaminated foods (Williams et al., 2004), giving rise to concerns that if not checked, aflatoxin contamination could impair food security and pose a great health risk to consumers, especially in developing countries (United States Agency for International Development [USAID], 2012). Prior to the review by Williams and colleagues (2004), aflatoxin was not accorded much attention, yet adverse effects may result from long term exposure even to low levels of the toxin in the food supply chain (Food and Agriculture Administration [FDA], 1997).

Many of the dietary staples like maize, rice, wheat, sorghum, millet, beans, corn, cassava, nuts, groundnuts, in developing countries could be contaminated with aflatoxin (Williams et al., 2004), although highest levels have been reported in maize and groundnuts (Wild & Gong, 2010). Studies carried out in Kenya reveal that more than 40% of diets in both rural and urban communities are likely to be contaminated by the aflatoxins (Daniel, 2011; Mwaura, 2011). This suggests that a big proportion of the Kenyan population risk exposure to aflatoxin and the associated health risk. High levels of aflatoxins in maize have been reported in Eastern Region of Kenya (Centre for Disease Control [CDC], 2004). Cases of aflatoxin contamination of staple foods have also been reported in Nyanza (Collins et al., 2010; Mwaura, 2011) and Western

Regions of Kenya (Mutegi et al., 2007; Alakonya et al., 2009). This points to widespread contamination of foods in other parts of the Kenya apart from Eastern Region. Maize, sorghum, groundnuts, cassava and rice constitute the major staple foods consumed in Kisumu County according to Department of Agriculture (DoA) Annual Report 2015 (DoA, 2015). Although focus on aflatoxin has been confined to maize, the possibility that other commonly consumed foods could be contaminated as suggested by Williams et al. (2004) remains largely unexplored and information on the contribution of other foods to aflatoxin exposure in Kisumu County is inadequate.

Pregnant women may be exposed to aflatoxin through consumption of aflatoxin contaminated foods, posing a health risk not only to the women but also to the unborn fetus and the young child (Castelino et al., 2014). Some of the studies focusing on maternal aflatoxin exposure have been reported by Oveisi et al. (2006); Bhat et al. (2003) and Ofori-Adjei, (2012). However, previous studies have used aflatoxin serum albumin adducts to determine aflatoxin exposure in pregnant women. Dietary intake, although a less sensitive indicator, has been recommended as one of the most practical ways of determining aflatoxin exposure (Williams et al., 2004), and was used to determine aflatoxin exposure in pregnant women in the current study. Actual information on aflatoxin intakes through diet has been shown in West Africa (Gong et al., 2004), the Gambia (Castelino et al., 2014) and Nigeria (Ibeh et al., 2014). In spite of aflatoxicosis outbreak in Kenya (CDC, 2004), information on aflatoxin intake that would enable estimation of exposure of pregnant women through diet in Kisumu County is lacking.

Many young children in the developing world risk exposure to aflatoxins through maternal breast milk and complimentary foods. Exposure of young children to aflatoxins is of great concern given that early childhood environment is critical for growth and disease risk in later life (Barker, 2002) and children who are stunted in growth do not grow to their full potential both physically and cognitively (Ismail et al., 2014). Exposure to aflatoxins has been associated with neurological impairment, immunosuppression and child mortality (World Health Organization [WHO], 2006). Knowledge of the effects of aflatoxins on the growth and health of infants and young children, as stated by Lombard (2014), is important for designing appropriate strategies for management of the toxins.

Globally, malnutrition is the underlying contributing factor in about 45% of the deaths of millions of children under the age of five years (Black et al., 2013), and more than 50% of deaths in this group occur in developing countries (WHO, 2006). According to the Global Nutrition Report by International Food Policy Research Institute (IFPRI), stunting and wasting remain a big challenge globally (IFPRI, 2016). Further, it is noted that out of the Eight Global Nutrition Targets for 2025 adopted by The World Health Assembly, three are geared towards improving child growth, namely; a achieve a 40% reduction in children under 5 years who are stunted; achieve 30% reduction in low birth weight; experiences no increase in overweight in children under 5 years (IFPRI, 2016). These targets can only be realized if measures are put in place to counteract factors that may hinder proper growth and development of young children.

Data by Kenya Demographic and Health Survey [KDHS] 2008-2009 show stunting rates of 26.9% (KNBS & ICF Macro, 2010) for Nyanza region, suggesting the need for more

intervention to meet the World Health Assembly targets by 2025 (IFPRI, 2016). To address factors contributing to malnutrition and morbidity, influence of many factors have been explored, including aflatoxin exposure. Aflatoxin has been known to potentially influence both malnutrition (Wu et al., 2011; Gong et al., 2002) and morbidity of infants (Jiang et al., 2005). Maternal aflatoxin exposure has been implicated in poor growth outcomes in infants, yet previous studies have focused on the period after complimentary feeding. Further, most studies addressing exposure of infants to aflatoxin prior to complimentary have mainly assessed exposure to aflatoxin in women and infants using serum aflatoxin albumin adducts (Shuaib et al., 2012; Jolly et al., 2005), and associations of aflatoxin with growth status in cross sectional studies (Okoth and Ohingo, 2004; Mahdavi et al., 2010), but not in appropriately designed longitudinal studies that are necessary for inference of causation. Conclusive evidence of the role of aflatoxin on growth of infants prior to complimentary feeding is therefore lacking.

Aflatoxin exposure has been associated with increased susceptibility to infections resulting from suppressed immune functions (Turner et al., 2003; Jiang et al., 2005) and death due to acute poisoning (Probst et al., 2007). Both low and high intakes of aflatoxin have been associated with poor health outcomes. Children who suffer from severe acute malnutrition after introduction of complimentary feeds, have been found to be prone to the hazards of dietary aflatoxins (Khlangwiset et al., 2011). Aflatoxin exposure may result in delayed rate of recovery from protein malnutrition due to its effect on protein synthesis, resulting in aflatoxin-induced disruption to Ribonucleic Acid (RNA) in children with severe acute malnutrition (Adhikari et al., 2006). Therefore, aflatoxin could be a setback to interventions aimed at reducing malnutrition in developing countries like Kenya. Kisumu County Integrated Development Plan (KCIDP)

highlights main causes of sicknesses in the County as malaria 44.7%, fever 11.2%, common cold (flu) 5.2% and diarrhea 2.4 % (CGOK, 2013). To reduce infant morbidity and mortality, information on whether or not aflatoxin may contribute to morbidity common in early infancy, especially infections that are common in regions such as Kisumu could provide insights that could be useful in designing appropriate mitigation strategies.

Certain characteristics make Kisumu County prone to aflatoxin contamination: higher levels of aflatoxin contamination have been recorded in foods in some areas of Nyanza Region (Kisumu County is one of the seven counties in Nyanza Region) compared to areas known for frequent outbreak of aflatoxicosis (Collins et al., 2010; Mwaura et al., 2011); some of the foods grown and consumed in Kisumu County, including maize, sorghum and groundnuts are high risk commodities for aflatoxin contamination (Williams et al., 2004); high stunting figures have been reported among children <5 years of age in Nyanza Region (Kenya National Bureau of Statistic [KNBS & ICF Macro, 2014; FAO, 2013) and a possible association has been reported between aflatoxin exposure and wasting in the former Kisumu District (Okoth and Ohingo, 2004); prevailing climatic conditions in Kisumu County including frequent floods, drought, erratic rainfall (1200mm and 1300mm), high temperatures (20^{o}C and 35^{o}C) and high humidity (40 % - 89 %) (County Government of Kisumu [CGOK], 2013), may provide a favorable environment for growth of mould and production of aflatoxins. Kisumu is therefore one of possible appropriate areas where the question of aflatoxins effect on infant growth may be investigated, but such information would be relevant to other aflatoxin-prone areas.

1.2 Statement of the Problem

Kisumu is aflatoxin-prone and levels of stunting of public health significance have been reported. Exposure to aflatoxin has been associated with poor health outcomes, including stunting in young children. The possible contribution of aflatoxin to stunting and other forms of malnutrition, which is implicated in high infant morbidity and mortality, remains inconclusive. Aflatoxin B_1 is the most common and most potent form of aflatoxin. Despite existence of aflatoxin regulatory limits for aflatoxin B_1 by the Kenya Bureau of Standards (KEBS), reports indicate that 1.8 million Kenyans may be chronically exposed to large amounts of aflatoxins through consumption of contaminated staple foods. Most studies assessing aflatoxin contamination in foods have focused on maize with isolated studies on groundnuts. However, evidence suggests potential contamination in other foods that could therefore be sources of human aflatoxin exposure. Information on potential dietary sources of aflatoxin exposure is limited.

Exposure of pregnant women to aflatoxin is of great concern because of the effect it will have not only on the health of the women, but also to the unborn fetus and the young infant. Aflatoxin is transferred from mother to child through breast milk. Data from which to evaluate the extent and severity of biological exposure of humans in Kisumu County is lacking. Further, there is lack of information on aflatoxin intakes in population sub groups, such as pregnant women, that would enable measurement of aflatoxin exposure.

Studies that have established a negative association between aflatoxin exposure and retarded growth in young children have focused on aflatoxin exposure in the complimentary feeding

period, yet exposure of children to aflatoxin may begin in the period prior to introduction to complimentary feeds, through breast milk. There is no conclusive evidence of the role of aflatoxin on growth of infants prior to complimentary feeding. It is not clear whether part of the stunting and other forms of malnutrition reported in Kisumu County could be attributed to aflatoxin exposure. Even though the Kenyan Government requires mothers to exclusively breastfeed their children up to 6 months, majority of the mothers exclusively breastfeed their children for 3 months and only few achieve the target of 6 months; hence the restriction of the assessment to 0-3 months.

Aflatoxin exposure is associated with increased susceptibility to infection in young children. Exposure to aflatoxins has been associated with increased risk of diarrhoea and pneumonia in children, suggesting that aflatoxin may lead to increased morbidity in this sub group. Main causes of sicknesses in the County as malaria, fever, common cold (flu) and diarrhea are the main forms of morbidity reported in infants in Kisumu County. However, the role of aflatoxin on morbidity in young children in Kisumu County has not been established nor the effect of aflatoxin exposure on infant morbidity during the first three months of age.

1.3 General Objective

The study aimed to investigate the sources of aflatoxin exposure and the effects of maternal exposure to aflatoxin on infant's growth and morbidity in the first 3 months of life in Kisumu, County, Kenya.

1.4 Specific Objectives

The specific objectives were to;

1. Determine sources of aflatoxin exposure and aflatoxin levels in selected foods (maize, sorghum, groundnuts, cassava, rice, *dagaa* and milk) in Kisumu County, Kenya.
2. Assess aflatoxin exposure in pregnant women in Kisumu County.
3. Assess the effect of maternal aflatoxin exposure on infant growth indicators (weight, length, WLZ, WAZ and LAZ,) at 3 months of age
4. Assess the effect of maternal aflatoxin exposure on infant morbidity.

1.5 Research Questions and Hypotheses

1.5.1 Research Questions

The central research question that the study endeavored to answer was: Did exposure to aflatoxin in infancy compromise growth in such infants? The study therefore answered the following associated research sub questions.

1. What are the major sources and levels of aflatoxin contamination in selected foods in Kisumu County?

2a. What are the levels of aflatoxin exposure among pregnant women in Kisumu County?

2b. What proportion of pregnant women in Kisumu County consumed aflatoxin levels above 10 ppb through their diet?

1.5.2 Research Hypotheses

1. Maternal aflatoxin exposure had no effect on infant's length, weight, length for age (LAZ), weight for age (WAZ) and weight for length (WLZ) in the first 3 months of life.
2. Maternal aflatoxin exposure had no effect on infant's morbidity at 3 months of age.

1.6 Justification of the Study

Reports indicated that about 4.5 billion people globally (CDC, 2004; Williams et al., 2004) were exposed to aflatoxins through dietary intakes and 1.8 million Kenyans were chronically exposed to large amounts of aflatoxins (WHO, 2006). Cases of aflatoxin contamination in foods and aflatoxicosis have been reported yearly in Eastern Region of Kenya the worst having occurred in 2004 where 317 cases and 125 deaths were reported. However, high levels of aflatoxin have been reported in maize in Nyanza compared to Eastern Region, which has been assumed to have the highest levels of aflatoxin contamination in food (Mwaura, 2011), causing concern that other parts of the country could be affected, other than Eastern Region. Data from KDHS 2014 survey indicate that 18% of children < 5 years in Kisumu County are stunted, and stunting has been linked to aflatoxin exposure. This is of concern given that exposure to aflatoxin may impact negatively on the health of consumers, especially young children who may be very susceptible to the effects of the toxin. A prospective cohort study was necessary to establish such relationship; the strength of the current study. Evidence from literature review suggest that 45% of households in Kisumu County could be exposed to aflatoxin, which is a threat to achievement of Sustainable Development Goals (SDGs)) 1, 2 and 3 on: end poverty in all its forms; eradication of hunger and achieve food security and improved nutrition; and ensure healthy lives and promote well-

being for all and at all ages respectively, as envisaged by Post 2015 Development Framework (FAO, 2013) and to the achievement of economic, social and political pillars of the Vision 2030. The Kenya Government formulated the National Food and Nutrition Security (NFSN) Policy (GoK, 2011), to support integrated, multi-intervention strategies to eliminate all micronutrient deficiencies for all ages and life stage groups. However, aflatoxin exposure could be a threat to the realization of objectives of this policy. The finding of this study would be useful for the County and National Government to design policies, programs and interventions for ensuring access to healthy and nutritious foods, especially for pregnant women and young children. The ultimate goal would be to achieve improved growth and reduced morbidity among infants and young children in Kisumu County and nationwide.

1.7 Significance of the Study

Findings from this study provided evidence that all the sampled foods were contaminated with varying levels of aflatoxin, but sorghum had the highest levels of contamination compared to other foods. The study also established that processed milk had higher levels of aflatoxin compared to raw milk. These findings are important because they provided evidence on the extent of aflatoxin contamination in foods that could spur action to reduce exposure to the toxins and the associated risks on growth and health outcomes of infants not just within the community in Kisumu County, but also in Kenya as a whole. The Agriculture and Health sectors could use the generated evidence to justify budgetary allocation for implementation of appropriate interventional measures against incidences of aflatoxin contamination in the Kisumu County and other parts of Kenya.

Aflatoxin exposure may lead to undernutrition resulting in poor growth, especially in young children. It is reported that failing to address under-nutrition may result in 2-3% losses in national Gross Domestic Product (GDP); reduced physical productivity of the work force; reduced cognitive development; and poor reproductive performance in women. Collectively, these factors could lead to the inter-generational transmission of under-nutrition and poverty (International Food Policy Research Institute [IFPRI] & the Enteric and Diarrheoal Diseases [EDD] & Agriculture and Nutrition Teams at the Bill & Belinda Gates Foundation [BMGF], [IFPRI, EDD, BMGF], 2012). Despite the aforementioned concerns, there was a paucity of human studies on the effect of aflatoxin exposure on growth in children 0 – 3 months of age, hence the findings of the current study provided a much-needed contribution of knowledge on aflatoxins and growth in infancy. Further, the findings of this study provided evidence based data that would inform government authorities on the levels of aflatoxin contamination in foods and the effects on infant growth. The findings of the current study further established that aflatoxin had an effect on morbidity of infants, and specifically, diarrheoa and malaria, which would influence appropriate mitigation measures.

1.8 Assumptions

This study assumed that establishment of dietary aflatoxin intake above 10 ppb translated to exposure to the toxin at levels that could influence growth in infants. This assumption was based on the 30 - 60 day half-life of serological aflatoxin albumin adduct levels observed in an earlier study (Wild et al., 1992), indicating that aflatoxin lingers in the body for relatively long periods of time.

1.9 Scope of the Study

This study investigated the effect of maternal aflatoxin exposure on growth and morbidity of infants 0-3 months of age. The study was conducted in Kisumu East Sub and Nyando Sub-Counties in Kisumu County, Kenya, and adopted both cross sectional survey and prospective cohort study design.

1.10 Limitations of the Study

The study used a prospective cohort design which according to (Creswell, 2009) is susceptible to confounding due to non-randomness in sampling. However, it is the only justifiable design when randomization is not feasible, such as in studies where the exposure of interest is harmful. Assessment of effects of toxins can only be compared in individuals who are already naturally exposed with those not exposed. Potential confounding was limited by matching infants of exposed women with those of non exposed women for age and monthly household income (factors which were found to predict aflatoxin intake at baseline). To further address confounding, other potential confounders were controlled for in the determinations of effect of aflatoxin on growth and morbidity of infants 0-3 months of age.

Exposure assessment using diet alone may limit the interpretation of an effect of aflatoxin. However, we used usual intake based on one day's meals combined with information on frequency of consumption, which reflects usual intake. Although a role of genetic factors may not be ruled out, our findings are consistent with those of studies that are based on aflatoxin-

albumin adducts in older children, hence support an effect. However, the absolute magnitudes of effect estimates should be interpreted with caution.

Though foods from the market were collected only once and therefore seasonality variations were not taken into account, this has been suggested as an area of future research. The study presented aflatoxin levels in foods available in the households and markets and did not establish whether the problem was from storage conditions or arrived in the markets already contaminated with aflatoxin.

CHAPTER TWO: LITERATURE REVIEW

2.1 Sources of Aflatoxin Contamination

2.1.1 An Overview of Aflatoxin Contamination in Foods

Aflatoxins are naturally occurring carcinogenic toxins associated with poor growth outcomes (Gong et al., 2004; Yu et al., 2004; Tang et al., 2009) and suppressed immune functions (Turner et al., 2003), especially stunting in young children. Aflatoxin are widely spread in foods and the environment (Gürbay et al., 2010) and are considered as unavoidable contaminants of foods. The toxins are produced by fungal action during food production, harvest, storage and processing (Williams et al., 2004) and affect a large proportion of world's staple foods especially maize and groundnuts (Wild and Gong, 2010; Wu and Khlangwiset, 2010; Wu, (2015). Contamination of food supplies by aflatoxin is of particular concern in rural communities of developing countries (Antonius et al., 2005; Bhat et al., 2003), where high levels have been found in staple foods, causing concerns about the quality of the food and the health implications to the consumers.

Aflatoxins are heat resistant and are not destroyed by normal industrial processing or cooking (Creppy, 2002) and the presence of the toxins in food products even at lowest levels may pose a health risk to consumers. Many of the developing countries dietary staples like rice, wheat, sorghum, millet, beans, corn, cassava, and groundnuts may be susceptible to aflatoxin contamination, however, highest levels have been established in maize, groundnuts and cotton seed (Williams et al., 2004). Due to high levels of food insecurity in developing countries, consumers feed on mouldy food especially during hunger periods (Gong et al., 2008). Therefore, if not checked, as stated by Miraglia et al. (2009), aflatoxin contamination could impair food security and health in developing countries.

Maize is a major staple food constituting a bigger proportion of the diet in many developing countries, including Kenya. Any contamination through maize may affect a large percentage of people, including young children, through the food chain. Maize, sorghum, groundnuts, cassava and rice constitute the major staple diet consumed in Kisumu County (DoA, 2015), and are likely to be contaminated with aflatoxins. However, emphasis in most researches on aflatoxin contamination levels in Kenya have been conducted in Eastern Region and mainly focused on maize (CDC, 2004; Lewis et al., 2005; Mwihia et al., 2008; Muthomi et al., 2009). Given that other foods could also be at risk of aflatoxin contamination and exposure even to low levels of the toxins may have long term effect on health, studies aimed at determining aflatoxin levels in staple foods that constitute the diet of Kisumu County, and other parts of the Kenya, ought to be encouraged.

Animals may feed on contaminated feeds, thereby exposing consumers to the toxin through contaminated products such as eggs, milk, meat and fish (Oveisi et al., 2006). The main sources of aflatoxins in animal feeds are groundnut meal, maize and cottonseed meal (Henry et al., 2001). Consumption of contaminated feeds by animals exposes consumers to the toxin through the animal based food chains. A study carried out to detect and quantify the amount of AFM_1 in raw cow's milk and AFB1 in dairy feed samples in the greater Addis Ababa milk shade, Ethiopia, found the presence of aflatoxin M_1 in all milk samples with contamination levels ranging from 0.028 to 4.98 μg/L and 26.3% of the samples exceeded 0.5 μg/L; all the feed samples were contaminated with aflatoxin ranging between 7 and 419 μg/kg (Gizachew et al., 2016). Another study carried out in Cameroon to determine aflatoxin contamination in food and body fluids in relation to malnutrition and cancer status in a four-year survey (1991–1995),

established that 45.2% of the egg samples, 15.9 % of the cow raw milk samples and 4.8% of breast milk samples were contaminated with aflatoxin (Tchana et al., 2010). Reports on aflatoxin contamination in animal feeds and foods have also been reported in Kenya (Lunyasunya et al., 2005; Kangethe and Langa, 2009; Dora et al., 2015). This implies that consumer in Kenya are likely to be exposed to aflatoxins though consumption of crop based and animal based food products; and levels of aflatoxin in this foods need to be established.

Research determining exposure of aflatoxin in aquatic species has reported that the toxins pose a risk to performance and health of fish; exposing consumers to the toxins through the fish food chain (Spring and Daniel, 2005). In a study carried out in Rio de Janeiro State, Brazil, to determine species of the fungal genera Aspergillus, Fusarium, Penicillium and Fumonisim B_1 (FB1), Aflatoxin B_1 (AFB_1) and Ochratoxin (OTA) from feed intended for fish farms, it was established that 55% of the sample of 60 tilapia fish were contaminated with AFB_1 (Barbosa et al., 2013). The aforementioned studies confirm the presence of aflatoxin in animal based foods and aflatoxin contamination through feed which may find its way into the human diet, potentially endangering the health of consumers; and the need to carry out studies to establish the levels of the toxins in other animal based foods.

2.1.2 Strains of Aflatoxins

There are about 20 strains of aflatoxins currently in nature but the four most common are; B_1, B_2, G_1 and G_2 and two additional metabolic products; M_1 and M_2 (Food and Agricultural Organization [FAO], 2004; Garrow et al., 2000) . Aflatoxin M_1 and M_2 were first isolated from milk of lactating animals fed on aflatoxin infected feeds, hence the M designation. Aflatoxin B_1

and B_2 designations resulted from exhibitions of blue fluorescence under UV-light, while G designation was derived from the yellow-green fluorescence exhibited under UV-light (Stroka and Anklam, 2002; .MoA, 2010). However, aflatoxin B_1 (AFB_1), found mainly in cereals and groundnuts is considered the most potent and most predominant and is produced by both *Aspergillus flavus* and *Aspergillus parasiticu*s (Lee et al., 2004; Wild et al., 2002).

Aflatoxin M_1 is the major metabolite found in human breast milk (Mahdavi et al., 2010; Sadeghi et al., 2009) and in milk of lactating animals (Keskin et al., 2009). Like other forms of aflatoxin, the metabolite is not affected by pasteurization and ultra-high-temperature (UHT) treatment or processing (Unusan, 2006; Yaroglu et al., 2005) and heat treatments do not change the amount of aflatoxin M_1 in milk products (Henry et al., 2001). Any intervention to reduce the effect of aflatoxin should therefore focus at the entire food chain to ensure minimal amounts of the toxins find their way into the foods. Aflatoxin B_1 and aflatoxin M_1 have been classified by the International Agency for Research on Cancer (IARC) as primary group (group 1A) and secondary group (group 2B) carcinogen agents respectively (IARC, 2002; Keskin et al., 2009). Aflatoxin B_1 is the most potent form common in solid food and aflatoxin $M_{1,}$ a metabolite of Aflatoxin B_1, is the most common in animal and human milk, and therefore the focus of the current study. The chemical structures of aflatoxin B_1, G_1, B_2, G_2 are shown in Figure 2.1.

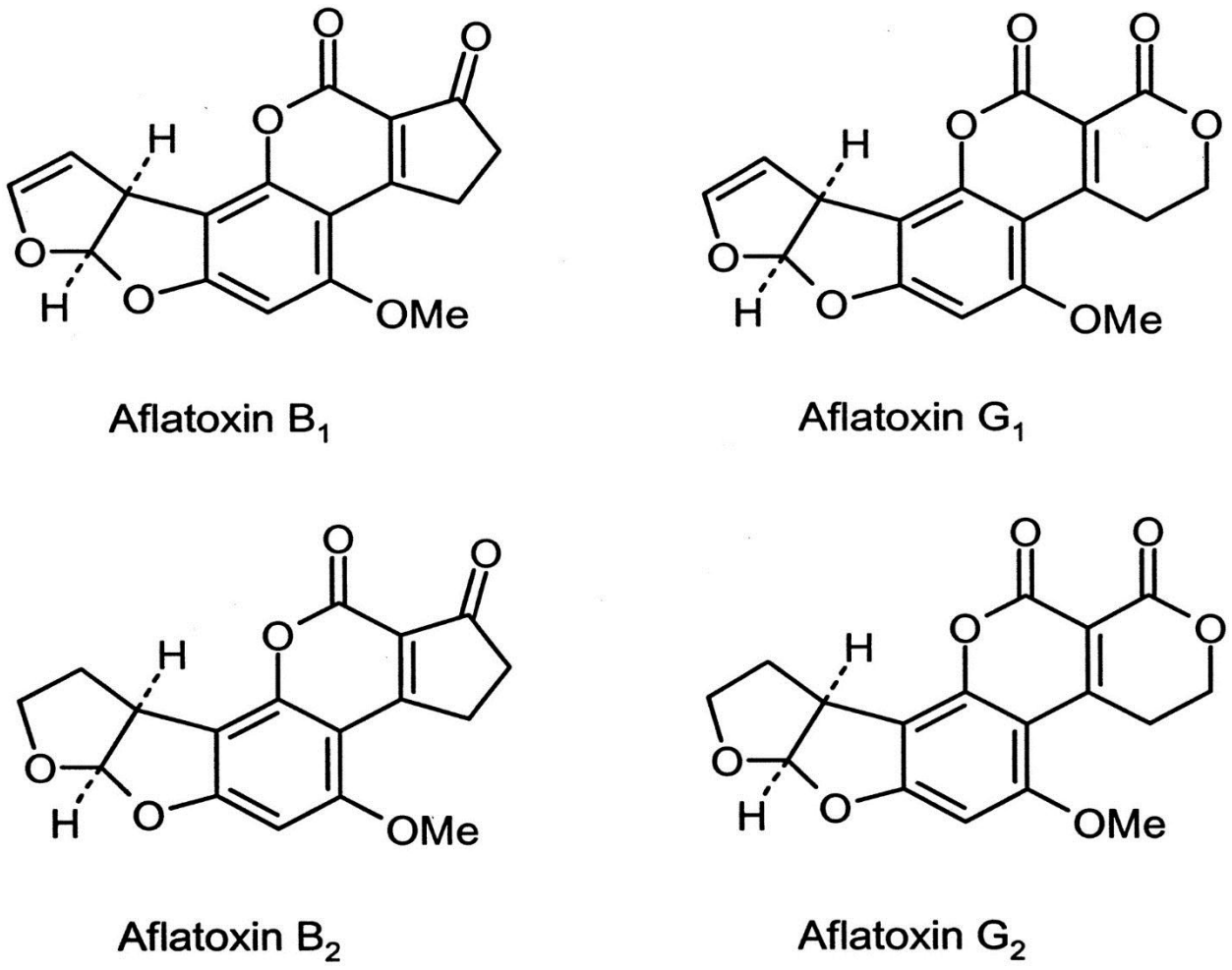


Figure 2.1 Chemical Structures of Aflatoxins (Williams et al., 2004)

2.1.3 Conditions Favoring Aflatoxin Production

Several factors play a key role in determining mould infestation and toxin production, among them; humid climate, moisture stress, high temperature stress, poor agronomic practices, wrong choice of varieties and insect damage of the host plant (IARC, 2002; Vellidis, 2006, Wagacha et al., 2008; Williams et al., 2004; Magda and Pavel, 2006). Temperatures of 10 - 40^{o}C; humidity of 65 - 70%; and drought conditions provide ideal environment for the growth of the fungi. Drought stress may affect proper development of maize kernels and other crops, increasing

susceptibility to aflatoxin contamination (MoA, 2010). This is because when there is inadequate moisture, the crop is unable to develop fully to maturity making it susceptible to pest and mould attack.

Changing weather patterns may be a factor in mould infestation of staple foods and may have a negative impact on food security of a country (Anderson et al., 2004; Luo et al., 2005; Van der Fels-Klerx et al., 2009; Russell et al., 2010). For instance, too much rain during harvesting period may present challenges in drying the produce to optimal moisture levels, which may make it susceptible to mould infestation. Season has also been identified as an important factor in determining aflatoxin exposure in foods (Wild et al., 2002; Cotty and Jaime-Garcia, 2007) and in some regions like The Gambia, aflatoxin has been reported to be highest at harvest and during dry seasons (Castelino et al., 2014); and in Egypt aflatoxin contamination was more prevalent during summer months of May-September, with highest rates in June (Nektaria, 2007). Aflatoxin outbreaks in Kenya in 1981 and 2004 occurred in areas where drought and food shortages were followed by unusual rainy season during harvest (CDC, 2004). The climatic conditions described herein are likely to be found in Kisumu County (CGoK, 2013); making it prone to contamination of food with aflatoxins. Seasonal variation may therefore determine levels of aflatoxin in market and household foods and a study focusing on effect of seasonal variations on aflatoxin exposure would be appropriate for Kenya and other parts of the Country.

Aflatoxin contamination cannot be easily prevented without significant investment in production, drying and storage facilities (Williams et al., 2004). This is a challenge for developing countries with limited and overstretched resources. Aflatoxin contamination may occur both at pre-harvest

and post-harvest of crops (MoA, 2010; Shuaib et al., 2012). Among the known causes of pre-harvest aflatoxin contamination are infestation of crops by fungal diseases, drought stress and floods, unsuitability of crop for particular climate, insect damage and poor agricultural practices (Cotty and Jaime-Garcia, 2007; Wu and Khlangwiset, 2010). On the other hand, postharvest contamination can result from inappropriate post-harvest practices such as poor storage, transportation and processing. Long term storage of food produce in hot humid conditions, as stated by Gong et al. (2008) and Magan et al. (2003), result in fungal proliferation and increased post-harvest toxin contamination.

Food needs to be properly dried after harvesting, treated with appropriate pesticides and stored in clean and well aerated stores in order to minimize attack by moulds and pests. A study carried out by Azziz-Baumgartner et al., (2004) in Eastern Region, Kenya, revealed that maize grain stored inside houses was more likely to be contaminated with aflatoxin than maize stored outside in granaries. The warm environment inside the houses and storage of maize on dirty floor may also precipitate fungal growth in maize kernels (MoA, 2007). A study carried out in Western Kenya established that groundnuts stored in polypropylene and polyethylene bags were more likely to be contaminated compared to those stored in jute bags (Wagacha et al., 2013). Therefore, both pre-harvest and post-harvest management of crop produce are important management factors in the preventing and controlling aflatoxin levels in food. Most households in Kenya, as reported by MoA (2010), are likely to store their grains inside the houses without proper treatment and to use polypropylene and polyethylene bags, exposing the food to contamination by aflatoxins.

Prevailing climatic conditions in Kisumu County including erratic rainfall ranging from 1200 mm and 1300 mm; temperatures of 20^{o}C and 35^{o}C, humidity of 40% – 89%, drought and frequent floods (County Government of Kisumu [CGOK], 2013) provide conducive environment for fungal growth and production of aflatoxins. However, limited data is available on levels of aflatoxin contamination in common staple foods that constitute the diet consumed in Kisumu County.

2.1.4 Aflatoxin Regulatory Limits for Human Food

Various countries and international bodies have developed maximum tolerable aflatoxin limits in human food. The purpose of this is to minimize levels of the toxins in foods and consequently reduce the effect of the toxin on human health. Aflatoxin B_1 regulatory limits in foods have been set as follows: Codex Alimentarius, 9 ppb (Codex Alimentarius, 2004); the European Commission [EC], 2 ppb (EC, 2001); Kenya 10 ppb (Kenya Bureau of Standards [KEBS], 2013); South Africa, 5 ppb (Medical Research Council [MRC], 2001). Regulatory limits have also been set for aflatoxin M_1: Codex Alimentarius, 0.5 ppb (0.05 ppb in countries with the most stringent regulations (Codex Alimentarius, 2000, 2004); Switzerland, 0.01ppb (Codex Alimentarius, 2004). Although the Kenya Bureau of Standards has set the regulatory limit for aflatoxin B_1 levels in solid foods at 10 ppb, there is no set regulatory limit for aflatoxin M_1 in milk. Strict measures need to be instituted to ensure that aflatoxin levels in foods are at a limit that may not be injurious to the consumers, especially young children who are vulnerable. Given that milk is the first complimentary feed for most children and almost forms part of the diet of many households in Kenya, the KEBS should set acceptable regulatory limits for aflatoxin M_1 in

Kenya to protect the health of consumers, especially the infants and young children who according to KNBS and ICF Micro (2014), may be weaned to cow's milk early in life.

2.1.5 Aflatoxin Contamination in Kenya

2.1.5.1 Summary of Aflatoxin Incidences in Kenya

Cases of aflatoxin outbreaks from consumption of highly contaminated foods have been reported in many countries among them; India and Thailand (CAST, 2003; CDC, 2004). Reports of aflatoxin contamination in foods have also been reported yearly in Kenya (Table 2.1). One of the highest aflatoxicosis outbreaks worldwide resulting from consumption of contaminated maize occurred in Kenya in 2004 (Azziz-Baumgartner et al., 2004; CDC, 2004; Lewis et al., 2005).

Some of the studies that have reported cases of aflatoxin contamination in foods in Eastern Region of Kenya include: Centers for Disease Control [CDC], (2004) investigated the outbreak of jaundice with case-fatality rate (CFR) in Sub-Counties of Makueni and Kitui, Eastern Region, high levels of aflatoxin (up to 8000 ppb) in foods collected from the affected areas and a total of 317 cases and 125 deaths were reported; Lewis et al. (2005) assessed the extent of maize contamination and evaluated the relationship between market maize and aflatoxicosis outbreak in Eastern Region; 55% of the maize products had aflatoxin levels above 20 ppb: Probst et al. (2007) undertook a study to determine the primary causal agents of the 2004 contamination events in Kenya; considering both fungal aflatoxin-producing potential and frequency of occurrence in the contaminated crop; aflatoxin contamination ranged from 0.27 ppb to 4,400 ppb total aflatoxin: Mwihia et al., (2008) carried a study to determine aflatoxin levels in household maize in Makueni Sub-County and correlate aflatoxin levels to maize drying and storage

practices; 35.5% of the maize samples had aflatoxin levels above the regulatory limit: Muture et al. (2005) assessed the magnitude of aflatoxin contamination of maize and maize products in the affected areas in Eastern Region of Kenya, 57.7% of cases and 21.7% of controls had levels beyond 1000µg/kg and the amount of aflatoxin observed in the food samples had a range of 0-58,000 µg/kg. These studies confirm that most of the research on aflatoxin in Kenya were carried out in Eastern Region and mainly focused on maize.

Studies on aflatoxin contamination in foods have also been carried out in Western and Nyanza Regions of Kenya: Okoth and Ohingo, 2004 carried out a cross sectional study to determine association between aflatoxin levels in weaning flours and nutritional state of children in Kisumu District. The results revealed an association between aflatoxin and wasting, but not stunting and underweight, in infants fed on aflatoxin contaminated weaning flours. A study carried out in South Nyanza and Eastern Region to assess aflatoxin levels in maize in the two Regions (Mwaura, 2011), revealed that 31% of maize from farmers' fields from Eastern compared to 40% from South Nyanza had aflatoxin levels above 10 ppb; and 38% of the samples from the farmers' stores in Eastern Region compared to 60 % from South Nyanza had aflatoxin levels above the regulatory levels. Wagacha et al., (2013) assessed the effect of storage bags, temperature, relative humidity on fungal population and aflatoxin contamination in groundnuts in Western Kenya; there were significant differences in moisture content of groundnut samples stored under different temperature and relative humidity conditions and storage bags. These findings suggest that Nyanza Region, where Kisumu County is located, could be at risk of aflatoxin contamination, yet the focus has been on Eastern Region. This also implies that other regions in

Kenya could also be at risk of exposure to aflatoxin,; therefore, the status of aflatoxin exposure in other regions need to be established and appropriate mitigation measures put in place.

The Kenya Ministry of Agriculture (MoA) has outlined some of the incidences of aflatoxin contamination in the country over the years as shown in Table 2.1.

Table 2.1 Summary of Aflatoxin Incidences in Kenya

Year	Aflatoxin Incidence
1981	The first 20 cases of human aflatoxicosis were reported in Eastern Region, resulting in 2 deaths.
2003	68 people died of aflatoxin poisoning in Eastern Region
2004	125 people in Eastern Region out of 317 reported cases died of aflatoxin poisoning.
2005	32 out of 75 of the reported cases of aflatoxin poisoning in Eastern Region were fatal.
2006	28 out of 71 cases reported in Makueni and Machakos Sub-Counties were fatal.
2007	21 cases were fatal in Makueni.
2008	2 cases were fatal in Kibwezi.
2009	Over 32,000 bags of stored maize were condemned in Ishiara and Bura irrigation schemes. However no fatal cases were identified.
2010	2.3 million bags of maize were contaminated by the aflatoxin.

Source: (MoA, 2010).

2.1.5.2 Aflatoxin in Staple Foods in Kenya

In an effort to curb the levels of aflatoxin in foods, the Kenya National Cereals and Produce Board (NCPB) in 2010 suspended purchase of maize after detecting high levels of aflatoxin in maize purchased in Machakos, Emali, Makueni, Konza, Thika and Kithimani depots in April 2010 (MoA, 2010). The mean levels of aflatoxin contamination in these areas were 74.3 ppb,

70.7 ppb, 64.3 ppb, 69.9 ppb, 14.4 ppb and 28.5 ppb respectively. This suggests the need to establish aflatoxin levels in staple foods in different parts of the Country in order to inform policy interventions by the National and County Governments.

Most households in Kisumu obtain their food products from own farms and from the markets (DoA, 2015). It is documented that any effort to combat exposure to aflatoxin must consider the potential role of the market system in sustaining exposure (Lewis et al., 2005, Okoth & Kola, 2012). Most staple foods in Kenya are marketed through informal marketing systems where products are seldom tested for aflatoxin contamination (Felicia, 2004). The major food markets in Kisumu County include; Kibuye whole sale market, Kibuye open air market, Oile market, Mamboleo market, Ahero market, Kiboswa market, Holo market (Personal communication with Kisumu East District Crops Officer, 2014). Investment in drying, and storage facilities is crucial for prevention of aflatoxin contamination in market foods (William et al., 2004). Most of the markets in Kisumu do not have appropriate drying and storage facilities, potentially exposing the food products to extreme weather conditions and aflatoxin contamination.

Some of the foods consumed in the Region come from the neighboring regions, which could be another source of aflatoxin contamination (Table 2.3). Further, testing of food for moisture content and aflatoxin levels is not carried out by the Agricultural Officers due to lack of the necessary equipment (Personal communication with Kisumu East District Crops Officer, 2014). The Kenya Bureau of Standards (KEBS) only carries out aflatoxin analysis of milled grains products, animal feeds and composite flours monthly through market surveillance, but not dry grain products (personal interview with KEBS Quality Assurance Officer, 2014). Routine daily

analysis is only carried out for clients who bring their products for analysis and certification. Maize, sorghum, groundnuts, cassava, *dagaa,* and rice constitute the major staple foods consumed in Kisumu County (DoA, 2015). These foods, including milk, are also used as complimentary foods in Kisumu County yet their potential to expose infants to aflatoxin has not been established. Priority in determining exposure to aflatoxin in Kisumu County should focus on staples foods and milk, given their high consumption and their use in infant complementary feeding.

2.1.5.3 Aflatoxin in Animal Feeds and Foods in Kenya

Consumption of contaminated animal feeds by animals may expose consumers to the toxin through contaminated animal products (Oveisi et al., 2006, Lunyasunya et al., 2005). Dairy animals also feed on improved pastures such napier grass, fodder shrubs and legumes, which can be contaminated with aflatoxins through soils (Lunyasunya et al., 2005). It is reported that most animal feed manufacturers rarely tests imported raw animal feed materials for aflatoxins and the Kenya Bureau of Standards does not remit results of aflatoxin in feeds regularly (Kangethe and Langa, 2009). A study carried out in Kenya to highlight existing danger of mycotoxin contamination of dairy feeds, found that aflatoxin B_1 was one of the most widely occurring and dangerous of all mycotoxins found in animal feeds (Lunyasunya et al., 2005).

Another study initiated to assess the knowledge and practices of urban dairy farmers and feed millers about aflatoxin in feeds and milk, determine the prevalence and quantify the levels of AFB_1 and AFM_1 in animal feeds and milk respectively from urban environs in Kenya, reported

that 86% percent of the feed samples from farmers were positive for aflatoxin B_1 and 67% of these exceeded the FAO/WHO level of 5µg/Kg-1, and 81% of the feed samples from feed millers and 87% from agrochemical shops were positive, while 58% and 66% of the positive samples exceeded the FAO/WHO limits respectively (Kangethe and Langa, 2009). The study by Kangethe and Langa further established that 72% of the milk from dairy farmers, 84% from large and medium scale farmers, and 99% of the pasteurized marketed milk were positive for aflatoxin M_1, and 20%, 35% and 31% of positive milk from dairy farmers, medium and large scale farmers, and market outlets respectively, exceeded the WHO/FAO levels of 0.05µ g/Kg-1.

According to report by Abila (2000), Nile Perch and *dagaa* together constitute over 90% of fish of Lake Victoria and fish is central to the lakeside communities as a rich protein food and a solution to the protein deficiency conditions affecting children in the lake area (Abila, 2000). A study carried out to investigate consumer fish preferences and trends in demand for Nile Tilapia and African catfish in five urban centres in Kenya, established that women consumed more fish than their male counterpart and recommended that the government should educate consumers about the safety, healthiness and nutritional value of aquaculture products (Githukia et al., 2014). The findings of the study by Githukia and colleagues implied that women could be at a higher risk of aflatoxin contamination from fish than their male counterparts and the need to ensure that fish consumption does not impact negatively on women health. In a recent study carried out in Winam Gulf of Kenya to establish determinants of carcinogenic polyclic aromatic hydrocarbons, aflatoxins and nitrosamines in processed fish, aflatoxins mean concentration of 0.33 – 1.58 ppb were found in sun dried *dagaa* and daily intake of aflatoxins through consumption of *dagaa* was estimated at 0.0079 ug/kg during rainy season (Dora et al., 2015).

The aforementioned findings confirm the presence of aflatoxin in animal based food and aflatoxin contamination through animal feed which may find its way into the human food chain, potentially endangering the health of consumers; and therefore the need to determine the aflatoxin in such foods.

2.1.6 Interventions on Aflatoxin Contamination in Kenya

The Kenya Ministry of Agriculture has put measures in place to minimize levels and effects of aflatoxin contamination in the country (MoA, 2010). These measures include: provision of aflatoxin surveillance and monitoring equipment, capacity building of extension workers and sensitization of farmers. Between 2004 and 2010, a total of 820 extension workers were trained and 236,877 farmers sensitized on sound grain and pulse conditioning techniques before storage. Further, 153,000 posters on aflatoxin awareness were produced and distributed in aflatoxin prone areas. Routine surveillance of food and feed stores is now mandatory and all grain handlers are required to be registered. Trained extension officers have been equipped with simple moisture detecting equipment for monitoring quality of grains. The MoA has equipped each Sub-County in aflatoxin prone areas with at least 10 moisture meters. A technical committee to assess aflatoxin contamination and develop possible intervention was formed in 2010, comprising of MoA, MoPHS, KEBS, KEPHIS, and KALRO (MoA, 2010).

In January 2010, the MoA undertook the following measures to counteract cases of aflatoxin contamination in the Country: issued a National Aflatoxin Alert to all Provincial Directors of Agriculture and District Agricultural Officers to mitigate against possible aflatoxin outbreak by

up scaling campaigns on proper post-harvest management of produce; conducted a national crop storage needs assessment survey in January 2010 necessitated by the good harvest and the prevailing wet weather conditions at that time; recorded 3 radio programmes on crop post-harvest management, one in *Kikamba* (the local language in the Region most affected by aflatoxin contamination) and 3 in Kiswahili *(Sikio la Mkulima),* the national language (MoA, 2010).

According to the National Food Security and Nutrition Policy (The Government of Kenya, GOK, 2011), the Kenya Government has undertaken to support integrated, multi-intervention strategies to eliminate all vitamin and mineral deficiencies for all ages and life stage groups. However, despite these measures, aflatoxin exposure continues to be a problem in Kenya. Further, the interventions on aflatoxin were only limited to the supposedly aflatoxin prone areas with an assumption that other areas in the country are not affected. As indicated, focus of aflatoxin intervention has not considered areas such as Kisumu which has shown to have comparable, if not greater, exposure to aflatoxin compared to Eastern Region.

2.1.7 Kisumu County Food Production Status

According to the Kisumu County, Department of Agriculture Annual report (DoA, 2015), maize, sorghum, rice and cassava are among the major food crops being promoted in the County and could therefore result in increased production and consumption of these foods. Although some of these foods have been found to be susceptible to aflatoxin contamination (Collins et al., 2010), aflatoxin levels in these foods in Kisumu County have not been established. The production figures for these crops for 2015 in Kisumu County are shown in Table 2.2.

There was an increase in rice production due to greater involvement by Food and Agriculture Organization (FAO), the National Irrigation Board (NIB), Economic stimulus package, the Agricultural Credit Facility by Equity bank, and currently (in 2016) by support from the Kisumu County Government, which has put food security as number one priority (CGOK, 2013). Cassava, rice and sorghum are being promoted as food security crops by the Department of Agriculture with expected increase in production and consumption. These foods were also likely to be contaminated with aflatoxin based on findings from literature review (Williams et al., 2004).

Table 2.2 Kisumu County Selected Food Production 2015 Figures

Crop	Annual food requirements (90 kg bags)	Annual production (90 kg bags)	Surplus/deficit (90 kg bags)
Maize	1,171,548	536,960	-634,588
Sorghum	429,240	201,300	-227,940
Rice	186,408	450,150	+263,743
Beans	214,932	51,450	-163,482
Cassava	134,420MT	32,250MT	-102,170MT

Source: DoA (2015).

The Kisumu County only produced 536,960 bags of maize against annual requirement of 1,171,548 bag million bags and the deficit had to be imported from neighbouring counties and countries. This could be a possible source of aflatoxin contamination. Cross border trade study carried out in Nyanza Region revealed an influx of contaminated grains through Busia and Esebania Borders into Kenya (Obade et al., 2007, unpublished). Some of these contaminated

foods end up in major markets in Kisumu County and could contribute to the levels of aflatoxin in foods consumed in the County. Table 2.3 shows other sources of some of the foods consumed in Kisumu County.

Table 2. 3 Other Sources of Food Consumed in Kisumu County

Food Item	Source of food
Dagaa	Homa Bay, Migori, Siaya, Bondo
Rice	Busia, Tanzania, Ahero, Dominion farms
Groundnuts	Uganda, Busia, Homa Bay, Kisumu East
Cassava	Busia, Tanzania, Kisumu East, Siaya
Maize	Rift Valley, Busia, Molo, Migori
Sorghum	Busia, Uyoma, Migori, Siaya
Processed milk	Super markets
Raw milk	Nandi, Kericho, Bomet

Source: DoA, 2015

Kenya Agricultural and Livestock Research Organization (KALRO) has been mandated to provide farmers with rice and sorghum seeds. This has resulted in increased productivity and consumption of these foods, which may be susceptible to aflatoxin contamination. A report from the then Ministry of Fisheries Development (MoFD) revealed that Nyanza produced 90% of the total fish in the Country with *Rastrienobola argentea* (*dagaa*) contributing 47% of the fish produced in Nyanza and d*agaa* is one of the cheap sources of protein that is consumed by many Kenyans (Abila, 2000; MoFD, 2012), as well as a major ingredient in animal feeds.. In 2010, 70,000 tons of *dagaa* was produced, 70% of which went to feed industry and 30% for human consumption (MoFD, 2012). Any contamination that finds its way into feeds ends up in human consumption through the food chain.

A variety of the staple foods produced and consumed in the region, which are also used as complimentary foods, could be contaminated with aflatoxin. Data on aflatoxin contamination in Kisumu County is lacking and only one study conducted a research on aflatoxin contamination of complimentary flours (Okoth and Ohingo, 2004). Consequently, there is paucity of information on aflatoxin levels in other commonly consumed foods including *dagaa,* maize, rice, groundnuts, sorghum, cassava and milk, which can inform the relevant authorities and policy makers on the magnitude of contamination from such foods.

2.1.8 Food Consumption Patterns in Kisumu County

Maize meal consumption remains the predominant preserve of majority of Kenyans, especially the poor (Muyanga et al., 2004). Maize consumption in Kenya has been estimated to be 98 kilograms per person per year (Nyoro et al., 2004). These views concur with the findings of a study carried out at Chulaimbo Sub-District Hospital, Kenya to assess the nutrient intake and nutrient status of HIV seropositive patients attending an AIDS outpatient clinic, which found that on average the respondents took three meals per day, and there was high consumption of carbohydrate sources like maize meal, with low nutritive value; monthly income was a strong and significant predictor of diet diversity and majority of the respondents with monthly income below one dollar a day had an inadequate dietary the intake (Onyango et al., 2012). Therefore, the poor are more likely to eat food of poor quality and of low nutritive values.

A study carried out to investigate consumer fish preferences and trends in demand for Nile tilapia and African catfish in five urban centres in Kenya, established that women consumed more fish than their male counterparts. The study recommended that in order to promote

preference and consumption of farmed fish in Kenya, the government should educate consumers about the safety, healthiness and nutritional value of aquaculture products (Githukia et al., 2014). Therefore, women are more likely to be exposed to aflatoxin from contaminated fish compared to the male counterparts. According to report by Abila (2000), Nile perch and *dagaa* together constitute over 90% of fish of Lake Victoria and fish is central to the food insecurity problem for lakeside communities as a rich protein food, and a solutions to the protein deficiency conditions affecting children in the lake area (Abila, 2000). Consumption of cereals and animal based foods maize has been associated with exposure to aflatoxin. Information on levels of aflatoxin in most of the staple food in Kisumu County has not been established.

Milk consumption in Kenya was projected at 4.1 billion litres in 2014, the highest in Sub-Sahara Africa (Njurui et al., 2009). The annual per capita consumption at household level in the rural areas was 45 litres for milk – producing households and 19 litres for 'milk – purchasing' households and 145 litres for urban areas. Per capita consumption in Central and Rift Valley Regions was 144 -152 litres and 38-54 litres in other Regions (MoLD, 2012). This translates to daily milk consumption of 0.128 litres (128 mls) in other Regions. Per capita milk consumption in Kisumu County was estimated at 31.2 litres which translates to 0.09 litres (90 mls per day), (MoLD, 2012). Table 2.4 shows daily intake of aflatoxin through milk in five Regions (Henry et al., 2001).

Table 2. 4 Daily Intake of Aflatoxin in Milk in Five Regions

Diet	Milk intake (kg/day)	Weighted mean Aflatoxin in milk µg/kg	Aflatoxin Intake ng/person/day	ng/kg/bw/day
European	0.29	0.023	6.9	0.11
Latin America	0.16	0.022	3.5	0.058
Far East	0.032	0.36	12	0.2
Middle East	0.12	0.005	0.6	0.10
Africa	0.042	0.002	0.1	0.002

Source: Adapted from (Henry et al., 2001).

2.2 Aflatoxin Exposure in Women of Reproductive Age

2.2.1 Exposure of Women of Reproductive Age to Aflatoxin

Women of reproductive age may be exposed to aflatoxin through consumption of contaminated staple foods. Evidence that aflatoxin-albumin adducts have a half-life of 30-60 days indicates that persons are likely to still be exposed over a period of several months, once identified as exposed. Exposure before delivery is therefore likely to translate to continued post-partum exposure. Aflatoxin B_1 ingested by the pregnant woman through dietary intake is converted to aflatoxin M_1 and passed over to the foetus through placental transfer (Turner et al., 2007; Partanen et al., 2010) and to the infant post-partum through breast milk (Polychronaki et al., 2008; Galvano et al., 2008; Gong et al., 2004; Oluwafemi, 2012).

A study carried out in The Gambia to determine aflatoxin exposure status during the early and later stages of pregnancy in rural Gambian women and explore possible interactions with seasonal influence on this relationship, confirmed that Gambian pregnant women were exposed to aflatoxin throughout the pregnancy (Castelino et al., 2014). Hence, aflatoxin exposure in pregnant women translates into exposure of the unborn fetus during pregnancy and the young child during early infancy. The findings by Castelino et al. (2014) concur with the findings of a study carried out in Adamawa state in North East Nigeria to determine the level of exposure of aflatoxin from mother to child and its mode of transfer where 570 pregnant women in the labour ward of The Federal Medical Centre Yola were investigated for aflatoxin exposure (Ibeh et al., 2014). The findings revealed that high levels of aflatoxin values above 20 ppb were obtained in over 65% of amniotic fluid, venous maternal blood and neonatal cord blood samples, further confirming exposure of infants through maternal dietary intake. Another study carried out in Egypt to determine the effect of aflatoxin exposure on child's growth, found a significant positive correlation between serum aflatoxin B_1 in the children and serum aflatoxin B_1 in their mothers (Shouman et al., 2012). The results suggested that aflatoxin present in maternal blood crosses the transplacental barrier, resulting in exposure of the fetus in *utero* through the amniotic fluid. Therefore, foetuses and infants risk exposure to aflatoxin through maternal exposure, placental transfer during pregnancy and breast milk intake.

Findings from a study carried out to investigate the association between birth outcomes and blood levels of aflatoxin B_1 (AFB1)-lysine adduct in 785 pregnant women attending antenatal clinic in Kumasi, Ghana, revealed that participants in the highest AFB_1-lysine quartile with 'very high' AFB_1-lysine level were more likely to have low birth weight babies and showed a trend of

increasing risk for low birth weight compared to participants in the lowest quartile (Shuaib et al., 2010). Although above evidence suggests that maternal aflatoxin exposure may affect weight of infants at birth and growth in general, further research is required to establish a cause-effect relationship. A similar study carried out to examine the association between aflatoxin B_1 (AFB_1)-lysine adduct (AF-ALB) levels in pregnant women in Kumasi, Ghana; and examine the association between AF-ALB levels and birth outcomes among the women, found that higher aflatoxin B_1 biomarker levels in the blood of pregnant women resulted in higher levels of adverse birth outcomes (Jolly et al., 2007). These findings further confirm that maternal exposure to aflatoxin during pregnancy exposes unborn foetus and the infant to the toxin and the associated health risks. Evidence of occurrence of aflatoxin in breast milk has been shown in Africa (Oluwafeni et al., 2012). This evidence confirms risk of exposure of infants to aflatoxin through breast milk, hence even with exclusive breastfeeding, infants may still be exposed. Studies in Kenya that have revealed existence of aflatoxin exposure in women, although providing a measure of exposure in lactating women, were restricted to the Eastern Kenya Region. Given that aflatoxin exposure may be dynamic, data that reflects such status should be generated regularly to reflect status at a given period.

In two studies carried out in pregnant women in Kumasi, Ghana, the socio-demographic determinants of aflatoxin B_1-lysine adduct in pregnant women were assessed (Shuaib et al., 2012; Ofori-Adjei, 2012). The findings of the studies revealed that high aflatoxin albumin-adduct levels in the women were inversely associated with indices of higher socio-economic status; implying that women in the lower socio-economic class were more likely to be exposed to aflatoxin compared to those in the higher socioeconomic class. These findings suggest that

women in the low socioeconomic are more likely to be exposed to aflatoxins because of consumption of poor quality food.

2.2.2 The Metabolism and Measurement of Aflatoxin Exposure

The main biomarkers used in human and animal aflatoxin exposure studies are aflatoxin B_1 (AFB_1) and its metabolites such as aflatoxin M_1 (AFM_1) in milk, AFM_1 and AFB-N^7- guanine (AFB-N^7-Gua) in urine and AFB_1 macromolecular adducts such as aflatoxin albumin adducts (AFB-albumin) in serum (Williams et al., 2004). Aflatoxin M_1 (AFM_1) is a major metabolite found in the milk of lactating animals and humans exposed to dietary AFB_1 (Navas et al., 2005).

Internal exposure to aflatoxins can be assessed by measuring aflatoxin M_1 in urine or in milk. Aflatoxin M_1 is excreted in urine at an amount between 1:2 and 2:2% of dietary aflatoxin B_1 and varying figures are given for estimated carryover of aflatoxin from dietary intake to milk in animals; 1% by Gong et al. (2008). Even though the conversion of aflatoxin B_1 to M_1 is low, the metabolite is potent even at low levels. This may explain why the tolerable limit for aflatoxin M_1 is lower than that for aflatoxin B_1. The half-life of aflatoxin B_1-albumin adducts is longer than that of aflatoxin B_1- N^7-guanine, and can reveal exposure over a longer time period. The aflatoxin-albumin adduct measured in peripheral blood has a half-life in the body of 30-60 days; hence is a measure of exposure over a longer period and a more reliable indicator of a person's chronic exposure to aflatoxin than other metabolites. Figure 2.2 highlights oxidative products of AFB_1 (Wild and Turner, 2002)

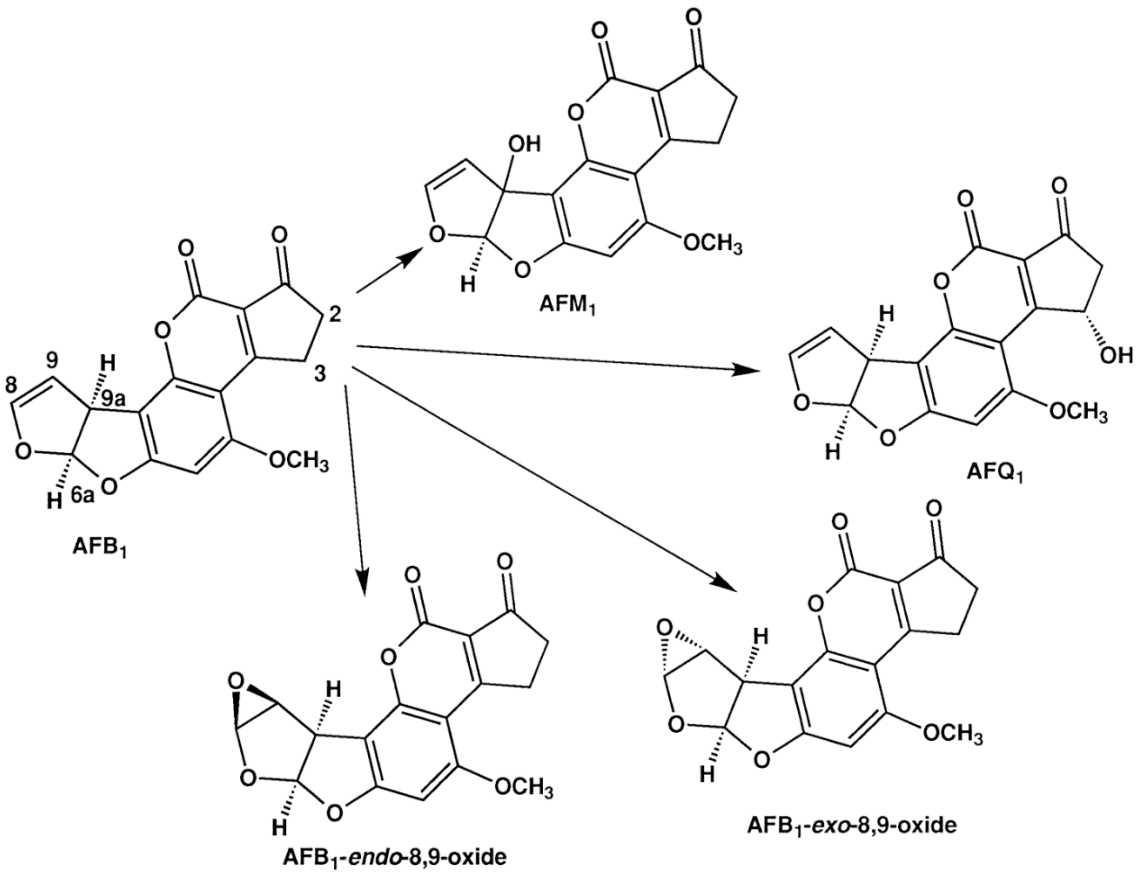


Figure 2. 2 Oxidation Products of AFB1 (Wild and Turner, 2002)

Most of the available studies of human exposure measured aflatoxin-albumin concentration which reflects long term exposure (Turner et al., 2007; Shuaib et al., 2010). Data from which to evaluate the extent and severity of biological exposure of humans in developing countries is lacking (Williams et al., 2004). Analysis of prepared meals has been cited as the most reliable source for determining aflatoxin exposure through diet because people may sort grain and remove those kernels that are considered unfit to eat (Williams et al., 2004). Several procedures have been suggested on how to determine aflatoxin exposure from foods consumed by individuals. In one approach, aflatoxin exposure can be determined by establishing the levels of aflatoxin contamination in a given food commodity and the quantity of the food consumed

(Gong et al., 2008). In a similar approach, aflatoxin intake through milk can be determined by assessing mean aflatoxin M_1 concentration in milk samples, then multiplying by milk consumption of the individual (Henry et al., 2001).

Another approach that has been applied to estimate dietary aflatoxin exposure is to relate aflatoxin level in foods consumed, amount of each food stuff consumed per day and body weight using the formula: Exposure (ng kg^- body weight/day) = (Contamination level) (amount consumed)/body weight in kg (Shephard, 2008). The approach by (Gong et al., 2008) was applied in this study to determine absolute aflatoxin intake by pregnant women in one day's diet as shown below.

> Absolute aflatoxin (µg)/day = Amount of aflatoxin (µg/kg) in analyzed food x total amount of food consumed in a day.

2.2.3 Effect of Aflatoxin Exposure on Human Health

Aflatoxin exposure has been implicated in adverse human health outcomes in areas where the toxin is prevalent in foods and many people are chronically exposed to large amounts of the toxin worldwide (CDC, 2004; Shephard, 2008; Strosnider et al., 2006). The effect of aflatoxin exposure is felt more in developing countries and this may be attributed to high levels of malnutrition coupled with chronic exposure to high levels of the toxin in their diets (Williams et al., 2004). Consumption of high levels of aflatoxins has been associated with aflatoxicosis resulting in death (Antonius et al., 2005; Fung and Clark, 2004), while chronic dietary exposure to aflatoxins is associated with liver and other cancers (IARC 2002) and poor growth outcomes in young children (WHO, 2006; Gong et al., 2008).

Most early studies on aflatoxin focused on its association with cancer (Medical Research Council (MRC), 2001; International Agency for Research on Cancer (IARC), 2002), and were mainly conducted in developed countries where exposure to the toxin is low (Kim et al., 2000; Henry et al., 2002). In developing countries, where exposure ranges from low to high, attention to effects of aflatoxins has majorly focused on aflatoxicosis, which involves extreme, acute exposure to the toxin over a short period of time. This is mainly because aflatoxicosis may result in immediate severe outcomes including death, hence is likely to attract attention. Chronic exposure to aflatoxins and its effects has received little attention, and only recently, have concerns that were raised by researchers such as Wild et al. (1992), been given due attention, especially in developing country contexts. This notwithstanding, evidence has pointed to a need for concern about exposure to aflatoxins in developing countries where it is evident that populations are exposed to varying levels of the toxins, for extended periods of time (Bandyopadhyay et al., 2007), and not just high exposure for short periods of time resulting in aflatoxicosis.

2.3 Maternal Aflatoxin Exposure and Infant Growth

2.3.1 Measurements of Growth in Infants

Anthropometry is an important method in the assessment of the health and nutritional status of a population of children and an individual child (WHO and UNICEF, 2009). Child linear growth can be measured using stature, length or weight. Stature refers to measurement made with the child erect or standing for children > 24 months. Length, also referred to as recumbent length is normally taken with the subject lying down and is recommended for children < 24 months of age who cannot stand erectly without support (Lee and Nieman, 2007; Rheeder, 2010). Therefore, length may refer to either recumbent length or stature measurements. Anthropometric indicator

gives a measure of an individual's growth status in relation to the reference median, expressed either as a percentile, a percentage of the reference median, or as a proportion of the standard deviation often referred to as a Z-score (WHO, 2006). The WHO proposed that a single anthropometric growth reference be used for both individual child growth monitoring and for assessing nutritional status of populations (WHO, 2006). The National Center for Health Statistics (NCHS) child growth reference database, NCHS/WHO, has been recommended as the reference growth standard for international use. In this database, weight-for-length, length-for-age and weight-for-age are interpreted by using the Z-score classification system. The Z-score system expresses the anthropometric value as the number of standard deviations or Z-scores below or above the reference mean or median value. Therefore, Z-scores describe how far (in standard deviations) a child's weight is from the mean or median weight of a child at the same length in the reference data.

According to World Health Organization [WHO], 2006 children whose length for age, weight for age and weight for length Z-scores are 2 standard errors or more below WHO growth standards (Z-scores $\leq$ -2) are considered to be stunted, underweight and wasted (WHO, 2006) respectively. These definitions were applied in this study in defining infant growth.

2.3.2 Effect of Maternal Aflatoxin Exposure on Infant Growth

Research findings indicate that early life exposures influence health and disease later in life (Barker, 2002; Terry and Sussex, 2001). Exposure of infants to aflatoxins is detrimental to their normal growth and other aspects of health later in life. Early linear growth retardation is associated with poor cognitive development, reduced physical productivity, a greater risk of poor

pregnancy outcomes later in life, including low birth weight babies and a greater risk of obesity, coronary heart disease, diabetes and hypertension later in life (Dusick et al., 2003; Ismail et al., 2014). Protection of fetuses, infants and young children from the effects of aflatoxins through maternal exposure would not only reduce the risk of them not realizing their cognitive potential, but also contribute to reducing the risk of non-communicable diseases whose prevalence is on the rise, not just in developed countries, but also in developing countries.

The World Health Assembly had adopted the Eight Global Nutrition Targets for 2025, five of which are geared towards improving child health: Achieve a 40% reduction in children under 5 who are stunted; Achieve 30% reduction in low birth weight; experiences no increase in overweight in children under 5 years; increase the rate of exclusive breastfeeding in the first 6 months up to 50%; reduce and maintain wasting in children under 5 at less than 5% (International Food Policy Research Institute [IFPRI], 2016). These targets can only be achieved if mechanisms are instituted to protect mothers and young children from growth insults including exposure to aflatoxin contamination through dietary intake. More information is needed on relations between nutritional factors and aflatoxin exposure, as reported by Williams et al., (2004), for the emergence of the full picture of when and how human nutrition is affected by aflatoxin.

Young children may be at risk of aflatoxin exposure right from the time of conception (Abdulrazzaq et al., 2002; Isaiah et al., 2014) through to complimentary food period (Gong et al., 2004). Exposure of the women to the toxin may, therefore, be a risk factor for infant aflatoxin exposure. Most young children in developing countries are weaned onto maize and other cereal

based porridge which may expose them to aflatoxins through diet early in life (Kimanya, 2008). Evidence from Kenya reveals that children are breastfed until at least the latter part of the second year, but begin to receive cereal-based gruel before the age of 3 months (Onyango et al., 2002). This means that infants risk double exposure; from maternal milk as well as from early cereal based complimentary feeds. Studies indicate that children are more vulnerable to effects of aflatoxin exposure than their mothers (Mocchegiani et al., 2001; Nektaria, 2007) and are also more likely to die from aflatoxin poisoning compared to adults (Williams et al., 2004). This has been linked to lowered capacity for biotransformation of carcinogens in infants than in adults, which may result in longer circulation time of the chemical.

Several studies have supported a possible association between aflatoxin exposure and reduced WAZ, LAZ and WLZ in young children: a study carried out among children in Benin confirmed a significant inverse correlation between aflatoxin albumin adducts and LAZ and WLZ-score but not WAZ-score (Gong et al., 2004); a study by Mahdavi et al. (2010) noted that children whose mothers were AFM_1 positive had lower LAZ and WAZ than children born to women with no detectable AFM_1. There is evidence that children from developing countries are born shorter than average (compared to WHO, 2006 standards), and their length-for-age z-scores (LAZ) continue to decline rapidly in their first two years of life (International Food Policy Research Institute (IFPRI) & the Enteric and Diarrheoal Diseases (EDD) & Agriculture and Nutrition Teams at the Bill & Belinda Gates Foundation (BMGF), (IFPRI, EDD, BMGF) (2012). These findings indicate that exposure to aflatoxin may result in cases of stunting (LAZ), underweight (WAZ) and wasting (WLZ), though most studies support the association between aflatoxin exposure and stunting, and therefore, length.

Studies have also supported a possible association between aflatoxin exposure and reduced length and LAZ-scores in young children; Turner et al. (2007) found a relationship between maternal aflatoxin exposure and lower height and weight gain in infants during the first year of life; a study carried out in Egypt showed a significant difference in length for age z-score (LAZ), but not weight for age z-score (WAZ) between aflatoxin B_1 negative and positive children (Shouman et al., 2012); a cross sectional study carried out among Iranian mothers, revealed that levels of AFM_1 in breast milk were inversely correlated with length of infants at birth (Sadeghi et al., 2009). The strength of the current study is that it is a cohort in design and therefore can determine a cause effect relationship between aflatoxin exposure and infant length.

High prevalence of childhood stunting has been reported in South and East Asia, and Sub-Saharan Africa, where food borne aflatoxin exposure is also high (Khlangwiset et al., 2011) and in West Africa, (Cardwell et al., 2004). Stunting during childhood has been associated with a reduction in adult size, reduced work capacity and adverse reproductive outcomes (Gibson, 2005). Collectively, these factors may lead to the inter-generational transmission of under-nutrition and poverty (IFPR, IDD and BMGF, 2012). In Kenya, high levels of stunting in young children have been reported in areas where aflatoxin levels in foods are also high (Appendix 9).

Several studies have also demonstrated a possible association between aflatoxin exposure and stunting and underweight in children under five years of age (Gong et al., 2002; 2003; 2008; Strosnider et al., 2006; Turner et al., 2007); a study carried out in Benin and Togo confirmed a dose-response relationship between aflatoxin exposure and the degree of stunting and

underweight in children <5 years old in Benin and Togo, where all members of the study population had aflatoxin exposure [aflatoxin-albumin adducts between 5 and 1064 pg/mg albumin in 99% of the children] (Gong et al., 2002); a prospective study carried out in Tanzania to investigate the association between child growth and aflatoxin exposure among 166 children 6 to 14 months revealed that prevalence of stunting was 44%, 55% and 56% at recruitment, 6th and 12th months after recruitment respectively (Shirima et al., 2015); a cross sectional study carried out in Kisumu District found an association between aflatoxin and wasting, but not underweight and stunting in infants fed on aflatoxin contaminated flour (Okoth and Ohingo, 2004). However, cause-effect relationship cannot be deduced from a cross sectional study, but from an appropriately designed study, such as a prospective cohort study. Another study carried out in West Africa found that young children who were chronically exposed to aflatoxin in foods were stunted and underweight, as measured by World Health Organization (WHO) z-scores (Cardwell and Henry, 2004). There are conflicting findings reported on association between aflatoxin exposure and infant growth, especially underweight and wasting, suggesting for more research in that area.

High rates of stunting have been reported in Nyanza region: according to KDHS, 2008/2009 figures, stunting rates among children < 5 years of age in Nyanza Province was 26.9% (KNBS & ICF Macro, 2010); data from FAO (2013) further show even higher stunting figures in all the counties in Nyanza Region; Kisii 35.3%, Homa Bay 37.0%, Kisumu 33.1%, Migori 46.2%, and Siaya 38.4%. A report from a workshop on prevention and control of aflatoxin contamination along maize value chain in Kenya, concluded that while the Country is aware of effects of aflatoxicosis, effects of chronic exposure to aflatoxin such as stunting in children are not

addressed (FAO/University of Nairobi, 2011). A study is on-going in Eastern Kenya to identify and utilize post-harvest storage technologies to reduce aflatoxin exposure and also study the impact this will have on child growth, especially stunting (Hoffman et al., 2013). Above findings point to an association between aflatoxin exposure and infant growth, the current study being longitudinal in design, aimed to establish an effect between maternal aflatoxin exposure and infant growth during the critical period of child growth in the first 3 months of life.

Child stunting has been treated as a nutritional problem, with interventions focusing on micro and macronutrients. However documented evidence reveals that most successful of these interventions achieved only a 0.7 increase in height-for-age Z-score, which is about one-third of the average growth deficit among children in Africa and Asia (Dewey and Adu-Afarwuah, 2008). Therefore, there is need to establish other factors that could have a role in retarded child growth, especially stunting; the focus of the current study. Studies have found similar results on the relationship between aflatoxin exposure and stunting (LAZ), but not with underweight (WAZ) and wasting (WLZ); the current study endeavors to fill this gap through its findings. Although maternal aflatoxin exposure has been implicated in poor birth outcomes and retarded growth in young children, previous studies have focused on the period after complimentary, yet children could be exposed to aflatoxin during pregnancy and through maternal breast milk.

2.3.3 Other Factors Affecting Child Growth

Several factors may affect the growth of infants apart from aflatoxin exposure. The environment in which the child grows, maternal nutrition during pregnancy and infant nutrition have been implicated in growth faltering in young children (Dusick et al., 2003). According to Jonsyn-Ellis

(2012), growth faltering is associated with many factors, among them; socio-economics status, insufficient food intake and episodes of diarrheoa. These factors may results in inadequate food and nutrient intake; as well as poor absorption and utilization of nutrients by the body, resulting in malnutrition and growth faltering. Poor maternal nutrition may compromise the amount of nutrients released to the infant during pregnancy and through breast milk, which may affect infant growth in general. Research findings have also correlated placental malaria with maternal anemia and reduced birth weight of the new born infants (Menendez et al., 2000) and the major risk factor for placental malaria is an age < 25 years old (Tako et al., 2005). On the contrary, it is recorded that aflatoxin may have some measure of protection against malaria because of direct effect of aflatoxin on parasitic multiplication (Williams et al., 2004). Some of the confounding factors were controlled for during data analysis.

2.4 Aflatoxin Exposure and Infant Morbidity

Malnutrition in young children is associated with many health problems, including increased rate of infectious illnesses, impaired learning capabilities, and reduced work productivity (Partnership for Aflatoxin Control in Africa [PACA], 2015). Children who suffer from acute malnutrition, have been found to be prone to the hazards of dietary aflatoxins (Khlangwiset et al., 2011; Tchana et al., 2010, Adhikari et al., 2006). A report by WHO Expert Group Meeting in 2005 indicated that exposure to aflatoxins may be a causative factor in neurological impairment, immunosuppression and child mortality (WHO, 2006). Aflatoxin exposure is also associated with delayed rate of recovery from protein malnutrition, due to its effect on protein synthesis (Williams et al., 2004), and increased infections in young children (Adhikari et al., 2006).

Exposure to aflatoxin in contaminated food results in suppression of the cell-mediated immune responses, due to the effect of aflatoxin on factors responsible for production of lymphokines and antigen processing by macrophages (Turner et al., 2003). Macrophages play a major role in host defenses against infection by presenting antigen to lymphocytes during the development of specific immunity and serve as supportive accessory cells to lymphocytes. Macrophages also increase their phagocytic activity and release various active products, such as cytokines and reactive intermediates, to carry out nonspecific immune responses. Aflatoxin has been shown in vitro to inhibit phagocytic cell function in normal human peripheral blood monocytes, resulting in reduced immunity (Williams et al., 2004; Jiang et al., 2008).

It has been established that the status of micronutrients in human body, may be compromised by aflatoxin exposure (Williams et al., 2004). Vitamin A has been found to play a protective role in human lymphocytes by inhibiting generation of AFB_1-induced reactive oxidative species. This may result in lower levels of retinol in individuals who are exposed to aflatoxin due to detoxification of aflatoxin derivatives that are potentially carcinogenic, by the micronutrient. The resulting vitamin A deficiency may increase the biological exposure to aflatoxin contamination (William et al., 2004), increasing liver AFB_1 microsomal activity, conversion of AFB_1 to its reactive metabolite and formation of DNA adducts.

A study by Tang et al. (2009) among 507 Ghanaian participants recorded a significant negative correlation between aflatoxin B_1 albumin adducts and vitamin A and vitamin E levels. Serum albumin adducts levels were statistically higher in subjects who had low levels of both vitamins A and E as compared with the subjects who had high vitamins A and E (Tang et al., 2009). Such

exposure may also affect micronutrient status in infants resulting in reduced immunity and increased morbidity. Research findings indicate that vitamin A and E supplementation significantly reduces aflatoxin induced toxicity and carcinogenesis, compromising the intended purposes of the nutrients (Alpsoy1, 2009; Pimpukdee et al., 2010). Aflatoxin exposure may also have an effect on availability of dietary zinc and selenium, which have antioxidant properties and are also essential for healthy immune function (Mocchegiani, 2001). Given the probable low-to-high exposure to aflatoxin in Kenya and other developing countries, efforts to improve micronutrient status of young children such as vitamin A supplementation and diet diversification may be compromised by aflatoxin exposure. Maternal exposure to aflatoxin, therefore, exposes the infant to the toxins, affecting the health of the infant by interfering with micronutrients status in the body.

Kisumu County Integrated Development Plan (KCIDP) 2013-2017, highlights main causes of sicknesses in the County as malaria 44.7%, fever 11.2%, common cold (flu) 5.2% and diarrhea 2.4 % (CGOK, 2013). Malaria is a common illness among infants and young children in developing countries and an endemic disease in this Region and is a leading cause of morbidity and mortality, especially in children (CGOK, 2013). Exposure to aflatoxins has been associated with increased risk of diarrhoea and pneumonia in children (WHO, 2006), but not malaria. A study carried out in Gambian children observed changes in immunity as a function of aflatoxin-albumin adducts, which were detected in 93% of the children; in a multivariable analysis, secretory immunoglobulin A (IgA) was markedly lower in children with detectable aflatoxin-albumin concentrations than in those with non-detectable concentrations and a weak antibody response to a pneumococcal challenge was observed (Turner et al., 2003). This suggests that

aflatoxin may lead to increased morbidity to illness such as diarrhoea and pneumonia, but may on the other hand result in reduced morbidities such as malaria.

The mechanism by which aflatoxin affects infant morbidity is not clear, and as reported by Khlangwiset et al. (2011), data on clinical aflatoxicosis in human is still limited although there is substantial evidence on human exposure in many areas of the world. Aflatoxin exposure may result in altered intestinal integrity through cell toxicity or immunosuppression, making the body susceptible to infections resulting intestinal malabsorption resulting and reduced food efficiency in infants (Cardwell and Henry, 2004; Gong et al., 2004). A study carried out in the Gambia, revealed that aflatoxin exposure led to a reduction in salivary sIgA in children resulting in alteration of the mucosal barrier and affecting resistance to intestinal infections (Turner et al., 2003). Exposure to aflatoxin may also cause impairment of the intestinal mucosa which may affect proper absorption and utilization of food in the body resulting in cases of diarrhea and other infections. Aflatoxin exposure has been associated with environmental enteropathy (EE), a sub clinical condition of the small intestine characterized by reduced absorptive capacity and increased intestinal permeability, which may be a cause of stunting (Smith et al., 2012).

The aforementioned views reveal the possible effect of aflatoxin exposure on infant morbidity in the study area and the need for more research and interventional measures. Exposure to aflatoxin may therefore affect the availability of micronutrients for body functions resulting in poor health outcomes. Therefore, although the role of aflatoxin in morbidity remains unclear, there are potential mechanisms that may support such a role. Further, data on association between aflatoxin exposure and infant morbidity is inadequate globally, in Kenya and in Kisumu County.

2.5 Summary of the Gaps in the Knowledge

The current study intends to address the following gaps; although focus on aflatoxin has been confined to maize, the possibility that other commonly consumed staple foods could be contaminated as suggested by Williams et al. (2004), remains largely unexplored and information on the contribution of other foods to aflatoxin exposure in Kisumu County is inadequate; in spite of aflatoxicosis outbreak in Kenya (CDC, 2004; Lewis et al., 2005), information on aflatoxin intake that would enable estimation of exposure of pregnant women through diet in Kisumu County is lacking; strong evidence on the role of aflatoxin on growth of infants prior to complimentary is lacking and data on possible effect of aflatoxin exposure on growth of infants through exposure to aflatoxin in this period in Kisumu Country is inadequate, yet young children could be exposed to aflatoxins prior to complimentary feeding through breast milk; knowledge on the effect of aflatoxin exposure on infant morbidity during the first three months of age in Kisumu County is lacking.

2.6 Conceptual Framework

Aflatoxins producing fungi affect a wide variety of food commodities including cereals, legumes, meat, groundnuts, milk, and dried fruit (Strosnider et al., 2006). Aflatoxin B_1 accumulates in foods and the contaminated food is consumed by humans. Aflatoxin M_1 is a major metabolite found in the milk of lactating animals and mothers exposed to dietary AFB_1 and estimated carryover of aflatoxin from dietary intake to milk in animals is around 1% (Gong et al., 2008). Pregnant women consuming contaminated foods expose their unborn children to the toxin through placental transfer during pregnancy and through breast milk after birth. In lactating mothers, the consumed aflatoxin B_1 is secreted into maternal breast milk as aflatoxin M_1, posing

a health risk to breast-fed infants. Infants may also be exposed to aflatoxin B_1 through introduction of complimentary foods. Infants of exposed mothers may develop adverse growth outcomes such as low birth weight, low weight-for-for age, low height/length-for-age and low weight-for-length/height and increased risk of morbidity. This is because aflatoxin exposure interferes with intestinal integrity leading to reduced food utilization efficiency, immunity and serum micronutrient levels. Figure 2.2 highlights the variables of interest from point of consumption of contaminated foods by the women to the point where the infants is exposed to the toxins and the effect of exposure on infant growth and morbidity. The conceptual framework model was adapted from (Wu and Khlangwiset, 2010) with slight modifications.

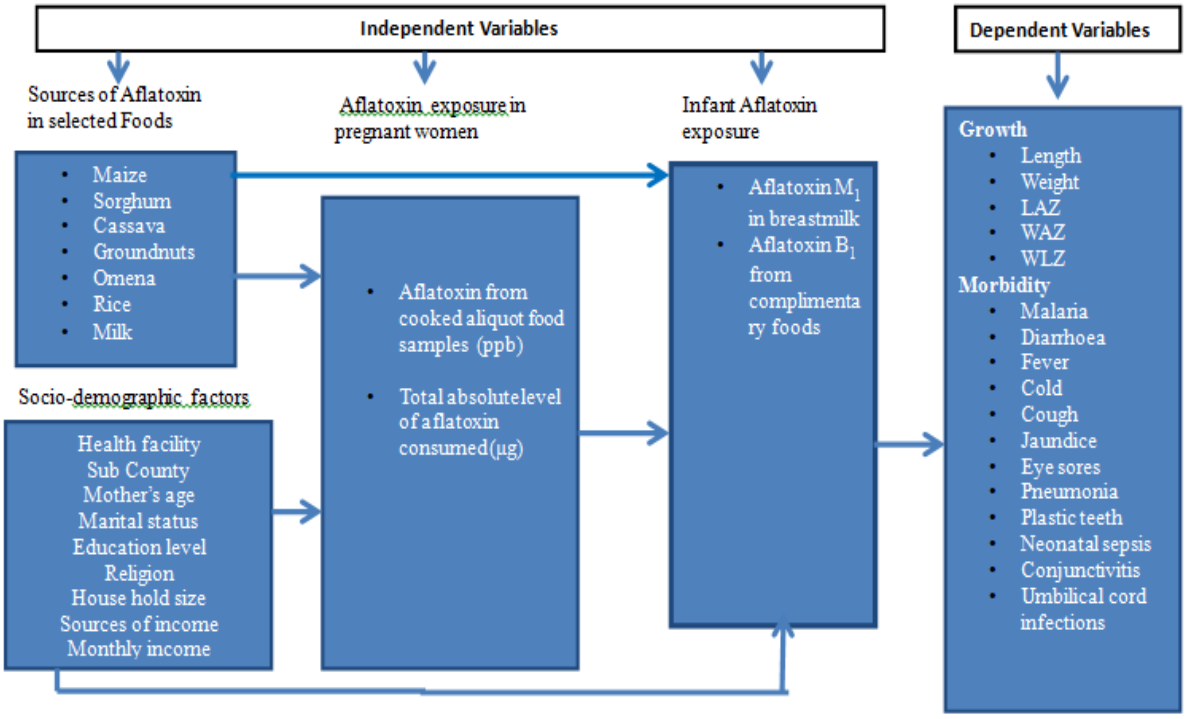


Figure 2.3 Conceptual Framework: Effect of Maternal Aflatoxin Exposure on Infant Growth and Morbidity

CHAPTER THREE: RESEARCH METHODS

3.1 Study Area

This study was conducted in Kisumu East and Nyando Sub-Counties, Kisumu County, Kenya (Appendix 1). Kisumu County was selected because of the following reasons: Studies carried out in South Nyanza and Homa Bay indicated that maize and groundnuts, respectively, from the farmers were contaminated with aflatoxin levels higher than reported in Eastern Region of Kenya; another study carried out on weaning flours in the former Kisumu District, were contaminated with aflatoxin (Okoth and Ohingo, 2004); the prevailing extreme climatic conditions including; frequent flooding, drought, erratic rainfall resulting in poor crop performance, were conducive for mould proliferation and aflatoxin production. Nyando Sub-County was selected because of its situation in the rural setting, and Kisumu East Sub-County because of the urban setup.

Kisumu County has seven Sub-Counties (Kisumu East, Kisumu West, Kisumu Central, Kisumu North, Nyando, Muhoroni and Nyakach), with an area of 2,085.9 km^2 and a population of 968,909 (Male, 48.9 %, Female, 51.1 %), 226,719 households and a population density of 465 people per km^2 (KNBS, & ICF Macro, 2010; Kenya National Bureau of Statistics (KNBS), 2011). Antenatal clinic attendance is pegged at 1372 and 2728 women at Kisumu County Referral Hospital and Ahero Sub-County Hospital per year respectively. Kisumu County has a poverty level of 45% and food insecurity index of 53.4%, (Ogola and Awange, 2006). Rainfall ranges between 1200 mm and 1300 mm with a mean annual temperature of 23^oC, ranging between 20^oC and 35^oC and humidity of 40 – 89% (CGOK, 2013). These climatic conditions are very favorable for mycotoxins growth. Common food crops grown in Kisumu County include

maize, sorghum, groundnuts, cassava, bananas, sweet potatoes and horticultural crops including fruits and a variety of indigenous and exotic vegetables (MoA, 2012). Due to the presence of a fresh water lake, one of the economic activities is fishing for local consumption and export (MoFD, 2012).

According to the Kenya Demographic and Health Survey 2008-09 (KNBS & ICF Macro, 2010), Nyanza region has a median duration of breastfeeding of 18.6 months and 0.6 months of exclusive breastfeeding; about 85% of breastfeeding children under 6 months living with the mothers were breastfed six or more times in the 24-hours preceding the last KDHS survey; majority of infants in Nyanza Province had birth weight of ≥ 2.5 kg (96.7%), 26.9% of children were stunted, 3.3% wasted and 13.7% were underweight. According to the recent results of Kenya Demographic and Health Survey (KDHS) 2014 (KNBS & ICF Macro, 2014): Nyanza Region had a median duration of exclusive breastfeeding of 3.4 months in the first 6 months of life and 58.4% of infants started breastfeeding within 1 hour of birth; 63% of infants 2-3 months were exclusively breastfed.

3.2 Study Design

A cross sectional design was used to assess aflatoxin levels in market foods and a prospective cohort design was used to assess the effect of aflatoxin exposure through maternal dietary intake on growth and morbidity of infants of exposed and non-exposed women in the first three months of life (Figure 3.1)

3.2.1 Cross Sectional Study

The cross sectional market study was conducted in Kisumu East and Nyando Sub-Counties, Kisumu County, Kenya. Based on data on food production and requirement in Kisumu County from the Department of Agriculture Annual Report 2015, and informal interviews with nutritionists, consumers and Department of Agriculture staff, common staple foods (maize, sorghum, millet, cassava, rice, *dagaa* and milk) were collected from five major markets (Kibuye wholesale market, Kibuye open market, Oile Market, Mamboleo market and Ahero market) and analyzed to determine the actual aflatoxin levels.

3.2.2 Cohort Study

In a prospective cohort study design, all pregnant women attending antenatal care clinics at Kisumu East County and Ahero Sub-County Hospitals, Kisumu County, were screened and recruited in the study between May and August 2013 (Table 3.3).

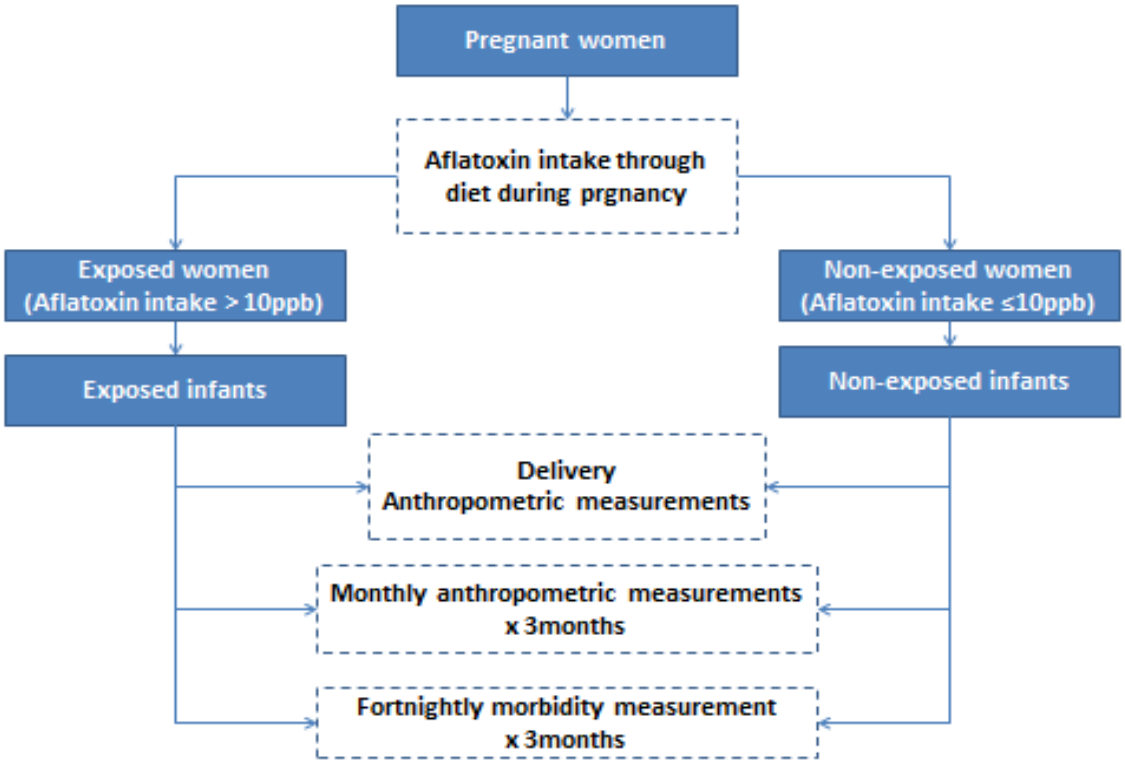


Figure 3.1 Study Design

3.3 Study Population

The target population was pregnant women and subsequently their infants. Being a cohort study, the population size is not relevant to the calculation of the sample size, which is solely dependent on selecting an adequate sample to determine effect.

3.3.1 Cross Sectional Study

The study population composed of 213 bags (90kg) of dry foods (*dagaa* 63 bags, rice 34 bags, groundnuts 23 bags, cassava 41 bags, maize 46 bags, sorghum 31 bags) from 5 markets (Kibuye wholesale market, Kibuye open market, Oile Market, Mamboleo market, and Ahero market) from Kisumu East and Nyando Sub-Counties, and raw milk samples from 3 milk bazaars (Ahero,

Mamboleo and Guba markets) and processed milk from 3 supermarkets in Kisumu County. The total population of milk samples in the supermarkets was unknown.

3.4. Cross Sectional Study

3.4.1 Sample Size

The sample size for the number of food samples to be collected from each market was calculated based on Israel (2009) formula:

$N/ (1+N (e)^2)$; .where N is the population size of total available bags of each food in the market at the time of survey and e is the margin of error set at 5% (0.05) at 95% Confidence level.

Only one sample of 500g was required per bag (Daniel et al., 2011, Njapau, 2008), hence the total number of bags was equal to the total number of the different samples. The total population of milk samples in the supermarkets was unknown. Two hundred and nineteen dried food samples (500g each) and 80 milk samples (50 samples of processed and 30 samples of raw milk) were collected as shown in Tables 3.1 and 3.2.

Table 3.1 Sample Size Distribution for Market Food Samples

FOOD SAMPLE	**Population** **N**	**Minimum sample** **N /(1+N(e^2))**	**Final Sampled (Table 8 below)**
Dagaa	63	54	60
Rice	34	31	31
Groundnuts	23	22	22
Cassava	41	37	37
Maize	46	41	41
Sorghum	31	28	28
Raw milk	30	30	30
Processed milk	50	50	50
TOTAL	238	213	299

Of the dry foods, *dagaa* had the highest numbers of bags (63) in the market followed by maize (41) and cassava (41), while groundnuts had the lowest number of bags (23) in the market.

Table 3.2 Number of Food Samples Collected from Markets

	No of Samples Collected from Each Market												
							Processed Milk Brands						
Market	***Dagaa***	**Maize**	**Rice**	**Groundnuts**	**Sorghum**	**Cassava**	**A***	**B***	**C***	**D***	**E***	**Raw Milk**	**Total**
	(n)	**(n)**	**(n)**	**(n)**	**(n)**	**(n)**						**(n)**	
Kibuye Whole sale	5	12	6	6	5	8	0	0	0	0	0	0	**42**
Kibuye Open Air Market	8	8	8	12	12	15	0	0	0	0	0	0	**63**
Oile Market	13	0	4	0	0	0	0	0	0	0	0	0	**17**
Mamboleo market	20	11	0	0	4	8	0	0	0	0	0	10	**53**
Ahero market	14	10	13	4	7	6	0	0	0	0	0	10	**64**
Guba Market	0	0	0	0	0	0	0	0	0	0	0	10	**10**
Supermarkets	0	0	0	0	0	0	10	10	10	10	10	0	**50**
Total	**60**	**41**	**31**	**22**	**28**	**37**	**10**	**10**	**10**	**10**	**10**	**30**	**299**

*** Represent the different brands of processed milk from the supermarkets**

The numbers of samples collected were calculated based on the number of bags available in the market at the time of data collection.

3.4.2 Inclusion Criteria

Six markets were purposively selected for inclusion in the survey. Based on their geographic locations, these markets served the largest population in their respective Sub-Counties. They were likely to have enough vendors with a variety of food products of interest to the study, including; maize, sorghum, cassava, rice, *dagaa* (*Rastrienobola argentea*) and milk. The markets selected were: Kibuye wholesale market, Kibuye open market, Oile Market, Mamboleo market, Guba and Ahero market. Foods that formed a big proportion of the staple food in Kisumu County according to MoA (2012) were chosen for inclusion in the study. The vendors were chosen if they had foods of interest to the study and gave an informal consent to have their foods sampled.

3.4.3 Sampling Procedure for Market Food Samples

Sampling of dry foods was done using a combination of cluster and systematic sampling. Five hundred grams each of available maize, sorghum, polished rice, cassava, groundnuts and *dagaa,* according to recommendation by Njapau (2008) and Daniel et al.(2011), was collected from each bag from the vendors. The European model, which recommends that a 500g sample composed of five 100 g portions of milk is taken from a batch, be used for the minimum sample size and sample selection method was applied (Codex Alimentarius, 2001).

3.4.4 Data Collection Procedures for Market Food Samples

Two hundred and nineteen dried food samples (Maize, sorghum, cassava, rice, groundnuts and *dagaa*) were scooped from selling bags at different points of the bags to ensure uniformity, using

respective vendor tools such as tins, and double packaged in brown paper envelopes to avoid cross contamination and moisture penetration (Daniel et al., 2011, Njapau, 2008). The packages were properly labeled, coded and the sources properly recorded and were transported to KALRO Kitale for aflatoxin analysis. Processed cow's milk samples were collected from three major supermarkets in Kisumu City and raw cow's milk samples from 3 market milk bazaars at Guba, Mamboleo and Ahero markets. Five 500 mls portions of milk were taken from each batch of milk (Codex Alimentarius Commissions, 2001) and put in 100 ml plastic bottles. A total of 80 milk samples were collected as follows: 50 processed milk samples from the 5 most common milk brands (coded for purposes of confidentiality) from three major supermarkets in Kisumu City; and 30 raw milk samples were collected from Ahero, Mamboleo and Guba market milk bazaars. The milk samples were immediately frozen and stored at -20^{o}C before being transferred to KALRO Kitale for analysis. Aflatoxin M_1 content was reported in parts per billion (ppb).

3.4.5 Aflatoxin Analysis

Aflatoxin B_1 levels in maize, sorghum, millet, cassava, rice, and *dagaa* were determined by Enzyme Linked Immunosorbent Assay [ELISA], as described by Kim et al. (2000) and Lee et al. (2004). The basic principle of ELISA lies in trapping the antigen on a solid surface or capturing the antigen with specific antibodies, and probing with specific immunoglobulin carrying an enzyme label. In this technique, about 200 g of food sample was ground into powder using a blender. Two grams of the ground samples was weighed into a screw cap glass vial and 10 ml of methanol/distilled water (70/30 v/v) was added and mixed for 10 minutes at room temperature

using a shaker. The extract was then filtered then 100 µl of the filtrate was diluted with 600 µl of distilled water and 50 µl of the diluted samples was used per well for quantitative analysis.

All reagents were brought to room temperature before use. Three micro-titre plate wells were inserted into the micro-well holder for all standards and samples to run in duplicates. Standard and sample positions were recorded. To each micro-well 50 µl of the standard solution or prepared samples, 50 µl of the enzyme conjugate and 50 micro-litres of the antibody solution was added. The mixture was shaken gently and incubated at room temperature for 30 minutes in the dark. The liquid was then poured out of the micro-wells and any excess liquid dapped off using absorbent paper. The wells were washed three times with washing buffer then 100 µl of substrate/chromogen was added to each well, mixed gently by shaking the plate manually. After incubating the wells at room temperature in the dark for 15 minutes, 100 µl of stop solution was added to each well, mixed gently by manual shaking. The microwells were measured optically by a microplate reader with an absorbance filter of 450nm (OD450) and a detection limit of < 1 ppb. Results were determined by comparing the optical density of the sample to the optical density of the kit standards. The sample had been diluted at a ratio of 5 to 1 with 70% methanol, and so the aflatoxin shown by the standard was multiplied by 5 in order to indicate the ng of aflatoxin per gram of commodity (Lee et al., 2004).

Aflatoxin M_1 levels in milk samples were analyzed using Helica Aflatoxin M_1 Assay (Aflatoxin M_1 ELISA), with high affinity for aflatoxin M_1 (Kim et al., 2000). In this procedure, an antibody with a high affinity for aflatoxin M_1 was coated onto polystyrene microwells and 200µL of milk sample was added to the appropriate microwell and aflatoxin M_1 was bound to the coated antibody. Subsequently, aflatoxin bound to horseradish peroxidase (HRP) was added and it

bound to the antibody not already occupied by aflatoxin M_1 present in the sample or standard. After 15 minutes of incubation, the contents of the wells were decanted, washed and an HRP substrate was added which developed a blue color in the presence of an enzyme. The intensity of the color was directly proportional to the amount of bound conjugate and inversely proportional to the amount of aflatoxin M_1 in the sample. An acidic stop solution was added which changed the chromogen colour from blue to yellow. The microwells were measured optically by a microplate reader with an absorbance filter of 450nm (OD450) and a detection limit of < 1 ppb. Results were determined by comparing the optical density of the sample to the optical density of the kit standards. Aflatoxin B_1 levels were reported in microgram per kilogram [μg/kg] (ppb).

3.5 Cohort Study

3.5.1 Sample Size Calculation for Pregnant Women

Sample size calculation was achieved in two steps. First, the number of women needed in order to determine a difference in growth between the exposed and non-exposed infants based a minimum difference obtained from a previous study on growth by Kramer et al. (2002) was determined. This formed the sample size for the cohort study. Next, the number of women needed to be screened in order to obtain sufficient exposed women to satisfy the sample size calculated for the cohort study was determined. This formed the sample size for screening for participation in the cohort study. Details of these procedures are indicated below.

3.5.1.1 Sample Size Calculation for Cohort Study

Sample size calculation for the cohort study was based on the minimum sample size required to detect a difference in growth of exposed and non-exposed infants. Based on the ability to detect a mean difference of 0.6 cm in length (Kramer et al., 2002) with 90% power, significance level of 5%, (α =0.05); two-sided test; a standard deviation (SD) of 1.5 and an estimated loss to follow-up of 10% (Kirkwood and Sterne, 2007); 132 mother-infant pairs were required (146 after inflating to cater for loss to follow-up).

$$n = \frac{(u+v)^2(\sigma_1^2 + \sigma_0^2)}{(\mu_1 - \mu_0)^2}$$

$\mu_1 - \mu_0 =$ Difference between the means

σ_1, σ_0 = Standard deviations of exposed and non exposed

u = Two sided percent point of the normal distribution corresponding to 100% minus the power e.g. if power = 90%, u is 1.28 (for all cases)

v = Percentage point of normal distribution corresponding to the (two-sided) significance level (95% confidence interval) e.g. if significance level = 5%, v = 1.96

u =1.28

v = 1.96

σ_1 = 1.5

σ_0= 1.5

$\mu_1 - \mu_0$ = 0.6 cm

$$n = \frac{(u+v)^2(\sigma_1^2 + \sigma_0^2)}{(\mu_1 - \mu_0)^2}$$

$$n = \frac{(1.28 + 1.96)^2 (1.5^2 + 1.5^2)}{(0.6)^2}$$

$$n = \frac{(1.28 + 1.96)^2 (1.5^2 + 1.5^2)}{0.36} = \frac{47.2329}{0.36} = 132$$ minimum sample size per group

Assuming an estimated loss to follow-up of 10% (10% of 132 =14), a total of 146 women was required per group (a total of 146 x 2 = 292 women).

3.5.1.2 Sample Size Calculation for Screening of Participants for the Cohort Study

To estimate the number of women needed to be screened to achieve the required sample size, it was assumed that 40% of the population could be exposed to aflatoxin. Based on research findings on prevalence of aflatoxin exposure in the study area which indicate that 40% of households in Nyanza Region are likely to be exposed to aflatoxin (Mutegi et al., 2007; Mwaura, 2011) and assuming that at most 40% of participants could therefore express indicators of aflatoxin exposure, a minimum of 365 women needed to be screened to identify 146 exposed women.

$$\frac{100 \times 146}{40} = 365$$ women needed to be screened.

However, because of the uncertainty about the 40% that would be exposed, double the minimum number required (365 x 2= 730) of pregnant women was screened. A total of 553 women were finally enrolled to participate in the study and of these, 137 were exposed and 416 were non-exposed. This was above the minimum required sample size of 132 for exposed and non-exposed. The sample size and distribution of women per health facility is as shown in Table 3.3.

Table 3.3 Sample Size Distribution per Health Facility

Health Facility	Population of pregnant women	Sample size	Total No. screened
Kisumu East	1372	185	244
Ahero	2728	368	486
Total	4100	553	730

Ahero County Hospital had a higher number of pregnant women attending antenatal clinic compared to Kisumu County Referral Hospital.

3.5.2 Inclusion Criteria

Pregnant women were eligible for inclusion into the study if they were registered attendees at Kisumu County Referral Hospital or Ahero County Hospitals, consented to participate in the study (Appendix 2 and 3); were up to 8 months pregnant as confirmed by the records in the mother -child health booklet; were residing in Kisumu East and Nyando Sub-Counties, Kisumu County, for the entire study period; were willing to deliver at designated health facilities; had no history of chronic illness such as diabetes and HIV and AIDS as indicated in mother child-health booklet; and were not on regular medication and had no pregnancy complications. The 'up to 8 months' pregnancy stage was chosen to give ample time for collection of meal samples and analysis in order to identify exposed and non-exposed women to participate in the study.

3.5.3 Sampling Procedure for Cohort Study Participants

In the Kenyan health system, Community Health Volunteers (CHVs) aid in health promotion and collection of health-related data from communities; this network was tapped into. Community Health Volunteers assisted to screen 730 pregnant women of up to the 8^{th} month of pregnancy

attending antenatal clinics and 553 were eligible to participate in the study. A sample of 185 pregnant women was required out of the target population of 1372 in Kisumu County Referral Hospital and 368 pregnant women out of the target population of 2728 from Ahero County Hospital (Table 3.4). All pregnant women attending antenatal clinics at Kisumu County Referral Hospital and Ahero County Hospital between May and August, 2013, were screened until the required sample size for each health facility was achieved. Aliquot samples of 10% of the foods eaten over 24-hours (breakfast, lunch, supper and snacks) by each of the 553 pregnant women were collected, packaged in plastic containers and taken to KALRO Kitale for aflatoxin analysis. Women with aflatoxin B_1 intakes >10 ppb were classified as exposed and those with aflatoxin B_1 intakes ≤10 ppb were categorized as non-exposed. The women were followed up to delivery and infants of both exposed and non-exposed women were followed for three months after birth. Anthropometric measurements and morbidity incidences of the infants were taken monthly and fortnightly respectively for 3 months postpartum (Figure 3.1). Three weight measurements and three length measurements of the infant were taken and recorded on the questionnaire sheet at each antenatal clinic visit and the average calculated (Appendix 4). At three months of age, 137 infants from exposed mothers and 137 infants from non exposed mothers, matched for maternal age and monthly household income using McNemar's test for proportions, were randomly selected for analysis of cohort study data. Data on women's socio-demographic factors, food frequency, 24-hour dietary recall, as well as infants' food and milk intake, anthropometry and morbidity were obtained through a questionnaire (Appendix 4).

3.5.4 Data Collection Instruments

Community Health Volunteers were trained on all procedures to be used in recruitment of participants and data collection using a developed field manual (Appendix 5). The women recruited into the study were required to attend post-natal clinic at Kisumu County Referral and Ahero County Hospitals for subsequent follow-ups. Those who attended post-natal clinic at other clinics closer to their residence were asked to indicate the clinic and were followed up there. A questionnaire on women's socio-demographic factors including residence, maternal age, education level, source of income, employment status, number of children; food frequency, 24-hrour dietary recall, as well as infants' data on gender, birth weight, sickness episode, feeding history and food frequency was used to collect study data (Appendix 4). Aliquot samples of the women's one day meal (breakfast, lunch, supper and snacks) were collected and analyzed for aflatoxin levels and actual consumption used to determine absolute aflatoxin intake. The women were followed up to delivery and postpartum period; infants of exposed and non-exposed women were followed for three months. Anthropometric measurements and morbidity incidences of the infants were taken monthly and fortnightly respectively for 3 months postpartum (Figure 3.1). Weights of infants were recorded using calibrated SECA Infant Weighing scales, model No. 7251021009 and lengths were determined using SECA 0 Child Length Boards. Infants' breast milk intakes were determined by the frequency of breastfeeding and recorded on infant food frequency questionnaire (Appendix 4). Infants' food intakes were determined from data obtained from women on other foods fed to infants using infant food frequency questionnaire (Appendix 4).

3.5.5 Validity and Reliability of Instruments

3.5.5.1 Validity

In this study research assistants with good knowledge of the local language were recruited and trained in the methods of data collection, recording and scoring of data in the questionnaires. A field manual was developed and used for orienting research assistants and other personnel involved in the study (Appendix 4). Data collection tools were pretested in a sub sample of 20 participants who were not part of the main study in a pre-test study at Nyakach County Hospital, Nyando Sub-County, before being applied in the main study. A sample size of 20 participants is considered appropriate for a pretest (Willis, 2005; Perneger et al., 2015). The equipment used for aflatoxin analysis in Kitale was purchased in 2012 from Biotech International, Germany, and therefore was still in good working condition. The Kenya Agricultural and Livestock Research Organization (KARLO) ensured proper functionality and accuracy of the equipment through annual calibration by qualified technicians. The infant weighing scales and length boards at the health facilities were frequently checked and calibrated after every measurement to ensure accurate results. The structured questionnaire was shared with 2 nutrition experts and 3 supervisors to review the variables to ensure clarity of the questions and their comments were used to improve on the quality of the questionnaire.

3.5.5.2 Reliability

A pilot study was conducted at Nyakach County Hospital to ensure instructions, questions and scale items were clear (Pallant, 2003; Webb et al., 2006). During the piloting the questions were posed to the respondents to assess consistency and ease of understanding. As per the recommendation of Willis (2005) and Perneger et al. (2015), a test-retest (reproducibility) of the

questionnaire was conducted two weeks apart to a group of 20 women at Nyakach County Hospital, Kisumu County. The aspects of the questionnaire subjected to test-retest were; maternal socio-demographic, food frequency, and 24-hour recall. The women were not included in the main study but were of the same study population. The results were correlated from the two questionnaire administrations using Pearson Product Moment Correlation and a reliability index of 0.80 at p value of 0.05 level of precision was attained, this being above 0.70, the least accepted value of reliability in research (Oso and Onen, 2009). In this study it was crucial that the aflatoxin intake measured reflected usual aflatoxin intake through diet. To achieve this actual foods consumed by participants in one day were collected and analyzed for aflatoxin levels, and weighed food records for 20 participants were taken to establish quantity of food consumed in one day (Appendix 15).

3.5.7 Measurement of Variables

3.5.7.1 Aflatoxin Intake by Pregnant Women

Aflatoxin intake by women through diet was determined by the levels of aflatoxin in the aliquot foods samples collected from participants and analyzed at KALRO Kitale. Pregnant women consuming foods with aflatoxin levels above 10 ppb were classified as exposed and those consuming foods with aflatoxin levels $\leq$ 10 ppb as non-exposed. Maternal aflatoxin exposure was determined by the levels of aflatoxin in women's diet and by the proportion of women whose intake was above 10ppb.

Daily absolute aflatoxin intake (µg) intake by pregnant women in a day was calculated using the formula:

Absolute aflatoxin (µg) = Amount of aflatoxin (µg/kg) in analyzed food x Total amount of food consumed in a day (Gong, 2008).

Information on aflatoxin levels in foods from the market outlets and foods cooked at home and consumed by pregnant women was generated from the amount of aflatoxin determined in analyzed food samples.

3.5.7.2 Infant Growth

Weight was measured as average of three weight measurements and length as average of three length measurements; lengths for age Z-scores (LAZ), weight for length Z-scores (WLZ) and weight for age Z-scores (WAZ) were computed using WHO Anthro 2005, w3.2.2 software programme [http://www.who.int/childgrowth/software/en/] (WHO, 2006; Blossner et al., 2006). The computed indices were used as indicators of stunting, underweight, and wasting, based on -2SD cut off point; stunting was measured by a child's length-for-age being 2 standard deviations below the WHO growth reference (LAZ ≤- 2); underweight was measured by a child's weight-for-age being 2 standard deviations below the WHO growth reference (WAZ ≤ - 2); and wasting was measured by child's weight-for-length being 2 standard deviations below the WHO growth reference [WLZ ≤- 2] (WHO, 2006).

3.5.7.3 Exclusive Breastfeeding

The variable on exclusive breastfeeding was generated using SPSS. Variables for those who consumed selected foods in a day, week and month (i.e. porridge, cow milk, NAN, water and

fruit juice) were generated, with 0 and 1 denoting did not consume and consumed the specified food respectively. Using the 'transform, 'recode into different variables' and 'if case' functions in SPSS, we computed a variable for exclusive breastfeeding. All the variables with porridge=0, and cow milk=0 and NAN=0 and water=0 and fruit juice=0, were computed to give a value of 1 for exclusive breastfeeding (ExclusiveBf=1). Variables with porridge >0 and cow milk >0 and NAN >0 and water >0 and fruit juice >0, were computed to give a value of 0 for exclusive breastfeeding (ExclusiveBf=0). This gave the total number of infants who were exclusively breastfed and those who consumed other foods apart from breast milk.

3.5.7.4 Infant Food Intake

Amount of other food consumed by the infant was determined from the food intake data collected using the infant food frequency questionnaire (Appendix 4).

3.5.7.5 Infant Morbidity

Infant morbidity was determined by recording the cumulative incidence of childhood illnesses reported in the first three months after birth. Malaria was defined as laboratory confirmed presence of malaria parasites at the respective health facilities. Fever was defined as body temperature at least 37.5^0c, while diarrhea was defined as child having 3 loose stools in 24-hours. Presence or absence of cough, cold, jaundice, conjunctivitis, plastic teeth, neonatal sepsis were defined as reported by the mother/care takers during antenatal clinic visits.

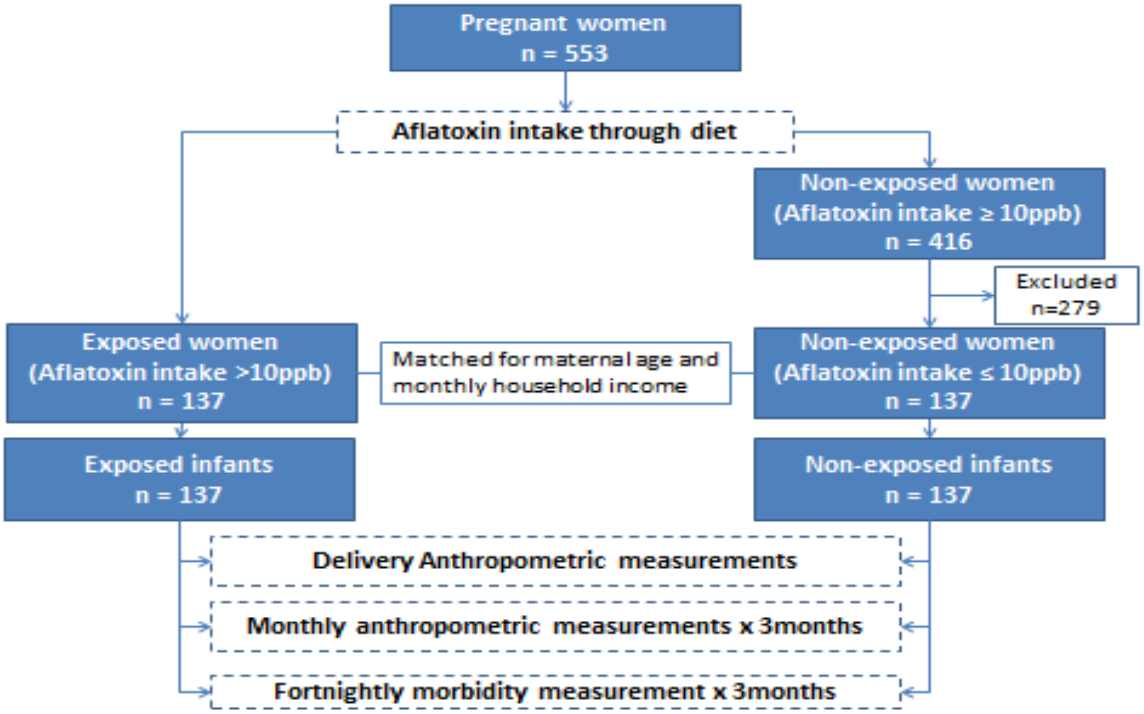


Figure 3.2 Participant Flow through the Study

3.6 Statistical Analysis

Data was entered in excel (version2010), cleaned and exported to Statistical Package for Social Sciences (SPSS) software (IBM SPPS Statistics®) version 20, where variables for analysis were generated. Continuous variables such as age of participant; size of household; weight of infant at birth; weight of infant at 1 month; weight of infant at 2 months, weight of infant at 3 months; length of infant at birth, length of infant at 1 month, length of infant at 2 months and length of infant at 3 months were tested for normality using frequencies and histograms to identify whether to use parametric or non-parametric test.

Prevalence of aflatoxin contamination levels in selected staple foods was determined by frequency of detectable AFB_1 in food samples and expressed as proportions. Levels of aflatoxin in market food were expressed as median and IQR. Levels of aflatoxin in cooked foods consumed by pregnant women and proportion of women exposed to aflatoxin levels > 10 ppb were used to determine aflatoxin exposure status. Predictors of maternal aflatoxin exposure were determined using multivariate logistic regression. WHO Anthro 2005, w3.2.2 software (WHO, 2006) was used to generate WLZ, WAZ and HLZ. Infants of exposed and non-exposed mothers were matched by maternal age and household income and logistic regression was used to compare differences between proportions of matched infant-mother pairs. Independent student T-tests were used to determine mean difference in infant weight and length at birth and logistic regression used to generate Odds Ratios of aflatoxin exposure at birth. Mean differences in infant length, weight, WLZ, HAZ and WAZ of exposed and non-exposed infants at 3 months after birth were assessed using Cox regression with constant time at risk. Relative Risks were calculated to determine the predictors of infant nutritional status; wasting, underweight and stunting using cox regression. P-values <0.05 were considered statistically significant results. Frequency distribution and prevalence of morbidity for each of morbidity outcomes were generated. Cross tabulations were performed on disease infection to determine frequency of episodes in the first 3 months after birth. Adjusted relative risk ratios with 95% CI were generated using Cox regression to establish the effect of aflatoxin exposure on morbidity at 3 months of age while adjusting for health facility, Sub-County of residence and maternal education. P-values <0.05 were considered statistically significant results. The exposed and non exposed women were already matched by maternal age and income levels. The adjusted analysis generated relative risk ratios and 95% confidence intervals.

3.7 Ethical Considerations

The research proposal was approved by the School of Graduate Studies, Maseno University approval process (Appendix 7). Permission to conduct study was sought from the Maseno University School of Graduate Studies. Ethical clearance was granted by the Maseno University Ethical Review Committee [MUERC] (Appendix 6). Ethical guidelines of anonymity, privacy and confidentiality of information gathered through the study were adhered to so the identity of participant was not disclosed to any unauthorized persons or to anybody for any purpose. Informed written consent was obtained from all the study participants. Participation in the study was voluntary and there were no direct benefits to participants. Participants benefited from education on aflatoxin awareness. All the necessary protocols were observed during entry into the study site (Appendix 8).

CHAPTER FOUR: RESULTS

4.1 Aflatoxin Contamination Levels in Market Foods

Aflatoxin in solid food and milk samples was assessed to determine contamination levels and identify samples with levels above the regulatory limits that could be potential sources of aflatoxin contamination in Kisumu Country (Table 4.1). The KEBS limits of 10ppb (KEBS, 2013) apply for solid foods and the Codex Alimentarius limits of 0.05 ppb (Codex Alimentarius, 2004) apply for milk samples.

Aflatoxin levels ranged from 0 ppb to 35.4 ppb aflatoxin B_1 in solid food samples and 0.00 ppb to 0. 13 ppb aflatoxin M_1 in milk samples. Of the solid food samples (n = 219) analyzed, 80.8% were contaminated with aflatoxin with 12.8% above the regulatory limit of 10ppb. All (100%) of the maize, groundnuts and sorghum samples were contaminated with aflatoxin. None of the cassava and *dagaa* samples had aflatoxin levels above the regulatory limit. Maize had the samples with the highest aflatoxin levels of 35.4 ppb, but they were isolated cases hence outliers. Of the dry foods, sorghum had the highest proportion of samples (71.4%) with aflatoxin contamination levels above the regulatory limit of > 10 ppb, with a median (IQR) contamination level at 14.2 (8.5, 19); while cassava had the lowest median (IQR) aflatoxin contamination levels at 0.5 (0.5, 0.5). All the processed milk (n = 50) and raw (n = 30) milk samples were contaminated with aflatoxin. Of the total processed milk samples (n=50)**,** 38% were contaminated with aflatoxin levels above regulatory limit of 0.05 ppb, while raw milk did not have any samples above the regulatory limit for milk. Detailed information on ranges of aflatoxin levels in specific foods and markets are shown in Appendix 13 and Appendix 14.

Table 4.1 Proportion of Food Samples Contaminated with Aflatoxin

Food Item	n	Contaminated n (%)	Above Aflatoxin regulatory Limit n (%)	Aflatoxin Levels	
				Median (IQR)***	ppb (Min, Max)
Dagaa	60	32 (53)	**0 (0)**	0.6 (0, 2.08)	(0.00, 2.76)
Rice	31	21(67)	1 (3)	0.5 (0, 1.2)	(0.00, 11.70)
Groundnuts	22	22 (100)	2 (9)	1.5 (0.5, 2.0)	(1.00, 27.60)
Cassava	37	33(89)	**0 (0)**	0.5 (0.5, 0.5)	(0.00, 3.50)
Maize	41	41(100)	5 (12.2)	0.5 (0.5, 1.0)	(0.50, 35.40)
Sorghum	28	28(100)	**20 (71.4)**	14.2 (8.5, 19)	(1.00, 24.50)
Total (AFB_1)	**219**	**177 (80.8)**	**28 (12.8)**	**0.70 (0.05, 2.08)**	**(0.00, 35.40)**
Processed milk	50	50 (100)	19 (38)	0.04 (0.03, 0.07)	(0.010, 0.13)
Raw milk	30	30 (100)	**0 (0)**	0.008 (0.005, 0.01)	(0.002, 0.012)
Total milk (AFM_1)	**80**	**80 (100)**	**19 (23.8)**	**0.03 (0.01, 0.50)**	**(0.0002, 0.13)**

*** Descriptive statistics were used to generate proportions, medians and interquartile ranges.

All *dagaa*, cassava and raw milk samples had aflatoxin levels below 10 ppb; with some of the samples having no detectable aflatoxin. Some maize samples had high aflatoxin levels, but all of these were outliers. When the median contamination levels were calculated, only sorghum had median aflatoxin levels above the regulatory limit of 10 ppb (Table 4.1).

Aflatoxin was assessed by market to determine potential source markets of aflatoxin contaminated foods in Kisumu County. Table 4.2 shows the overall median and IQR aflatoxin levels in total food samples for each market.

Table 4.2 Aflatoxin Levels (Ppb) by Market Place

Market	Number of samples analyzed	Median (IQR)***
Ahero**	64	0.7 (0.5, 2.08)
Mamboleo	42	0.5 (0.01, 1.8)
Kibuye Wholesale	36	0.5 (0.5, 1.75)
Kibuye Open Air market	65	0.5 (0.5, 7.3)
Oile Market	19	0 (0, 0.7)
Guba*	10	0.004 (0.002, 0.006)
Supermarkets*	51	0.04 (0.03, 0.07)

* Only milk samples.
** Dry foods and milk samples.
*** Descriptive statistics were used to generate medians and interquartile ranges.

Results in table 4.2 indicate aflatoxin levels in foods by market place. Food samples from Kibuye open air and Ahero markets had the highest overall aflatoxin median (IQR) levels, 0.5 (0.5, 7.3) and 0.7 (0.5, 2.08) respectively, compared to 0 (0, 0.7), 0.5 (0.01, 1.8) and 0.5 (0.5, 1.75) for former Oile, Mamboleo and Kibuye wholesale markets respectively.

4.2 Aflatoxin Exposure in Pregnant Women

4.2.1 Maternal Socio-demographic Characteristics

Maternal socio-demographic characteristics are shown in Table 4.3. Of the 553 participants recruited from two Sub-Counties, 185 participants (33.5%) were from Kisumu East Sub-County and 368 participants (66.5%) from Nyando Sub-County. Majority of the participants (83.2%) were aged between 15 – 19 years, with a mean age of 24.6 ± 5.2 years. Most households (91.1%) comprised of 1 to 6 persons. About eighty one percent of the women were married and 92.8% had either primary (55.4%) or secondary (37.4%) level of education. The major sources of income were business (49.7%) and from husband (23.3%), with most of the households (56.1%) having a monthly earning of Kshs. 2001- 5000.

Table 4.3 Maternal Socio-demographic Data

Variable Description	% (n)*
Health Facility:	
KDH	33.5 (185)
ADH	66.5 (368)
Sub-County of residence	
Kisumu East	32.5 (180)
Nyando	66.5 (368)
Kisumu West	0.9 (5)
Woman's age in Years	
15-19	17.3 (96)
20-24	37.8 (209)
25-29	28.3 (156)
30-34	11.6 (64)
35-39	4.2 (23)
40-44	0.9 (5)
Mean (SD)	24.6 ± 5.2
Participant's marital status	
Single	16.8 (93)
Married	81 (448)
Separated	0.9 (5)
Divorced	0.4 (2)
Widowed	0.9 (5)
Participant's education level	
None	0.4 (1)
Primary	55.4 (304)
Secondary	37.4 (207)
College	5.2 (29)
University	1.8 (10)
Participant's religion	
Christian	99.6 (551)
Muslim	0.4 (2)
No of people in the household	
1 – 6	91.1 (504)
7 – 12	8.9 (49)
Participant's source of income	
Farming	9.2 (51)
Employed	8.9 (49)
Business	49.7 (275)
Husband	23.3 (129)
Parents	7.6 (42)
Sibling	1.3 (7)
Household's monthly earnings	
<=2000	16.8 (93)
2001 – 5000	56.1 (310)
5001 – 10000	17.4 (96)
>10000	(54)

* Descriptive statistics were used to produce frequency distributions and proportions.

4.2.2 Aflatoxin Intake by Pregnant Women at Baseline

Aliquot food samples composed of 3 meals and snacks from cooked foods consumed by each of the 553 pregnant women in the previous 24-hours were analyzed for aflatoxin content (Figure 4.1). Of the 553 women, 75.2 % had dietary aflatoxin intakes ranging from 0 ppb to 9 ppb, 5.9% had dietary aflatoxin intakes ranging from 10 to 21ppb and 18.9% had aflatoxin intakes ranging from 22 to 39.5ppb.

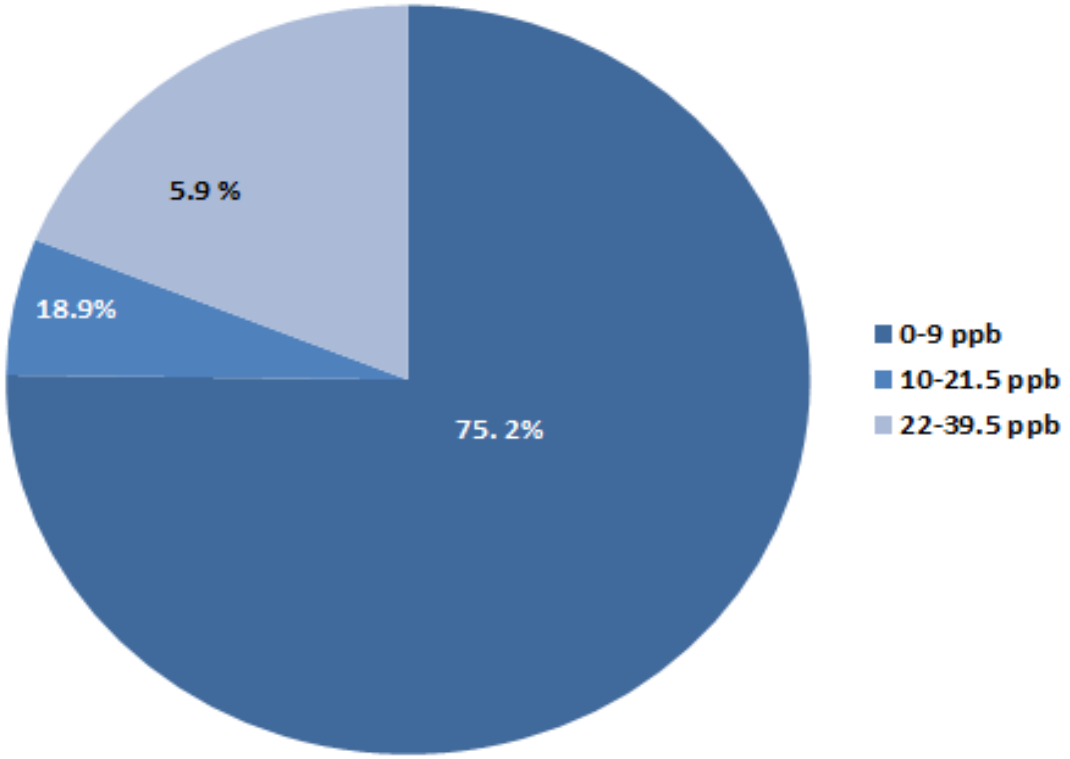


Figure 4.1 Aflatoxin Levels in Aliquot Food Sample Meals

Data is presented as tertiles of aflatoxin intake in this population of women. Of the 553 aliquot samples from foods consumed by pregnant women in the previous 24-hours, about 25% had aflatoxin levels ranging from 10 ppb to 39.5 ppb (Figure 4.1)

Daily absolute intake was calculated by multiplying amount of aflatoxin (µg/kg) in the total amount of food consumed by each woman in a day by the total amount of food consumed in a day (Gong et al., 2008). The aflatoxin levels were derived from the levels in aliquot samples of cooked meals consumed by each woman in a day and the amount of food consumed was calculated using 24-hour dietary recall and supported by data from food frequency questionnaires. The aliquot samples comprised of 3 main meals and any snack taken by the women in the previous 24 hours. Data on 24-hour recall was supported by information gathered from the food frequency questionnaire and weighed food records for selected sample of twenty participants (Appendix 15).

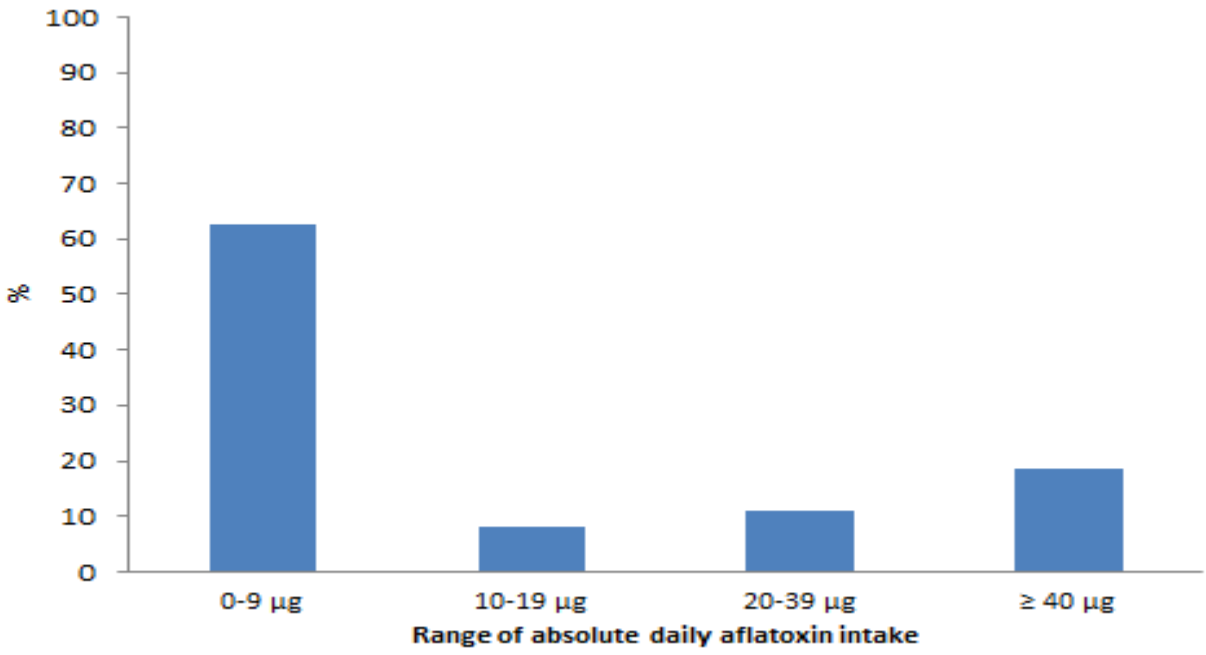


Figure 4.2 Daily Absolute Aflatoxin Intake by Pregnant Women in Kisumu County

Out of the 553 women recruited in the study, about 37% had daily absolute aflatoxin intake ranging from 10 µg to ≥40 µg (Figure 4.2).

The proportion of pregnant women consuming aflatoxin levels above 10 ppb was determined using data on aflatoxin level in food samples consumed by the women in one day (Figure 4.3).

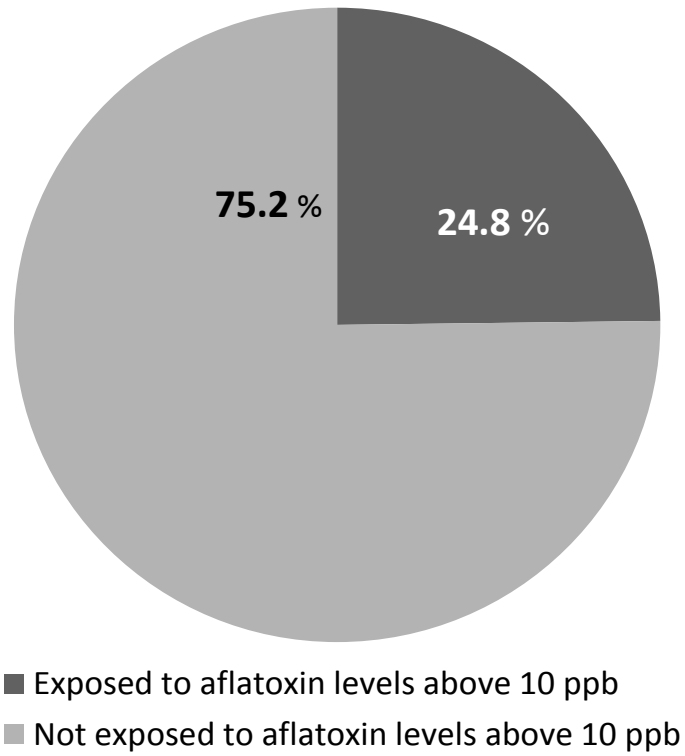
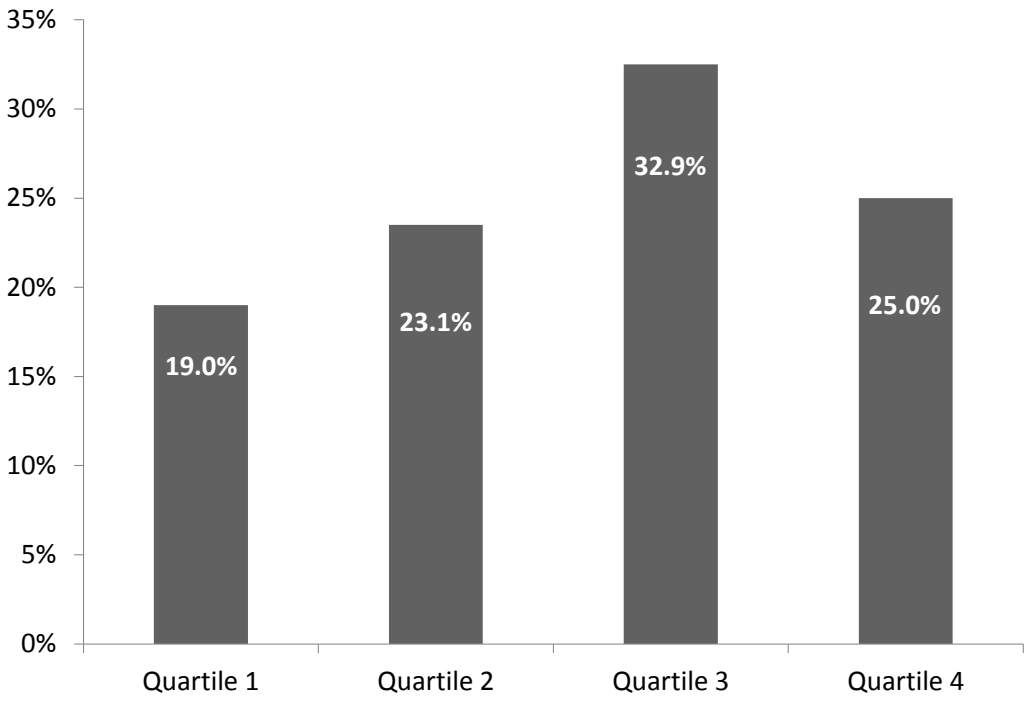


Figure 4.3 Proportion of Pregnant Women Exposed to Aflatoxin Levels >10 ppb

Of the cooked aliquot food samples from pregnant women who participated in the study, 24.8% had aflatoxin levels above 10 ppb (Figure 4.3), indicating that about 25% of the pregnant women in Kisumu County are exposed to aflatoxin levels above the recommended regulatory limit of 10 ppb.

Data on aflatoxin intake by pregnant women were stratified to determine the proportion of exposure by quartiles (Figure 4.4).



Quartile 1 = 0 – 0.9 ppb, Quartile 2 = 1.0 – 1.9 ppb, Quartile 3 = 2.0 – 8.75 ppb, Quartile 4 = > 8.75 ppb.

Figure 4.4 Quartiles of Exposure of Women to Aflatoxins

Results indicate that 19% of the pregnant women were in the 1st quartile, 23.5% were in the 2nd quartile, 32.5% in the 3rd quartile and 25% in the 4th quartile. More than 30% of the women were in the 3rd quartile with aflatoxin exposure ranging from 2 ppb to 8.75 ppb and 25% were in the fourth quartile with exposure ranging from 8.75 ppb to 39.4 ppb (Figure 4.4).

Table 4.4 Association between Aflatoxin Exposure and Socio-Demographic Factors at Baseline

Variables	N	Exposed n (%)	Not exposed n (%)	Crude Odds Ratio* [95%CI]	p-value
Age categories					
15-19	96	25(26.0)	71(74.0)	Ref	
20-24	209	63(30.1)	146(69.9)	1.22(0.71, 2.11)	0.463
25-29	156	35(22.4)	121(77.6)	0.82(0.45, 0.48)	0.514
30-34	64	6(9.4)	58(90.6)	0.29 (0.11, 0.76)	**0.012**
35-39	23	7(30.4)	16(69.6)	1.24 (0.46, 3.37)	0.670
40-44	5	1(20.0)	4(80.0)	0.71(0.08, 6.67)	0.764
Health facility					
Kisumu	185	26 (14.1%)	169 (85.9)	Ref	
Ahero	368	111(30.2%)	257 (69.8)	2.64 (1.65, 4.23)	**0.001**
Marital status					
Single	93	22 (23.7%)	71 (76.3)	Ref	
Married	448	113 (25.1%)	335 (74.8)	1.10 (0.60, 1.80)	0.750
Separated	5	1 (20.0%)	4 (80.0)	0.80 (0.09, 7.60)	0.851
Divorced	2	1 (50.0%)	1 (50.0)	Excluded	
Educational level					
None	2	1 (50.0%)	1 (50.0)	Ref	
Primary	306	82 (26.8%)	224 (73.2)	0.37 (0.02, 5.92)	0.48
Secondary	207	47 (22.7%)	160 (77.3)	0.29 (0.18, 4.79)	0.390
College	28	5 (17.9%)	23 (82.1)	0.22 (0.12, 4.09)	0.308
University	10	2 (20.0%)	8 (80.0)	0.25 (0.10, 5.99)	0.392
Occupation					
Housewife	153	39 (25.5%)	114 (74.5)	Ref	
Self employed	300	76 (25.3%)	224 (74.7)	0.99 (0.63, 1.55)	0.971
Employed	39	8 (20.5%)	71 (79.5)	075 (0.32, 1.78)	0.520
Other	61	14 (23.8%)	47 (77.0)	0.87 (0.43, 1.75)	0.698
Religion					
Christian	551	136 (24.7%)	415 (75.3)	Ref	0.434
Muslim	2	1 (50.0%)	1 (50.0)	0.33 (0.20-5.28)	0.431
Source of income					
Farming	57	14 (27.5%)	37 (72.5)	Ref	
Employed	48	10 (20.8)	38 (79.2)	0.70 (0.28, 1.76)	0.444
Business	276	70 (25.4%)	206 (74.6)	0.90 (0.46, 1.76)	0.754
Husband	129	31 (24.0%)	98 (76.0)	0.84 (0.40, 1.75)	0.633
Parents	42	12 (28.6%)	30 (71.4)	1.00 (0.43, 2.62)	0.905
Siblings	7	0 (0.0%)	7 (100.0)	Exclude	
Household monthly earnings Kshs)					
≤2000	93	39 (41.9%)	54 (58.1)	Ref (1.0)	
2001-5000	310	77 (24.8%)	233 (75.2)	0.46 (0.28, 0.74)	**0.002**
5001-10000	97	16 (16.5%)	81 (83.5)	0.27 (0.14, 0.54)	**0.001**
>10000	53	5 (9.4%)	48 (90.6)	0.14 (0.05, 0.41)	**0.001**

*Odds ratio and 95% CI were generated using binary logistic regression.

The odds of being exposed was not different in age categories 20-24 (OR=1.22, 95% CI: 0.71, 2.11, p=0.46), 35-39 (OR=0.82, 95% CI: 0.45, 0.48, p=0.54) and 40-44 (OR=0.29, 95% CI: 0.11, 0.76, p=0.01) years compared to the reference group of 15-19 years. However the odds of being exposed was 71%[1] lower in women aged 30-34 years (OR=0.29, 95% CI: 0.11, 0.76, p=0.012) compared to women of age category 15- 19 years. The odds of being exposed was 2.64 times higher in women from Ahero County Hospital (OR=2.64, 95% CI: 1.65, 4.23, p>0.001) compared to women from Kisumu County Referral Hospital. The odds of being exposed was not different in those who were married (OR=1.10, 95% CI: 0.60, 1.80, p=0.750), separated (OR=0.80, 95%: 0.09, 7.60, p=0.851) compared to who were single. The odds of being exposed to aflatoxin was not different in those with primary (OR=0.37, 95% CI: 0.02, 5.92, p=0.48), secondary (OR=0.29, 95% CI: 0.18, 4.79, p=0.390), college (OR= 0.22, 95% CI: 0.12, 4.09, p=0.308) and university (OR=0.25, 95% CI=0.10, 5.99, p=0.392) education compared to those with no education. The odds of being exposed to aflatoxin was not different in those who were self- employed (OR=0.99, 95% CI: 0.63, 1.55, p=0.971), employed (OR= 0.75, 95% CI: 0.32, 1.78, p=0.520) and others (OR=0.87, 95% CI: 0.43, 1.75, p=0.698) compared to those were housewives. The odds of being exposed to aflatoxin was not different in those who were Muslims (OR=0.33, 95% CI: 0.20-5.28, p=0.431) compared to those who were Christians. The odds of being exposed to aflatoxin was not different in those whose source of income was employment (OR=0.70, 95% CI: 0.28, 1.76, p=0.444), business (OR=0.90, 95%CI: 0.46, 1.76, p=0.754), husband (OR=0.84, 95% CI: 0.40, 1.75, p=0.633), parents (OR=0.84, 95% CI: 0.40, 1.75, p=0.905) compared to farming. The odds of being exposed was 54%, 73% and 86% less in women from households earning 2001-5000 (OR=0.46, 95% CI:0.28, 0.74, p=0.002), 5001-

[1] Calculated as (Odds Ratio – 1) x 100

10000 (OR=0.27, 95% CI:0.14, 0.54, p=0.001 and >10000 (OR=0.14, 95% CI:0.05, 0.41, p=0.001) respectively, compared to women in households with monthly income < 2000. The odds of being exposed decreased with increased income (Table 4.5).

The participants were matched for aflatoxin exposure by maternal age and monthly household income and their baseline characteristics determined by exposure (Table 4.4).

Table 4.5 Maternal Baseline Characteristics by Aflatoxin Exposure for Matched Women

Characteristic	Exposed (n=137) n (%)	Non-exposed (n=137) n (%)	All (n=274) n (%)
Sub-County of residence			
Kisumu East	25 (18.2)	42 (30.7)	67 (24.5)
Nyando	111 (81.0)	94 (68.6)	205 (74.8)
Kisumu West	1 (0.7)	1 (0.7)	2 (0.7)
Household Monthly Earnings (Kshs.)			
≤2000	39 (28.5)	39 (28.5)	78 (28.5)
2001-5000	77 (56.2)	77 (56.2)	154 (56.2)
5001- 10000	16 (11.7)	16 (11.7)	32 (11.7)
>10000	5 (3.6)	5 (3.6)	10 (3.6)
Woman's age*	23.6 (5.0)	24.1 (5.4)	(5.2)

*Descriptive statistics were used to generate Mean (SD), frequency distribution (n) and proportions (%).

Of the exposed women, a bigger proportion (81%) was from Nyando Sub-County, compared to Kisumu East (18.2%) and Kisumu West Sub-Counties (0.7%). Of the non exposed women, 30.7% were from Kisumu East Sub-County, 68% from Nyando Sub-County and 0.7% from Kisumu West Sub-County. Of the exposed women, 28.5% had household income ≤ Kshs. 2000,

56.2% had household income ranging from Kshs. 2001-5000, 11.7% had household income ranging from 5001-10000 and 3.6% had household income of > Kshs. 10000. Of the non-exposed women, 28.5% had household income ≤ Kshs. 2000, 56.2% had household income ranging from Kshs. 2001-5000, 11.7% had household income ranging from 5001 -10000 and 3.6% had household income of > Kshs. 10000.

4.3 Effect of Maternal Aflatoxin Exposure on Infant Growth

4.3.1 Infant Food Intake in the First 3 Months after Birth

Given that the premise of this study was to determine the effect of aflatoxin intake by the mother on infant growth, the possibility that other sources of food apart from breast milk could have been consumed by the infant hence introducing sources of aflatoxin other than breast milk, was first assessed. All infants who consumed foods other than breast milk in the first three months were considered as not having been exclusively breastfed (Table 4.6)

Table 4.6 Infant Food Intake (n=553)

Food Item and Frequency of Consumption	Quantity	n (%)*
Porridge (ml) consumed by infant in a day	0	540 (97.6)
	50 – 300	13 (2.4)
Cow's milk (ml) consumed in a day	0	517(93.5)
	20 – 300	16(2.9)
NAN(g) consumed in a day	0	552(99.8)
	20 – 300	1 (0.2)
Water consumed in a day	0	520 (94)
	5- 300	33 (6)
Water consumed in a day	0	547 (99)
	20 – 140	6 (1)
Exclusively breastfed	-	333(85.4)

0 implies not consumed.
*Descriptive statistics were used to generate frequency distribution (n) and proportions.

Of the infants who participated in the study, 2.4% (n=13) consumed porridge in a day, 2.9% cow consumed milk, 7% consumed water and 0.2% consumed NAN (Table 4.11).

Data on breast feeding frequency was collected to determine how many times an infant was breastfed in a day, because this could translate into amount of milk consumed in a day and also amount of aflatoxin exposure through breast milk.

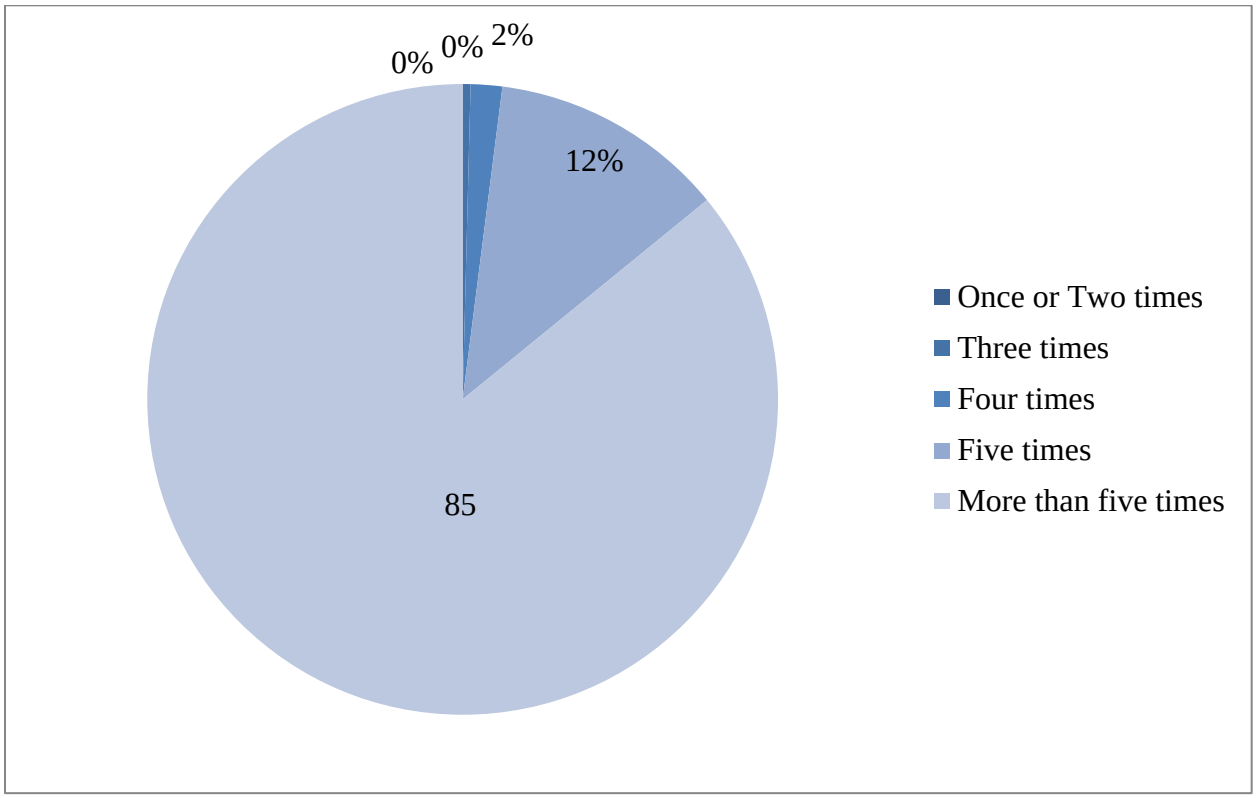


Figure 4.5 Breast Feeding Frequency per Day (%)

Of the 274 infants matched for mother's marital age and household income, 86% were breastfed more than five times in a day; 12% of the infants were breastfed five times a day, while 1.6% were breastfed four times a day and 0.4% were breastfed three times a day.

4.3.2 Balance of Baseline Characteristics in Exposed and Non-exposed Infants

Infant characteristics that could influence growth in exposed and non-exposed infants were assessed at birth to identify factors that could influence growth at birth, for adjustment in the effect analyses. Being a cohort study, such balance was determined by assessing mean difference between groups or odds ratios where appropriate (Table 4.7). Data on infant baseline characteristics and WLZ, HLZ and WAZ among 553 unmatched infants at 3 months of age in Kisumu County are presented Appendix 11 and Appendix 12, respectively.

Table 4.7 Infant Baseline Characteristics by Aflatoxin Exposure at Birth

	All Infants n=274	**Not Exposed n=137**	**Exposed n=137**	**Group Comparison (95% CI)**	**Pvalue**
				Mean difference*	
Birth mean weight[1](Kg)	3.26 ± 0.49	3.53 ± 0.43	2.99 ± 0.37	-0.54 (-0.61, -0.46)	<0.001
Birth meam length[1](cm)	49.45 ± 3.93	51.14 ± 3.49	47.84 ± 3.64	-2.99 (-3.71, -2.28)	<0.001
				Odds Ratios**	
Wasting (%)	18.8 (	23.5	13.3	0.500 (0.25, 1.01)	0.05
Stunting (%)	13.8	4.2	23.0	6.88 (2.56, 18.45)	<0.00
Underweight(%)	3.3	0.7	5.8	8.43 (1.04, 68.39)	<0.00
Exclusive Breastfeeding (%)	85.4 (234)	85.4 (117)	85.4 (117)	1.00 (0.51, 1.96)	=1.00
Low birth weight (%)	4	0.7	7.3	10.71 (1.35, 84.85)	=0.03

[1]Mean±SD; *Generated using t-test; **Generated using logistic regression.

The average birth weight was 0.54 kg lower in infants of exposed women than in infants of non-exposed women (95% CI: -0.61, -0.46, p< 0.001). The average birth length was 2.99 cm lower in infants of exposed women than in infants of non-exposed women (95% CI:-3.71, -2.28; p< 0.001). The odds of wasting was 50% lower in infants of exposed women compared to infants of

non-exposed women (95% CI:-3.71, -2.28; p<0.001). The odds of stunting was 6.88 times higher in infants of exposed women compared to infants of non-exposed women (95% CI: 2.56, 18.46; p<0.001). The odds of being underweight was 8.43 times higher in infants of exposed women than in infants of non-exposed women (95% CI: 1.04, 68.39; p<0.001). Of the 234 exclusively breastfed infants, equal numbers of 117 (85.4%) were from both exposed and non-exposed women. Exclusive breast feeding was not different in infants of exposed women and those of non-exposed women (p=1.00). The odds of lower birth weight was 10.71 times higher in infants of exposed women than in infants of non-exposed women (95% CI: 1.35, 84.85; p=0.05), Table 4.7.

Difference in weight and length between exposed and non-exposed infants at birth, 1, 2 and 3 months was determined using t-tests to establish existence of potential trends in growth over the study period (Table 4.8)

Table 4.8 Mean Weight and Length of Infants by Aflatoxin Exposure

Time (Months)	Mean Weight (kg)*				Mean Length (cm)*			
	Birth	**1**	**2**	**3**	**Birth**	**1**	**2**	**3**
Exposed	2.99±0.37	3.82±0.65	4.72±0.60	5.54±0.54	47.84±3.64	50.93±3.24	53.98±2.58	57.67±2.39
Not Exposed	3.52±0.41	4.72±0.64	5.70±0.73	6.65±0.74	50.84±3.40	54.43±3.22	58.15±3.27	62.83±3.59
P-value**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Overall	3.39±0.46	4.50±0.75	5.46±0.82	6.39±0.84	53.56±3.56	53.56±3.56	57.13±3.60	61.56±4.00

* Mean±SD generated using descriptive statistics.
**Based on results of difference between means of exposed and non-exposed children determined by t-test.

Both length and weight appeared to increase over the study period in both exposed and non-exposed groups with significant differences between the exposed and non-exposed groups at

each of the four time points (Table 4.8). Results of trend analysis for weight and length from birth to 3 months are presented in Figures 4.6 and 4.7.

Maternal aflatoxin exposure was assessed to determine the effect on infant weight at month 1, 2, and 3 in 137 exposed and 137 non-exposed matched infants

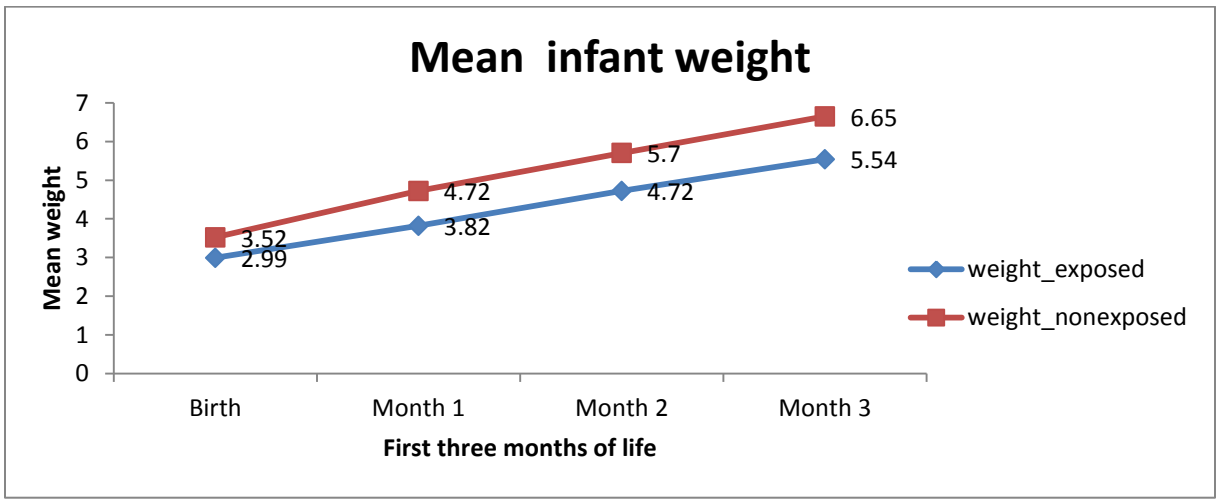
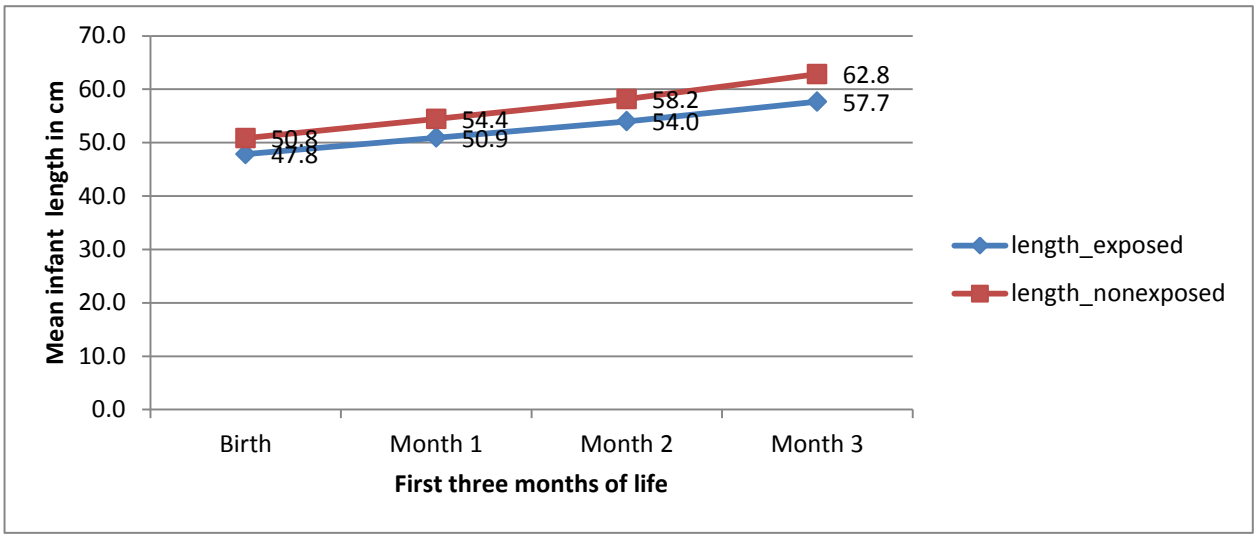


Figure 4.6 Mean Weight of Infants at the First 3 Months

Mean weights increased consistently from birth to 3 months. Mean weights of infants of exposed women were consistently lower than those of non-exposed women at birth, 1 month, 2 months and 3 months (Figure 4.7)

Maternal aflatoxin exposure was assessed to determine the effect on infant length at month 1, 2, and 3 in exposed and non-exposed infants. Mean length of infants of exposed women were consistently lower than mean length of non-exposed women at birth, 1 month, 2 months and 3 months (Figure 4.8)



n= exposed, 137, non-exposed, 137.

Figure 4.7 Mean Length of Infants at the 1st 3 Months

4.3.3 Effect of Aflatoxin Exposure on Infant Growth at 3 Months

Crude and adjusted analyses were carried out to determine whether aflatoxin exposure had an effect on infant anthropometric indices including weight, length, WLZ, LAZ and WAZ. Results in Table 4.9 show the effect of maternal aflatoxin exposure on infant LAZ and WAZ at 3 months of age. Infants of pregnant women exposed to aflatoxin levels above 10 ppb had lower mean weight, length, LAZ and WAZ, at 3 months of age compared to infants of women who were not exposed $p<0.001$. However, there was no evidence of any difference in mean WLZ between infants of exposed and infants of non-exposed women ($p = 0.071$).

Table 4.9 Effect of Aflatoxin Exposure on Infant Growth at 3 Months (Matched Analysis)

Variable	Crude Analysis		Adjusted Analysis	
	Mean (95% CI)	p-value Test	Mean (95% CI)	p-value *
Weight (kg)	-1.06 (-1.21, -0.92)	<**0.001**	-0.69 (-0.85, -0.53)§	<**0.001**
Length (cm)	-4.88 (-5.57, -4.20)	<**0.001**	-4.08 (-4.08, -3.36)◊	<**0.001**
WLZ	0.36 (0.04, 0.75)	=**0.022**	0.35 (-0.03, 0.74)†	=0.071
LAZ	-1.92 (-2.29, -1.55)	<**0.001**	- 1.54 (-1.93, -1.16)‡	<**0.001**
WAZ	-1.23 (-1.48, -0.99)	<**0.001**	- 0.79 (- 1.03, -0.54)^	<**0.001**

*Cox regression with constant time at risk was used to determine effect of aflatoxin exposure on infant growth.
§Adjusted for maternal age and infant weight at birth; ◊Adjusted for maternal age and infant length at birth; †Adjusted for maternal age and infant WLZ at birth; ‡Adjusted for maternal age and infant LAZ at birth; ^Adjusted for maternal age and infant WAZ at birth.

Relative risk for wasting, underweight and stunting was assessed using Cox regression with constant time at risk to determine the predictors of infant nutritional status; wasting, underweight and stunting (Table 4.10). Risk of stunting and underweight in infants of women who were exposed to aflatoxin levels above 10 ppb was 4.07 times higher (95% CI: 1.35, 12.29; p=0.013) and 6.61 times higher (95% CI: 0.80, 54.33; p=0.079), respectively, than in infants who were not exposed. There was no evidence of a difference in risk of wasting between infants of exposed and non-exposed women (RR 0.38, 95% CI: 0.040, 3.39; p=0.377). Results are presented in Table 4.10.

Table 4.10 Risk of Aflatoxin Exposure on Wasting, Underweight and Stunting at 3 Months in Matched Infants

Nutritional status	RR*	95% CI		Pvalue
		Lower	Upper	
Wasting (LAZ < -2)	0.37	0.04	3.39	0.38
Underweight (WAZ < -2)	6.61	0.80	54.33	0.08
Stunting (LAZ < -2)	4.07	1.35	12.29	**0.01**

*Cox regression analysis used to generate RR.

The risk of wasting was 63% lower in infants of exposed women than in infants of non-exposed women (95% CI: 0.04, 3.39; p=0.38). The risk of underweight was 6.6 times higher in infants of exposed women than in infants of non-exposed women (95% CI: 0.08, 54.33; p=0.08). The risk of stunting was 4.07 times higher in infants of exposed women than in infants of non-exposed women [95% CI: 1.35, 12.29; p=0.01] (Table 4.10).

4.4 Aflatoxin Exposure and Infant Morbidity

4.4.1 Frequency of Infant Morbidity

Cross tabulation was conducted to determine frequency of illness among exposed and non-exposed infants from birth to 3 months of age (Table 4.11). The most common sicknesses among infants in the first three months after birth were fever, malaria, common cold, diarrhea and plastic teeth.

Table 4.11 Frequency of Disease Infection in the First 3 Months in 137 Exposed and 137 Non-exposed Infants

	Month 1		Month 2		Month 3	
	Exposed n (%)	**Not Exposed n (%)**	**Exposed n (%)**	**Not Exposed n (%)**	**Exposed n (%)**	**Not Exposed n (%)**
Malaria	22(16.1)	10 (7.3)	4(2.9)	2 (1.5)	1(0.7)	1(0.7)
Fever	15(10.9)	17 (12.4)	15 (10.9)	9 (6.6)	0	4(2.9)
Jaundice	0 (0)	1 (0.7)	1 (0.7)	0	0	0
Umbilical cord infection	0	1 (0.7)	1 (0.7)	0	0	0
Diarrhea	7 (5.1)	0	4 (2.9)	2(1.5)	1 (0.7	0
Common Cold	11 (8.0)	10 (7.3)	6 (4.4)	5(3.6)	1 (0.7)	2 (1.5)
Cough	10 (7.3)	9 (6.6)	6 (4.4)	2(1.5)	1(0.7)	1(0.7)
Eye sores	0	1 (0.7)	0	1(0.7)	0	1(0.7)
Pneumonia	1(0.7)	0	0	0	0	0
Plastic teeth	4 (2.9)	4 (2.9)	2 (1.5)	0	0	0
Neonatal sepsis	0	0	0	0	0	0
Conjunctivitis	0	1(0.7)	0	0	0	0
Total Sick	**70**	**45**	**39**	**21**	**4**	**8**

*Descriptive statistics were used to generate frequency distribution (n) and proportions (%).

Of the 137 exposed infants, 70, 39 and 4 fell sick in the 1st, 2nd, and 3rd months, respectively compared to 45, 21 and 8 infants of the 137 non exposed infants who fell sick in the 1st, 2nd, and 3rd months, respectively. Of the infants of exposed women, 16%, 2.9% and 0.7% suffered from malaria in the 1st, 2nd and 3rd month after birth, respectively, compared to 7.3%, 1.5% and 0.7% infants of non-exposed women. Of the infants of exposed women, 10.9%, 10.9% and 0% suffered from fever in the 1st, 2nd and 3rd month after birth, respectively, compared to 12.4%, 6.6% and 2.9% infants of non-exposed women. Of the infants of exposed women, 5.1%, 2.9%

and 0.7% suffered from diarrheoa in the 1st, 2nd, and 3rd month after birth, respectively, compared to 0%, 1.5% and 0% infants of non-exposed women. Of the infants of exposed women, 8.0%, 4.4% and 0.7% suffered from common cold in the 1st, 2nd, and 3rd month after birth, respectively, compared to 7.3%, 3.6% and 1.5% infants of non-exposed women. Of the infants of exposed women, 7.3%, 4.4% and 0.7% suffered from cough in the 1st, 2nd, and 3rd month after birth, respectively, compared to 6.6%, 1.5% and 0.7% infants of non-exposed women.

4.4.2 Association between Maternal Aflatoxin Exposure and Infant Morbidity

The crude effect of maternal exposure on infant morbidity in the first 3 months of age was determined using bivariate analysis. Adjusted relative risk was determined using multivariate Cox regression with constant time at risk to control for potential confounding factors (Table 4.12)

Table 4.12 Association between Maternal Aflatoxin Exposure and Infant Morbidity

			Crude analysis		Adjusted analysis[1]	
	Not Exposed n (%)	**Exposed n (%)**	**RR (95% CI)**	**p-value**	**RR (95% CI)**	**p-value**
Malaria	13 (9.5)	23 (16.8)	2.08 (1.07, 4.03)	**p=0.03**	2.04 (1.05, 3.99)	**p = 0.04**
Fever	23 (16.8)	30 (21.9)	1.30 (0.76, 2.25)	p=0.34	1.25 (0.72, 2.16)	p = 0.43
Jaundice	1 (0.7)	1 (0.7)	1.0 (0.06, 15.99)	P=1.00	0.66 (0.04, 12.56)	P=0.79
Umbilical cord	1 (0.7)	1 (0.7)	0.15(0, 1.4x 10^5)	P=0.61	0.02(0.0, 1.4x10^5)	P=1.00
Diarrhoea	2 (1.5)	12(8.8)	4 (1.13, 14.18)	**P=0.03**	4.13 (1.16, 14.76)	**p = 0.03**
Common Cold	17 (12.4)	18 (13.1)	1.06 (0.55, 2.05)	P=0.87	1.96 (0.49, 2.86)	P=0.89
Cough	12 (8.8)	17 (12.4)	1.42 (0.68, 2.97)	P=0.36	1.34 (0.64, 2.83)	0.44
Pneumonia	0	1 (0.7)	-	-	-	-
Plastic Teeth	4 (2.9)	7 (5.1)	0.56 (0.16, 1.95)	P=0.36	0.53 (0.15,1.89)	P=0.33
Neonatal sepsis	0	0	-	-	-	-
Conjunctivitis	1 (0.7)	1(0.7)	0.15(0, 1.4 x10^5	P=0.61	0.15 (0, 164.78	P=0.38

RRs generated by Cox regression with constant time at risk; [1]Adjusted for health facility, Sub-County of residence and maternal education, monthly household income and age.

Results of crude analysis showed that the risk of malaria was 2.08 times higher in infants of exposed women compared to those of non-exposed women (95% CI: 1.07, 4.03, p=0.03). The risk of diarrhea was 4.00 times higher in children of exposed mothers than in children of non-exposed women (95% CI: 1.13, 14.18, p=0.003). In the adjusted analysis, aflatoxin exposure still had an effect only on malaria (RR=2.04, 95% CI: 1.05, 3.99, p=0.04), diarrhea (RR= 4.13, 95% CI: 1.16, 14.76, p=0.03). There was no difference in the risk of fever, jaundice, umbilical cord infection, common cold, cough, plastic teeth and conjunctivitis in exposed and non-exposed infants.

CHAPTER FIVE: DISCUSSION

5.1 Aflatoxin Contamination Levels in Selected Market Foods in Kisumu County, Kenya

In this study, selected market foods were analyzed to identify foods which could be potential sources of aflatoxin exposure in pregnant women in Kisumu County. All the samples of maize, sorghum, groundnuts and milk were contaminated with varying levels of aflatoxin, indicating that they are all sources of dietary aflatoxin exposure. All the foods except *dagaa*, cassava and raw milk had samples with aflatoxin levels above the regulatory limits; therefore they are sources of high aflatoxin exposure as defined in this study. Sorghum had the highest number of samples contaminated with aflatoxin and also the highest median aflatoxin levels reflecting the most consistent contamination of all the foods. Although maize had the samples with highest aflatoxin contamination levels, they were isolated samples. This study established that although sorghum, maize, groundnuts and rice are sources of aflatoxin contamination at levels above the Kenya regulatory limit, such contamination occurs in less than 50% of total samples and only translates to consistent contamination in sorghum. These results are of concern for several reasons:

First, the foods analyzed form a major component of staple foods produced and consumed in Kisumu County (MoA, 2012). Whereas previous studies on aflatoxin contamination internationally (Gong et al., 2008) and in Kenya (Collins et al., 2010; CDC, 2004; Mutegi et al., 2007) have focused on maize and groundnuts, little attention is given to other foods which could be potential sources of aflatoxin contamination. Results from our study show that rice, *dagaa*, sorghum and processed milk may also expose individuals to aflatoxins above regulatory limits and hence may pose a health risk to consumers in Kisumu County. This is especially so if

individuals purchase even isolated samples with high contamination levels, given that aflatoxin persists in the system for a considerable period of time. In a simulation study from the data in this study, it was indicated that if the food items assessed were used as complimentary foods for infants, assuming consumption of cereal and tubers and milk, infants could have aflatoxin intakes as high as 110 ng (0.110µg)/kg body weight per day (Obade et al., 2015a); and pregnant women 150 µg/ kg body weight per day (Obade et al., 2015b). These figures are higher than the findings from a study carried out in The Gambia, West Africa, where residents of Keneba, West Kiang, were found to be exposed to aflatoxin originating from several foods with an intake ranging from 0 to 29 µg/day (Wild et al., 1992).

Secondly, the findings of this study reveal that about 90% of the foods were contaminated with aflatoxin compared to the reported 25% of the world food contamination (FAO, 2011). However, unlike the FAO's 25% that takes into consideration aflatoxin contamination of all foods consumed, this study only focused on selected samples comprising of the commonly consumed foods in Kisumu County, therefore bias cannot be ruled out in the high percentage. Nonetheless, these foods form a big proportion of the foods consumed in Kisumu County and their flours are commonly used in complimentary foods by most households in the County (MoA, 2012, Okoth and Ohingo, 2004), hence the results of this study may be a good reflection of average consumption of aflatoxin. Data from weighed food records from one day dietary intakes by 20 participants showed that 19 consumed at least one meal of ugali, 9 at least one meal of porridge, 10 consumed at least one meal of rice, 20 at least one meal of tea with milk, and 9 at least one meal of omena in one day. These data showed that cereals, milk, as well as omena were commonly consumed in Kisumu County and could be possible sources of aflatoxin exposure,

when considered together with the results of the market survey. The foods found to be commonly eaten in this study are similar to those shown in other studies (Muyanga et al., 2004; Nyoro et al., 2004; Onyango et al., 2012).

Third, maize (Mwaura, 2011) and groundnuts (Wagacha et al., 2013) have been considered the major sources of aflatoxin and the results of this study showed that sorghum may also require equal attention given its production and wide consumption in the region (DoA, 2015). In this study, the proportion of sorghum samples with detectable aflatoxin levels was higher than that of maize and groundnuts, indicating more widespread contamination in sorghum than in other foods. This suggests that sorghum could be an emerging possible source of high levels of aflatoxin compared to maize as reported by previous studies (Lewis et al., 2005; Mwihia et al., 2008; Mwaura, 2011). Therefore, although the findings of this study are based on cross-sectional data, only reflecting contamination at one time point, the findings of this and the other studies, in combination, point to a need to confirm whether or not this contamination persists over time. Furthermore, from the food frequency and 24-hour dietary recall surveys, sorghum was used in preparation of *ugali* (cooked paste made from flour and water) and porridge consumed by both adults and young children (Appendix 4). Data from literature review showed that although groundnuts have been reported among the foods with high levels of aflatoxin in Nyanza Region (Mutegi et al., 2007; Wagacha et al., 2013); at the time of data collection, groundnuts were scarce in the market resulting in low consumption during the study period, indicating that overall aflatoxin contamination could be even higher than reported in our study, hence our results could be conservative.

Fourth, sorghum, rice and cassava are among the major food crops being promoted as food security crops by the National and County Governments under the Traditional High Value Crops and Rice Promotion Programmes (DoA, 2015). This has resulted in increased production and by extension, consumption of these foods. At the levels detected through the analysis, sorghum and rice could be among major contributors to aflatoxin exposure in Kisumu County, other than groundnuts and maize. Cassava and *dagaa* (*Rastrineobola argentea*) had the lowest aflatoxin levels among dried foods.

The levels of aflatoxin in *dagaa* reported in this study were similar to those reported in samples of *dagaa* in Winam Gulf of Kenya Kisumu (Dora et al., 2015). Given that Nile Perch and *dagaa* together constitute over 90% of fish of Lake Victoria and that 70% of *dagaa* from Nyanza region is used as animal feed (MoFD, 2012) and 30% as human food; and that women consumed more fish than their male counterparts (Githukia et al., 2014), there is need to ensure that fish consumption does not expose women and young children to aflatoxins . Further, given that the detection limit for both aflatoxin B_1 and aflatoxin M_1 was < 1 ppb, even samples with non-detectable levels could still contribute to the total aflatoxin exposure in Kisumu County.

Processed milk, but not raw milk, had samples with contamination levels above the Codex Alimentarius regulatory limits. The findings of this study concur with results from the study by Kangethe and Langa (2009) who established that 72% of the milk from dairy farmers, compared with 99% of the pasteurized marketed milk were positive for aflatoxin M_1, and 20%, and 31% of positive milk from dairy farmers, and market outlets respectively, exceeded the WHO/FAO

levels of 0.05µ g/Kg-1. High levels of aflatoxin in processed milk could have resulted from aflatoxin contaminated animal feed concentrates consumed by the animals.

Codex Alimentarius recommends a regulatory limit of 0.05µg/kg for countries with strict regulatory measures for aflatoxin $M_{1;}$ and 0.5µg/kg for other countries (Henry et al., 2001). Currently, Kenya does not have set minimum regulatory limit for aflatoxin M_1 for milk (KEBS, 2013), whose consumption is high especially in infants and young children. Given that mean aflatoxin M_1 concentrations of 0.023 µg, 0.05 µg and 0.5 µg in milk are associated with 9.4, 20 and 200 cancer cases per year per 10^6 people (Codex Alimentarius, 2001 and 2004), the levels in processed milk in Kisumu County should spur some action from respective authorities. Therefore, based on the levels of aflatoxin established in processed milk, there is need to institute quality control measures to ensure continued safety of milk consumed by infants and the entire community; given that milk forms a big proportion of the first food an infant is given before introduction of other foods.

In considering food contamination by source, food samples from Ahero and Kibuye open air markets had the highest median aflatoxin levels, while food samples from Oile market had the lowest aflatoxin levels. Ahero area is characterized by frequent flooding during the rainy seasons and prolonged drought during the dry periods (KCIDP, 2013), conditions which favor growth of moulds. Ahero market is also situated in the rural areas of Nyando Sub-County and according to the findings by Antonius et al.(2005) and Bhat et al.(2003), contamination of food supplies by aflatoxin was greater in rural communities of developing countries. Kibuye Open Air Market did not have properly constructed shelters for dry foods; the foods were therefore exposed to extreme

weather conditions making them vulnerable to aflatoxin contamination and proliferation of aflatoxins. Oile Market, on the contrary, had temporary structures which provided some form of protection against harsh weather conditions; which could explain the low levels of aflatoxin in foods from that market.

Kisumu County is not food secure and some of the foods consumed in the County are sourced from outside the County based on the figures on food production and requirements [Table 2.2] (MoA, 2015). Some of the sources of the food consumed are as indicated: *Dagaa* (Homa Bay, Migori, Siaya and Bondo); Rice (Busia, Tanzania, Siaya); Groundnuts (Uganda, Busia, Homa Bay); Maize (Rift Valley, Busia, Molo, Migori); Sorghum (Busia, Uyoma, Migori, Siaya), Raw milk (Nandi, Kericho, Bomet) (Table 2.2). Traceability of the food sources is important if efforts to minimize aflatoxin levels in the foods in Kisumu County are to succeed.

5.2 Aflatoxin Exposure in Pregnant Women in Kisumu County

About a quarter of pregnant women attending antenatal care clinics at Kisumu County Referral and Ahero County Hospitals consumed food with aflatoxin levels above the recommended Kenyan regulatory limit of 10 ppb. The results of the analysis of cooked food consumed by pregnant women in the previous 24-hours showed detectable levels of aflatoxin in most food samples. Aflatoxin levels in cooked food samples consumed by pregnant women in one day indicated that pregnant women consumed up to 39.5 ppb of aflatoxin in their daily diets; and 24.8 % of these women consumed food with aflatoxin levels above the recommended Kenyan regulatory limit of 10 ppb. This is of concern because of the cumulative effect of such exposure on the health of consumers over time, given that aflatoxin in the blood has a half-life of 30 to 60

days (Cardwell & Henry, 2004) and both low and high levels of exposure are associated with negative effect on human health.

The Kenya Bureau of Standards recommends 10 ppb as maximum permissible levels of aflatoxin in foods for human consumption (KEBS, 2013), Codex Alimentarius recommends aflatoxin allowable limit of 9 ppb (Codex Alimentarius, 2004), and the European Commission [EC] 2 ppb (EC, 2001). This means that pregnant women in Kisumu County consume higher amounts of aflatoxin than their counterparts in Europe where there are stringent aflatoxin allowable limits. Data from this study showed that overall, 92% of the food consumed by women in the study area had detectable aflatoxin levels. This implied that a big proportion of women and their infants were exposed to varying levels of aflatoxin through dietary intake. In a study carried out in the Gambia, a strong correlation was established between serum levels of AF-albumin and dietary aflatoxin (Wild et al., 1992). The Gambian study supports dietary intake as indicator of aflatoxin exposure, further giving strength to our study.

About 38% of the pregnant women had daily absolute aflatoxin intake ranging from 10 µg/day to 142 µg/day. These figures are higher than the findings from the Gambian study conducted in West Kiang (Wild et al., 1992). Findings from the study support a positive correlation between dietary aflatoxin intake and levels of serum aflatoxin-albumin adducts. This implies that pregnant women in Kisumu County have nearly twice as much aflatoxin intake as women in The Gambia, suggesting that exposure to aflatoxin in women in Kisumu County could potentially be reduced by up to a half if lessons were draw lessons practices in The Gambia.

Women recruited from Ahero County Hospital in Nyando Sub-County were almost 3 times more likely to be exposed to aflatoxin compared to women recruited from Kisumu County Referral Hospital in Kisumu East Sub-County. Climatic and environmental conditions in Nyando Sub-County favor growth of aflatoxin producing moulds (CGOK, 2013). Much of Nyando Sub-County is rural set up and contamination of food supplies by aflatoxin is reported to be greater in rural communities of developing countries (Antonius et al., 2005; Bhat et al., 2003). Therefore, based on the findings of this study, Nyando Sub-County could be at a higher risk of aflatoxin exposure compared to Kisumu East Sub-County because of the prevailing weather conditions and the rural location.

The results of this study also indicate that more than half of the exposed women were from households with low monthly income. Higher monthly household income was associated with reduced prevalence of aflatoxin exposure. These results are consistent with findings of two studies carried out in Kumasi, Ghana, to investigate socio-demographic determinants of aflatoxin B_1-lysine adduct levels among pregnant women which found that mean aflatoxin, as well as the percentage of women having high aflatoxin levels were inversely associated with indices of higher socioeconomic status (Shuaib et al., 2012; Ofori-Adjei, 2012). The findings of this study are also in line with those of a study carried out in Chulaimbo District Hospital, Kenya, which established that monthly income was a strong and significant predictor of diet diversity and majority of the respondents with low monthly income had inadequate dietary intake (Onyango et al., 2012). This could be attributed to the fact that women in the lower socio-economic status do not have the financial capacity to enable them purchase quality foods, and may therefore only afford food of lower quality which may be contaminated aflatoxins and other food contaminants.

The findings of this study indicate that households with low monthly income and mean age of 30-34 were more likely to consume aflatoxin contaminated foods than their higher income counterparts. These women should be targeted for any interventions on aflatoxin management and information on effect on maternal aflatoxin exposure should be incorporated in nutrition information given during antenatal clinics. Data from literature review on relationship between maternal age and aflatoxin exposure was lacking.

The amount of aflatoxin consumed through diet might not have been determined with high degree of accuracy in the current study due to variations in individual daily food intake. Individuals differ in their daily foods intake and food preferences may also vary on different days, whereas data for this study was collected in one day for each participant. In addition, food samples were collected between June and August 2013. This is normally the period when food harvesting is taking place in the Kisumu County. Aflatoxin contamination has been reported to increase with storage time (MoA, 2010, Gong et al., 2008; Magan et al., 2003). This could have contributed to the levels of aflatoxin observed in the current study. Groundnuts, one of the foods consumed in Kisumu County, is normally planted during the short rains in the months of August to October and harvested in December. This commodity was scarce in the market during the time of data collection, implying low consumption at household level. In a study carried out in The Gambian children, it was observed that aflatoxin albumin adduct concentration, an indicator of aflatoxin exposure, was strongly influenced by the month of sampling (Turner et al., 2003). Further, in a follow up cohort study carried out to assess levels of aflatoxin exposure among nursing Egyptian mothers and their children, the most dominant factor affecting the presence of AFM_1 in breast milk was the seasonal effect (Polychronaki, 2007). Therefore, seasonality is an

important factor in determining aflatoxin levels in foods. Given that this study was carried out in a season when aflatoxin levels could have been low, levels presented could have been lower than usual, hence seasonal variations in Kisumu County should be assessed. This study was conducted at one point in time, and therefore seasonal variations in aflatoxin exposure were not reflected. For conclusive information relevant to policy, data on seasonal variation is relevant and should be generated in a suitably designed study.

5.3 The Effect of Maternal Aflatoxin Exposure on Infant Growth

Infants of exposed women had lower birth weight and low birth length than infants of non-exposed women; and these variables were adjusted for in the analyses of the effect of aflatoxin exposure on infant growth. Association between weight and length and aflatoxin exposure has been shown in other studies. A cross-sectional hospital based survey conducted in Ghana, which assessed birth weight outcomes of 785 infants in relation to maternal aflatoxin exposure; found that mothers with the highest AFB-albumin quartile were more likely to have low birth weight babies, with a trend of increasing risk for low weight, compared to participants in the lowest quartile (Shuaib, 2010); similar findings are also reported by Ofori-Adjei (2012). The results of this study were consistent with those of previous studies which had reported a negative relationship between aflatoxin exposure and infant length: In a study among Iranian mothers, levels of AFM1 in breast milk were inversely correlated with length of infants at birth (Sadeghi et al., 2009), indicating an association between the two; a longitudinal study conducted in Benin to assess the effects of exposure on growth in children over a period of 8 months, found a strong negative correlation between serum aflatoxin albumins adducts and length increase (Gong et al., 2004). Data on infant baseline characteristics by aflatoxin exposure at birth (Table 4.7) from this

study concurred with these studies; that maternal aflatoxin exposure was a significant predictor of infant weight and length. However cross-sectional data can only infer associations and not causation which can only be determined in longitudinal studies, a strength of the current study.

There was no difference in exclusive breast feeding and frequency of breast feeding between infants of exposed and non-exposed women. The KDHS 2008-2009 report indicated that about 85% of breastfeeding children under 6 months in Nyanza Region were breastfed six or more times, hence our data aptly reflected the situation in Kisumu County. The KDHS 2014 did not provide information on breastfeeding frequencies. The KDHS 2014 data further indicated that Nyanza Region had a median duration of exclusive breastfeeding of 3.4 months in the first 6 months of life. Data from this study supported exposure of infants 0 to 3 months to aflatoxin being more likely to be through maternal intake than through influence of other foods, confirming the assumption that infants may be exposed to aflatoxin through breast milk.

Results from this study indicated that infants of women exposed to aflatoxin weighed less, and were shorter than infants of non-exposed women; and were at higher risk of stunting in the first 3 months of life. Because any differences at birth were adjusted for, these findings suggested that maternal exposure to aflatoxin affected growth of their infants during this period. The results of this study further showed that although mean weight of all infants increased over the study period, mean weights of infants of women exposed to aflatoxin were consistently lower than those of infants of non-exposed women at all time points of the study. However, although based on the data from this study, this did not translate to increased risk of being underweight in exposed relative to none exposed infants; a possible effect of exposure on underweight could not

be ruled out. This is because the difference found in this study is close to the SD in mean differences of all the groups at all the time points as reflected in Table 4.8. Given that underweight was based on standard deviation (SD) differences, any difference in underweight between the groups could have been too small to detect in this study on the basis of sample size. This argument is supported by large confidence interval reflected in Table 4.9 which supported the possibility of the sample size not being able to detect a difference should it have existed. This could have been due to the sample size calculation which was based on the most important indicator of growth - length.

The findings of this study on effect of aflatoxin exposure on infant weight were consistent with results of a longitudinal study conducted in The Gambia, examining the role of maternal aflatoxin exposure during pregnancy, early infancy, and infant growth velocity over the first 52 weeks of life; in which AF-albumin in maternal blood was a strong inverse predictor of infant weight gain over the first year, with lower gains in those with higher exposure (Turner et al., 2007). The results of The Gambian study showed that a reduction of maternal exposure from 110 ppb to 10 ppb would lead to 0.8 kg increase in weight over one year. Whereas the study by Turner and colleagues covered a period of one year, indicating that it accommodated exposure of aflatoxin from breast milk and other sources, this study focused on exposure from breast milk alone hence isolated the effect of breast milk alone; to determine whether or not women's exposure to aflatoxin could affect growth of their infants in early infancy. The findings of this study showed that an increase of 1 ppb in maternal aflatoxin exposure led to reduction of 52 g infant weight by 3 months of age. This showed that insults on weight of infants exist in the pre-complimentary period.

The results of this study further showed that each ppb increase in aflatoxin intake by a woman leads to a 0.2 cm decrease in length within the first 3 months of life of the infant. A study by Turner et al., (2007) established that a reduction of maternal AF-alb from 110 pg/mg to 10 pg/mg would lead to a 2 cm increase in height within the first year of life. At the intakes in the current study, this reduction in length could be as great as 4 cm on average. Given that by 6 months, length increases by 40% (Garrow et al., 2000); this reduction observed in this study is large. This is of great concern given that infant growth and development is highest during the first 4 months of life. Similar to changes in weight, although mean lengths of all infants increased over the first 3 months of life, they were consistently lower in infants of exposed women throughout the 3 months.

Similarly, the longitudinal study conducted by Turner et al., (2007) showed that AF-albumin in maternal blood was a strong inverse predictor of length gain of the infant over the first year, with lower gains in those with higher exposure. Given the previous argument, the findings of this study, therefore, indicated that aflatoxin consumed by an infant through breast milk alone could result in reduced length and stunting. Although a study by Okoth and Ohingo (2004) suggested an association between aflatoxin exposure and wasting, this study, like studies by Mahdavi et al., (2010) and Gong et al., (2004), found no evidence of a relationship between maternal aflatoxin exposure and infant WLZ. If not checked, aflatoxin exposure could hinder efforts by the Kenyan Government, as well as Kisumu County to achieve the Eight Global Nutrition Targets for 2015, among them; achieving a 40% reduction in children under 5 who are stunted and achieving 30% reduction in low birth weight (IFPRI), 2016). This study confirmed that

maternal aflatoxin exposure led to reduced infant growth (length, weight and stunting); however, it could not provide information on whether or not there was a threshold above which such an effect occurred. This would require measurement of effect at different levels of maternal exposure.

The mechanism by which aflatoxin exposure affects growth has not been clearly understood, but there is suggested possibility of compromised intestinal integrity and immune suppression (Gong et al., 2008). The toxins are ingested through food and pass through the digestive system and are absorbed into the circulatory system to the liver (Cupid et al., 2004). In the liver, aflatoxin may be transformed to the highly reactive intermediate aflatoxin exo-8, 9-epoxide. This molecule may bind to liver proteins and lead to their failure, resulting in acute aflatoxicosis or it may bind itself to nucleophilic sites in DNA, causing aflatoxin albumin adducts. Exposure to the toxin may result in delayed recovery from protein malnutrition, due to aflatoxin-induced disruption to RNA, or intestinal malabsorption. Aflatoxin exposure may also compromise intestinal integrity leading to impaired nutrient uptake through reduced epithelial uptake capacity (Gong et al., 2008). Chronic exposure to aflatoxin may affect micronutrient metabolism, by diverting the role of these nutrients to protecting the body against the toxins, resulting in deficiency and susceptibility to infections (Wu et al., 2011). Consequently, these may result in poor growth outcomes including underweight, wasting and stunting. Although this information supported plausibility of a mechanism for an effect of aflatoxin on growth, there were no clear mechanisms to elucidate how aflatoxin affected weight, length or growth indicators derived from these two anthropometric measures.

The findings of this study supported aflatoxin effect on infant length, weight and stunting, but not on underweight or wasting. However, given the inability of this study to give a conclusive statement on a possible effect on underweight, further study of ample sample size should be conducted to give a conclusive statement. A limitation of this study was that dietary intake was used, a proxy indicator, which could not be sensitive, to determine exposure. However, because the results were consistent with those of other authors who used sound study designs and a sensitive indicator of aflatoxin exposure, the results of this study reflected an effect of aflatoxin exposure on infant growth. This implied that measuring maternal aflatoxin exposure during period of exclusive breast feeding could serve as a proxy indicator of infant exposure to aflatoxin.

5.4 The Effect of Maternal Aflatoxin Exposure on Infant Morbidity

According to the findings of this study, the four most common illnesses among infants in Kisumu County were malaria, fever, common cold and diarrhea. These findings are in line with the report by Kisumu County Integrated Development Plan 2013-2017, which indicated that the main illnesses in Kisumu County were malaria, fever, common cold and diarrhoea (CGOK, 2013). Results of crude bivariate analysis showed that maternal aflatoxin exposure was associated with malaria and diarrhea, but not with other infections. After adjusting for health facility, Sub-County of residence, maternal education, maternal age and household monthly income, maternal aflatoxin exposure still had an effect on malaria and diarrhea. Studies have established that at lower levels, mycotoxins are immune suppressive, and could result in increased incidences of sicknesses (Turner et al., 2003; Adhikari et al., 2006; WHO, 2006) However, current studies focusing on effect of aflatoxin exposure on malaria from literature

review were lacking and therefore no comparison was made. Research findings have also correlated placental malaria with maternal anemia and reduced birth weight of the new born infants (Menendez et al., 2000) and the major risk factor for placental malaria was an age < 25 years old (Tako et al., 2005). Maternal age was one of the confounding factors adjusted for during analysis on effect of aflatoxin exposure on morbidity.

Results of this study further indicated that infants of exposed women had a higher risk of suffering from diarrheoa, compared to infants of non-exposed women. The findings of this study are supported by the WHO report, which showed that aflatoxin exposure increased the risk of diarrhoea especially in children; and that growth faltering in young children was associated with long term intestinal lesions resulting from diarrhea (WHO, 2006); further, data from KDHS 2014 revealed that 15.5% of children< 5 years of age in Kisumu County suffered from diarrheoa in two weeks preceding the survey (KNBS & ICF Macro, 2014).

Although WHO (2006) report indicated that aflatoxin exposure increased the risk of pneumonia, the results of our study showed that only one infant had pneumonia infection in the first 3 months after birth, confirming low incidence of the disease in the target population. The findings of this study further showed no evidence of a relationship between aflatoxin exposure and incidences of jaundice, umbilical cord infections, neonatal sepsis and conjunctivitis. The results of this study showed a relationship between aflatoxin exposure and malaria and diarrhea, which may result in reduced nutrient utilization by the infant.

The mechanism by which aflatoxin affects infant morbidity has not been clearly explained. However, chronic exposure to aflatoxin has been implicated in poor micronutrient metabolism in young children (Williams et al., 2004; Wu and Tingco, 2011), yet micronutrients are essential for normal growth and development of the fetus, the infant and the young child. Vitamins and minerals, especially serum zinc and selenium, are critical for proper functioning of the immune system of young children (Garrow et al., 2000; Mocchegiani et al., 2000). If these micronutrients are interfered with, the health of the young child will be compromised leading to increased infections. Exposure to aflatoxin has also been associated with reduced salivary IgA, which according to Turner et al., (2003) and Smith et al., (2012) is a vital component which binds to bacterial and viral surface antigens and provides an important component of mucosa barrier protection and resistance to intestinal infections.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study made the following conclusions with confidence.

1. Maize, sorghum, rice, groundnuts, cassava, *dagaa* and milk, which form the bulk of staple and frequently consumed foods in Kisumu County, are contaminated with aflatoxin. Sorghum had the highest levels of contamination compared to maize or groundnuts which have been known to have the highest levels. Although focus has been on maize and groundnuts, sorghum is a source of aflatoxin exposure and should be included among crops of concern for aflatoxin exposure.

2. Almost a quarter of pregnant women in Kisumu County are exposed to aflatoxin intakes above the regulatory limit of 10 ppb through their diet, with more than one third having daily absolute intake ranging from 10 µg to 142 µg; and therefore risk exposing their infants to the toxin.

3. Maternal exposure to aflatoxin increases risk of reduced weight, length and risk of stunting and underweight in infants, but not risk of wasting.

4. Maternal aflatoxin exposure increases risk of malaria and diarrhea in infants. There was no evidence of increased risk of fever, pneumonia, common cold, cough, jaundice, umbilical cord infection, neonatal sepsis and conjunctivitis resulting from aflatoxin exposure.

6.2 Recommendations

6.2.1 Recommendations to the Policy Makers

1. The National Food and Nutrition Security Policy 2011 which is silent on aflatoxin control and management should be reviewed to incorporate this element in order to minimize levels of aflatoxin in market and household foods.

2. The National and County Governments should identify and address sources of aflatoxin contamination in foods.

3. Stringent routine surveillance of foods in the markets outlets should be enhanced to ensure quality of foods consumed in Kisumu County, given that markets were identified as source of dietary aflatoxin.

4. The Kenya Bureau of Standards should set and reinforce minimum aflatoxin regulatory limits for aflatoxin M_1 in milk to ensure safety of milk consumed by Kenyans, especially young children.

6.2.1 Suggestions for Further Research

1. A longitudinal study to assess the effect of seasonal variations of aflatoxin levels in household and market foods, to identify when individuals are most likely to be exposed.

2. A study to establish the threshold of maternal aflatoxin exposure for an effect on infant growth.

3. To quantify the exposure of children through breastmilk in Kenya, prevalence studies to establish aflatoxin M_1 levels in maternal breast milk is recommended.

4. A study with ample sample size to give a conclusive statement on the effect of maternal aflatoxin exposure on underweight.

5. Studies to confirm whether there is a relationship between aflatoxin exposure and diarrheoa and malaria and potential mechanisms where associations are confirmed.

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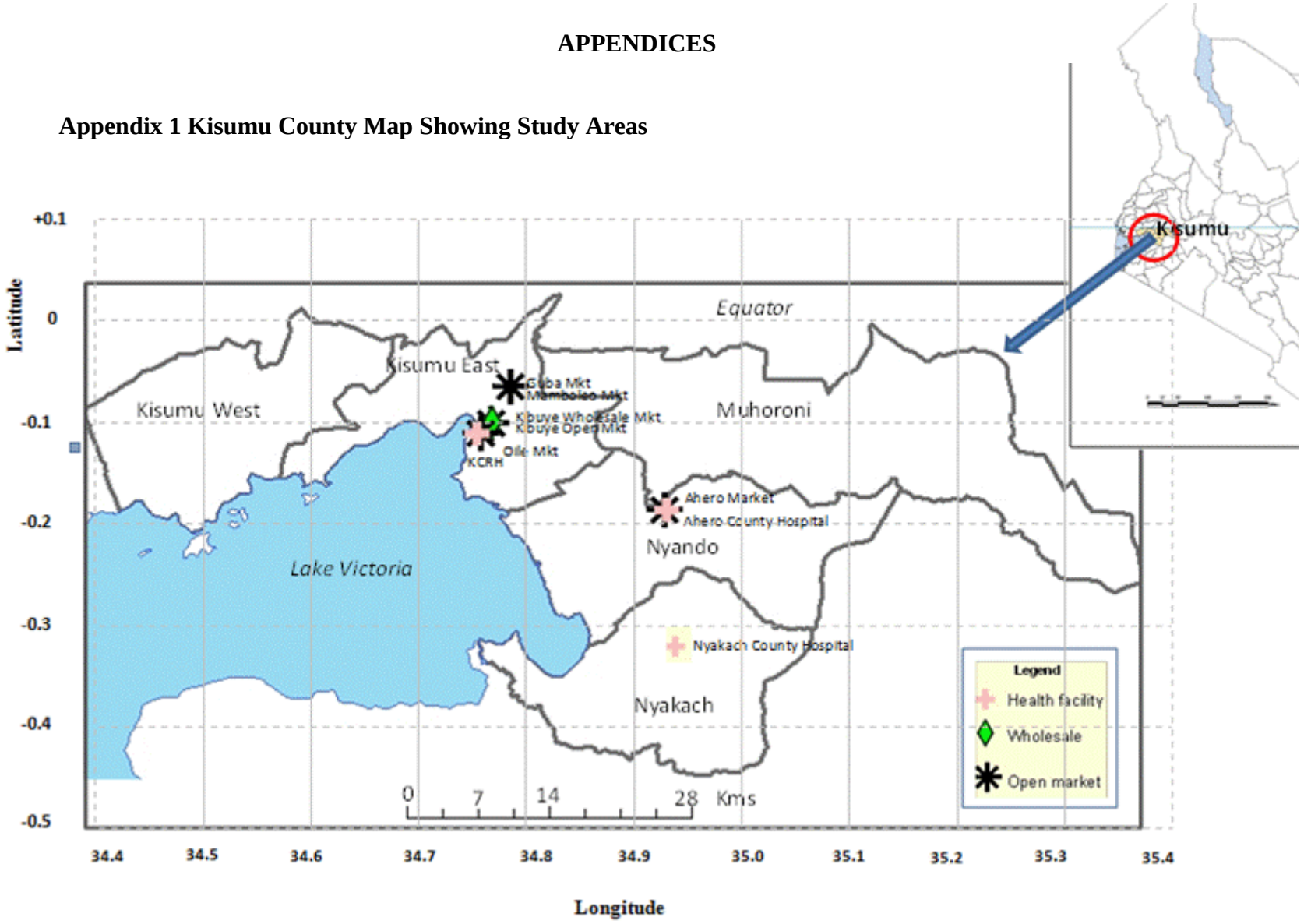
APPENDICES

Appendix 1 Kisumu County Map Showing Study Areas



Appendix 2 Consent Letter for Participants

MASENO UNIVERSITY

SCHOOL OF PUBLIC HEALTH AND COMMUNITY DEVELOPMENT

(Ecole de la Sante Publique et du Development Communataire)

(ESPUDEC)

Informed Consent for Participation in Research Study

Title of Research: The Effect of Maternal Aflatoxin Exposure through Diet on Growth of Infants 0 - 3 Months in Kisumu County, Kenya

Principal Investigator

Mary Ibayo Obade- PG/PhD/085/2010

Co-investigators

Dr. Pauline Andang'o

SPHCD, Maseno University

Dr. Charles Obonyo

Kenya Medical Research Institute (KEMRI), Kisumu

Dr. Francesca Lusweti

Kenya Agricultural and Livestock Research Organization (KALRO), Kitale

Study Location

This study will be conducted in Kisumu County, Kenya. Kisumu County has six Sub-Counties (Kisumu East, Kisumu West, Kisumu North, Nyando, Muhoroni and Nyakach) and 7 constituencies (Kisumu East, Kisumu West, Kisumu Central, Seme, Nyando, Nyakach and Muhoroni). The study aims to investigate the effects of women's intake of aflatoxin on their infants' health and growth in the first 3 months of life in Kisumu, County, Kenya and will culminate in a postgraduate thesis.

Participation in the Study

We request your participation in this study, to enable us find out whether consuming aflatoxin in the food we eat affects the growth and health of children. The study will consist of two parts: In the first part, your participation will involve responding to a questionnaire on foods you eat, and giving us a small sample of some of the foods so that we can test it to see if it contains any aflatoxin. Every participant will receive information on the effect of aflatoxins on health and how to minimize intake of aflatoxin in food. We will then request those of you who are eligible to participate in the second part, to continue with the study. The second part of the study entails comparing the growth and health of children born to women whose intake of aflatoxin is low and those whose intake is above the amount allowed. For those who are eligible to participate in the second part of the study, we will request that you deliver your child at the Kisumu and Ahero Sub-County Hospitals. We will take measurements of weight and length of your infants at delivery and over a period of 3 months, and we will also ask you some questions on how the child feeds, as well as questions concerning your child's health during that time. We aim to ask these questions during your postnatal clinic visits, at whichever health facility you attend for these services.

We do not expect that conducting this study will harm you or your child in any way, and will only require that you provide a sample of foods you eat at one time point. We will advise you on how to ensure that the aflatoxin levels in the food you eat are low, because although we do not know to what extent they may affect your child; we do know that the less you take of these substances the better, for your health. The information we find will be important for making decisions on how to ensure that the food we eat is safe. We will keep any information we collect from you, confidential, and will not link it to you individually, but will use it collectively to assess whether the foods we eat affect our children's health. Participation in this research study is voluntary; refusal to participate will involve no penalty. You are free to withdraw consent and discontinue participation in this research study at any time without prejudice.

Contact Information

Any questions and concerns may be addressed to Mary Ibayo Obade, C/o Office of the Provincial Director of Agriculture, P. O. Box 1700 -40100, Kisumu. Mobile: 0715261604, 0733443228. E-mail address: mobade2002@yahoo.com

For any questions pertaining to rights as a research participant, please contact: **The Secretary, Maseno University Ethics Review Committee, Private Bag, Maseno; Telephone numbers: 057-51622, 0722203411, 0721543976, 0733230878; Email address:** muerc-secretariate@maseno.ac.ke; muerc-secretariate@gmail.com.

Appendix 3 Agreement

The Effect of Maternal Aflatoxin Exposure through Diet on Growth of Infants 0 - 3 Months in Kisumu County, Kenya

I have received a copy of this informed consent and consent to participate in the study titled: *The Effect of Maternal Aflatoxin Exposure through Diet on Growth of Infants 0 - 3 Months in Kisumu County, Kenya*. The information contained in the consent form has been read to me and explained fully, and I understand what my participation in the study entails. I have freely taken the decision to participate in the study without coercion and I understand that I am free to withdraw my participation at any time without prejudice.

Signature of Participant…… Date……

Printed Name of Participant……..

Signature of study personnel responsible for explaining the study information:

Appendix 4 Research Questionnaire

Title of Research: The Effect of Maternal Aflatoxin Exposure through Diet on Growth of Infants 0 - 3 Months in Kisumu County, Kenya

Aflatoxin is a public health problem which is more pronounced in developing countries, and may be a problem in Kisumu County. This study aims to establish the effect of maternal aflatoxin exposure on growth of infants 0 – 3 months in Nyanza Region. Please take time and provide information required in the questionnaires. The information provided through these questionnaires will provide useful information on maternal aflatoxin exposure in the Kisumu County and effect of such exposure on infant growth and morbidity. The questionnaire should not take more than 10-30 minutes to complete. All information provided will be treated as confidential.

Section A: Social Demographic Indicators

Date of interview_____ Code of participant_____

Sub-County: _____ Address_____

Mobile_____

Enumerator: _____

Please answer all questions as honestly as you can. You may leave blank any question that you do not feel comfortable answering.

1. Residence: (1) = Kisumu East (2) = Kisumu West (3) =Kisumu North (4) = Nyando

(5) = Other (specify) _____

2. Age: _____

3. Marital status: (1) = Single (2) = Married (3) = Separated (4) = Divorced (5) = Widowed

4. Educational level: (1) = None (2) = Primary (3) = Secondary (4) = University (5) = Other (specify) _____

5. Occupation: (1) = Housewife (2) = self-employed (3) = Employed (4) = other (specify) _____

6. Religion: (1) = Pagan (2) = Christian (3) = Muslim (4) = Traditional (5) = other (specify) _____

7. Size of the household: _____

8. Source of income: (1) = Farming (2) = Employed (3) = Business (4) = Others (specify) _____

9. Monthly earnings? (1) <2000 (2) = 2001 – 5000 (3) = 5001 – 10000 (4) >10000

Section B: Food Frequency Questionnaire: Women

FOOD	Amount	HOW OFTEN		
		Daily	Weekly	Monthly
		Times per day (Indicate)	Times per week (indicate)	Times per month (indicate)

Section C: Food Frequency Questionnaire: Infant

FOOD	Amount	HOW OFTEN		
		Daily	Weekly	Monthly
		Times per day (Indicate)	Times per week (indicate)	Times per month (indicate)

Section D: Infant Breast Milk Intake

How often do you breast your baby in a day?

(1)= Once (2) =Two times (3) = Three times (4) =Four times (5) = Five times

(6)=More than five times

Do you have face any difficulties with breastfeeding your baby? (1)=Yes (2) = No

If yes, please explain_____

Weight of infant before breast feeding: _____

Weight of infant after breast feeding: _____

Section E: Infant Anthropometric Data

Date: _____ Name: _____

Sex: _____ Age: _____

Date	Visit	Weight 1	Weight 2	Weight 3	Average

Date	Visit	Length 1	Length 2	Length 3	Average

Section F: Infant Morbidity

Has the child been ill in the last 14 days (2 weeks)? (1)Yes (2) No

If yes, what illness (list if more than one illness)

_____

_____

How many times was the child ill? _____

How did you know that the child had the illness? Please tick as appropriate.

_____

(1)=self (2) =Clinical officer (3) =Nurse (4) =Doctor (5) =Community health worker

(6) Other (Please specify) _____

Section G 24-Hour Recall of Dietary Intake

Date_____

Name_____

Time	Food/drink item	Description of food or drink	Amount
E.g. 7.00 am	Porridge	E.g. (3L prepared from 2L water and X g millet-cassava-maize flour)	1 500ml cup

Appendix 5 Field Manual

MASENO UNIVERSITY

SCHOOL OF PUBLIC HEALTH AND COMMUNITY DEVELOPMENT

(ESPUDEC)

DEPARTMENT OF NUTRITION AND HEALTH

FIELD MANUAL

SOURCES AND EFFECT OF MATERNAL AFLATOXIN EXPOSURE THROUGH DIET ON

GROWTH AND MORBIDITY OF INFANTS 0 - 3 MONTHS IN KISUMU COUNTY, KENYA

By

Name: Mary Ibayo Obade

Registration No: PG/PHD/085/2010

Purpose of Manual

This manual would be used for orienting research assistants and other personnel involved, and to serve as a reference guide during the implementation of this study.

Introduction

Aflatoxins are naturally occurring group of mycotoxins that are produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin contamination has been reported in many staple foods worldwide as well as in maize and groundnuts in Homa Bay, Rongo and in complimentary flours in the former Kisumu Sub-County. Exposure to the toxin is associated with many health problems, especially stunting and underweight in children. This study aimed to establish the effect of maternal exposure to aflatoxin through diet on growth of infants 0 – 3 months. In a prospective cohort study, pregnant women up to 8 months pregnancy from Kisumu County, Kenya and their infants 0 – 3 months born during the study period formed the study population. Five hundred and thirty three women were recruited to participate in the study out of which 137 exposed and 137 non-exposed women were matched for maternal age and household income. Food samples collected from five market outlets and aliquots of food samples collected from households were analyzed to determine aflatoxin levels. Women were categorized as "exposed" or "non-exposed" based on an aflatoxin intake cut-off point of ≤10ppb. The two groups of women were followed up to delivery. Monthly data was collected on infants' anthropometric indices and fortnightly on morbidity. The findings of the proposed study would shed light on levels of aflatoxin exposure in the study area and the effect of maternal exposure on growth and morbidity of infants 0-3 months old.

Objective of the Study

The main objective of the study was to investigate the sources of aflatoxin exposure and the effects of maternal exposure to aflatoxin on infants' morbidity and growth in the first 3 months of life in Kisumu County, Kenya

Study Area

The study would be conducted in Kisumu County, Kenya. Kisumu County is one of the six counties in Nyanza Region. The County has six Sub-Counties (Kisumu East, Kisumu West, Kisumu North, Nyando, Muhoroni and Nyakach) and 7 constituencies (Kisumu East, Kisumu West, Kisumu Central, Seme, Nyando, Nyakach and Muhoroni). Kisumu County has an area of 2,085.9 km^2 with a population of 968,909 (Male 48.9 %, Female 51.1 %), 226,719 households and a population density of 465 people per km^2. Rainfall ranges between 1200mm and 1300mm with a mean annual temperature of 23^oC, ranging between 20^oC and 35^oC and humidity of 40 – 89%. These climatic conditions may be favorable for mycotoxin growth. Common crops grown in Kisumu County include maize, sorghum, cassava, sweet potatoes and a variety of indigenous and exotic vegetables (MoA, 2012). Due to the presence of a fresh water lake, one of the economic activities is fishing for local consumption and export. The cohort study will be conducted at Kisumu and Ahero Sub-County Hospitals. Food samples will be collected from Kibuye Wholesale Market, Kibuye Open Market, Oile Market, Mamboleo Market and Ahero Market.

Study Population

The study would target 4100 pregnant women up to 8 months pregnancy attending antenatal clinic at Kisumu County Referral and Ahero County Hospitals yearly.

Inclusion Criteria

Pregnant women would be eligible for inclusion into the study if they: consented to participate in the study; are up to 8 months pregnant; resided in Kisumu County, Kenya; were willing to deliver at health centers; had no history of chronic illness such as diabetes and HIV/AIDS; were not on regular medication and had no pregnancy complications.

Participant Selection

Collection of food samples from markets

Agricultural extension officers would assist in collection of food samples from market outlets; double pack them in 1 kg paper envelopes to avoid cross contamination and moisture penetration before transporting to KALRO Kitale laboratory for aflatoxin analysis. The sample size for the number of food samples to be collected from each market would be calculated based on Israel (2009) formula $N/ (1+N (e)^{2})$ where N is the population size of total available bags of each food in the market at the time of survey and e is the margin of error set at 5% (0.05) at 95% confidence level

Selection of Participants for the Cohort Study

Community Health Workers would assist to recruit pregnant women of up to the 8^{th} month of pregnancy who would be screened for eligibility to participate in the study. Dietary intakes of 553 women will be determined and aliquots of 10% of the food samples eaten over 24-hours by

each participant would be collected and taken to KALRO Kitale for aflatoxin analysis. Women with usual intakes above 10ppb would be classified as exposed and those with usual intakes below 10ppb would be categorized as non-exposed. One hundred and forty six (minimum =132) women exposed and 132 non-exposed women as closely matched for age as possible with the exposed women would be selected to participate in the study.

Procedure for Selection of Respondents

- Participants would be requested to voluntarily participate in a research study to investigate the sources of aflatoxin exposure and the effects of maternal exposure to aflatoxin on infants' growth in the first 3 months of life in Kisumu County, Kenya.
- Only participants who would meet the selection criteria would be selected as respondents in the study.
- Respondents would sign a participant's consent form indicating that they had read and understood the content of the study and were willing to participate in the study.

Instruments and Instructions for Data Collection

Introduction

Aflatoxin is a public health problem which is more pronounced in developing countries, and may be a problem in Kisumu County. This study aims to establish the effect of maternal aflatoxin exposure on growth of infants 0 – 3 months in Kisumu County. A questionnaire comprising of the following sections: Socio-demographic indicators; the 24-hour dietary recall, food frequency questionnaires, Infant morbidity and anthropometric measurements, would be used in this study to collect data. The questionnaire would be administered by Research Assistants and the

Principal Researcher. The Research Assistants and the Principal Investigator would interview the participants to provide information required in the questionnaire. Data provided through this questionnaire would provide useful information on maternal aflatoxin exposure in the Kisumu County and effect of such exposure on infant growth and morbidity. The questionnaire should not take more than 45 minutes to complete.

Research Questionnaires

Section A: Socio-Demographic Indicators

Participant would be asked by Research Assistants to provide information on socio-demographic characteristics including; maternal age, education level, annual income, employment status, residence, number of children; as well as infant data on prematurity and gender using the instruction given below

Date of interview_____

Name of participant_____

Sub-County: _____

Address_____

Mobile_____

Enumerator: _____

Please answer all questions as honestly as you can. You may leave blank any question that you do not feel comfortable answering.

1. Residence: (1) = Kisumu East (2) = Kisumu West (3) =Kisumu North (4) = Nyando
(5) = Other (specify) _____

2. Age: _____

3. Marital status: (1) = Single (2) = Married (3) = Separated (4) = Divorced (5) = Widowed

4. Educational level: (1) = None (2) = Primary (3) = Secondary (4) = University (5) = Other (specify) _____

5. Occupation: (1) = Housewife (2) = self-employed (3) = Employed (4) = other (specify) _____

6. Religion: (1) = Pagan (2) = Christian (3) = Muslim (4) = Traditional (5) = other (specify) _____

7. Size of the household: _____

8. Source of income: (1) = Farming (2) = Employed (3) = Business (4) = Others (specify) _____

9. Monthly earnings? (1) <2000 (2) = 2001 – 5000 (3) = 5001 – 10000 (4) >10000

Section B: Food Frequency Questionnaire: Women

The food frequency questionnaire would provide information on the frequency (daily, weekly or monthly) of consumption of foods in the recent past by the participants. The participant would be presented with a questionnaire and would be required to indicate the food and how often each food is eaten in broad terms such as the number of times per day/per week/per month. The number of servings per consumption frequency would be asked in natural or household units using standard portion sizes such as a cup (150 ml), jars, glasses etc

Food	**Amount**	**How Often**		
		Daily	Weekly	Monthly
		Times per day(Indicate)	Times per week (indicate)	Times per month (indicate)

Section C: Food Frequency Questionnaire: Infant

The food frequency questionnaire for the infant would provide information on the frequency (daily, weekly or monthly) of other foods consumed by the infant other than breast milk in the recent past. This would provide information on other sources of aflatoxin exposure other than breast milk. The research Assistants would interview the women using the questionnaire below to collect this information.

FOOD	Amount	HOW OFTEN		
		Daily	Weekly	Monthly
		Times per day (Indicate)	Times per week (indicate)	Times per month (indicate)

Section D: Infant Breast Milk Intake

Research Assistants would estimate amount of milk consumed based on estimated intake amounts calculated by weighing infants before and after breast feeding and also based on the frequency of feeding. It is documented that mothers can breastfeed their infants up to 5 times in 24 hrs with an estimated mean breast milk intake of 500ml to 800 ml. The questionnaire below would be administered to collect more information on breast milk intake.

How often do you breast your baby in a day?

(1)= Once (2) =Two times (3) = Three times (4) =Four times (5) = Five times

(6)=More than five times

Do you have face any difficulties with breastfeeding your baby? (1)=Yes (2) = No

If yes, please explain_____

Weight of infant before breast feeding: _____

Weight of infant after breast feeding: _____

Infant Anthropometric

The Research Assistants would take routine anthropometric measurements (weight and length) of infants born to study participants at birth and monthly for 3 months, using accurately calibrated instruments. Weights of infants would be measured using infant weighing scales and lengths determined using infant length boards.

Research Assistants would use the questionnaire below to collect data on infant weight and length. Three measurements would be done on every day of visit to the health center and the average calculated.

Date: _____ Name: _____

Sex: _____ Age: _____

Date	**Visit**	**Weight 1**	**Weight 2**	**Weight 3**	**Average**

Date	**Visit**	**Length 1**	**Length 2**	**Length 3**	**Average**

Length for age Z-scores (LAZ), weight for age Z-scores (WAZ) would be calculated WHO Anthro software. Children whose lengths for ages, weights for ages, and weights for lengths are

2 standard errors or more below WHO growth standards (z-score ≤−2) would be considered to be stunted, underweight, and wasted respectively.

Section F: Infant Morbidity

Information on infant morbidity would be taken fortnightly for 3 months. Morbidity rates and anthropometric indices of growth would be compared in infants of exposed and non-exposed women 3 months postpartum. Research Assistants would use the questionnaire below to gather data on infant morbidity.

Has the child been ill in the last 14 days (2 weeks)? (1)Yes (2) No

If yes, what illness (list if more than one illness)

_____

_____

How many times was the child ill? _____

How did you know the child had the illness? Please tick as appropriate.

_____

(1)=self (2) =Clinical officer (3) =Nurse (4) =Doctor (5) =Community health worker

(6) Other (Please specify) _____

Section G: 24-Hour Recall of Dietary Intake

Research Assistants would ask participants to recall and provide information on all the food consumed over the previous 24-hours. The information would include everything that they would have eaten or drunk on the previous day between midnight and midnight. Water and

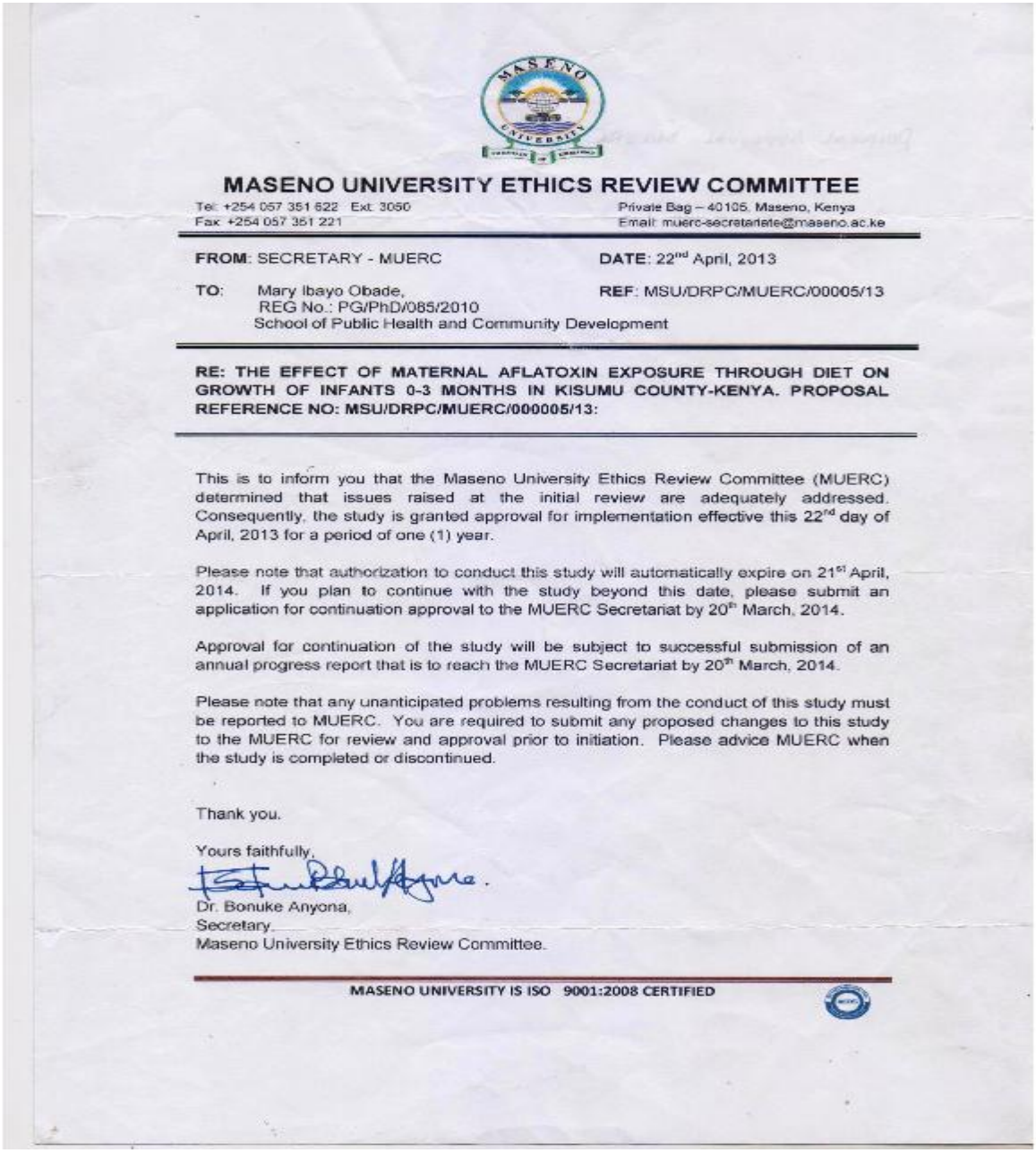
plasticine would be used to estimate quantities of liquid and solid foods taken by participants. At the end of the recall, respondents would be invited to add any additional items not initially recalled. This would be followed by collection of detailed information concerning the items listed. Lastly, there would be a recall review in which respondents would be given an opportunity to provide additional information and for the interviewer to prompt for information about foods or drink not mentioned. The table below would be used to collect the required information.

NAME_____

DATE_____

Time	Food/drink item	Description of food or drink	Amount
E.g. 7.00 am	Porridge	E.g. (3L prepared from 2L water and X g millet-cassava-maize flour)	1 500ml cup
Do you consider these meals typical or were they or any of them a special meal?			

Appendix 6 Ethical Clearance by MUERC



MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

Tel: +254 057 351 622 Ext. 3050
Fax +254 057 351 221
Private Bag – 40105, Maseno, Kenya
Email: muerc-secretariate@maseno.ac.ke

FROM: SECRETARY - MUERC

DATE: 22nd April, 2013

TO: Mary Ibayo Obade,
REG No.: PG/PhD/085/2010
School of Public Health and Community Development

REF: MSU/DRPC/MUERC/00005/13

RE: THE EFFECT OF MATERNAL AFLATOXIN EXPOSURE THROUGH DIET ON GROWTH OF INFANTS 0-3 MONTHS IN KISUMU COUNTY-KENYA. PROPOSAL REFERENCE NO: MSU/DRPC/MUERC/000005/13:

This is to inform you that the Maseno University Ethics Review Committee (MUERC) determined that issues raised at the initial review are adequately addressed. Consequently, the study is granted approval for implementation effective this 22nd day of April, 2013 for a period of one (1) year.

Please note that authorization to conduct this study will automatically expire on 21st April, 2014. If you plan to continue with the study beyond this date, please submit an application for continuation approval to the MUERC Secretariat by 20th March, 2014.

Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 20th March, 2014.

Please note that any unanticipated problems resulting from the conduct of this study must be reported to MUERC. You are required to submit any proposed changes to this study to the MUERC for review and approval prior to initiation. Please advice MUERC when the study is completed or discontinued.

Thank you.

Yours faithfully,

Dr. Bonuke Anyona,
Secretary
Maseno University Ethics Review Committee.

MASENO UNIVERSITY IS ISO 9001:2008 CERTIFIED

Appendix 7 Proposal Approval

MASENO UNIVERSITY
SCHOOL OF GRADUATE STUDIES

Office of the Director

Our Ref: PG/PHD/085/2010

Private Bag, MASENO, KENYA
Tel: (057) 351 622/351008/351011
FAX: 254-057-351153/351221
Email: sgs@maseno.ac.ke

Date: 20th February, 2013

TO WHOM IT MAY CONCERN

RE: PROPOSAL APPROVAL FOR MARY IBAYO OBADE—PG/PHD/085/2010

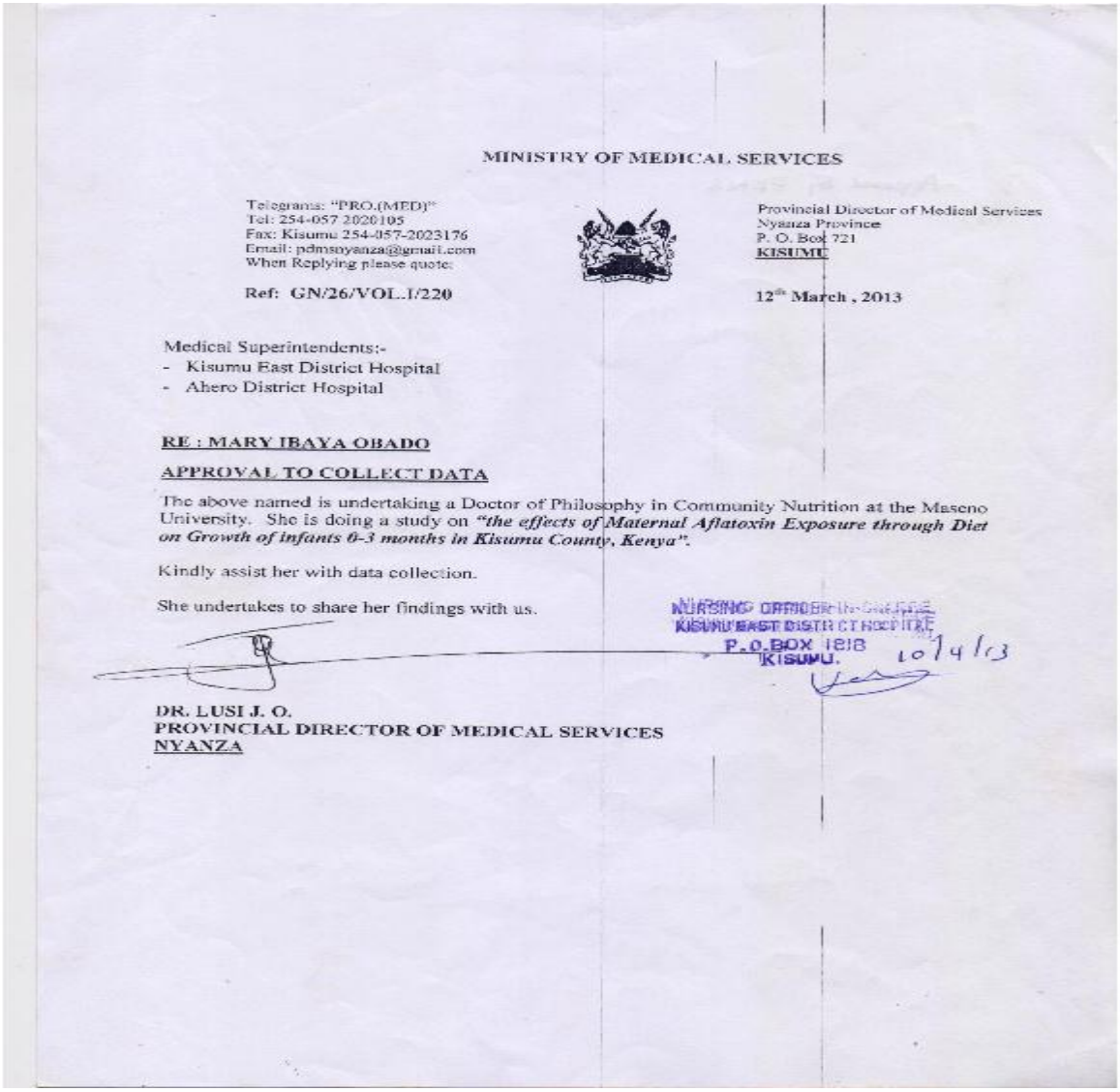
The above named is registered in the Doctor of Philosophy in Community Nutrition and Development Programme of the School of Public Health and Community Development, Maseno University. This is to confirm that her research proposal titled "The Effect of Maternal Aflatoxin Exposure through Diet on Growth of Infants 0-3 Months in Kisumu County, Kenya" has been approved for conduct of research subject to obtaining all other permissions/clearances that may be required beforehand.

MASENO UNIVERSITY RECEIVED 20 FEB 2013 SCHOOL OF GRADUATE STUDIES

Dr. Pauline Andang'o
ASSOCIATE DIRECTOR, SCHOOL OF GRADUATE STUDIES

MASENO UNIVERSITY ISO 9001:2008 CERTIFIED
FOUNTAIN OF EXCELLENCE

Appendix 8 Data Collection Approvals



MINISTRY OF MEDICAL SERVICES

Telegrams: "PRO.(MED)"
Tel: 254-057 2020105
Fax: Kisumu 254-057-2023176
Email: pdmsnyanza@gmail.com
When Replying please quote:

Ref: GN/26/VOL.I/220

Provincial Director of Medical Services
Nyanza Province
P. O. Box 721
KISUMU

12th March , 2013

Medical Superintendents:-
- Kisumu East District Hospital
- Ahero District Hospital

RE : MARY IBAYA OBADO

APPROVAL TO COLLECT DATA

The above named is undertaking a Doctor of Philosophy in Community Nutrition at the Maseno University. She is doing a study on ***"the effects of Maternal Aflatoxin Exposure through Diet on Growth of infants 0-3 months in Kisumu County, Kenya".***

Kindly assist her with data collection.

She undertakes to share her findings with us.

NURSING OFFICER In-Charge
KISUMU EAST DISTRICT HOSPITAL
P.O.BOX 1818
KISUMU.
10/4/13

DR. LUSI J. O.
PROVINCIAL DIRECTOR OF MEDICAL SERVICES
NYANZA

OFFICE OF THE PRESIDENT
PROVINCIAL ADMINISTRATION AND INTERNAL SECURITY

Telephone: Kisumu 2022219/Fax: 2022219
Email: ckisumucounty@gmail.com

COUNTY COMMISSIONER
KISUMU COUNTY
P.O. BOX 1912-40100
KISUMU

Ref: CC/KC/2 (35)

12th March, 2013

The District Commissioner
KISUMU EAST DISTRICT

The District Commissioner
NYANDO DISTRICT.

RE: PROPOSAL APPROVAL FOR MARY IBAYO OBADE – PG/PHD/085 2010

The bearer of this letter is a civil servant with the Ministry of Agriculture and now on study leave pursuing a PHD at Maseno University. She wants to undertake research on the effect of Maternal Aflatoxin Exposure through Diet on Growth of Infants 0-3 months in Kisumu County.

Please give her the necessary support. Enclose is her a copy her letter of approval from the university.

L. A. ODERO (OGW)
COUNTY COMMISSIONER
KISUMU COUNTY

Request approved by Dr Omonyo
Chief. DPHN for MOH.

KISUMU MUNICIPAL COUNCIL
DEPT. OF HEALTH
06 MAY 2013
P. O. Box 105, KISUMU

Appendix 9 Aflatoxin Levels Versus Stunting in Selected Counties in Kenya.

County	Stunting (%) (FAO, 2013)	Highest reported aflatoxin Level & Source	
		Aflatoxin Level	Source
Nairobi	22.7	100 ppb	Okoth and Kola (2012)
Kisumu	33.1	82 ppb; 39.4 ppb	Okoth and Ohingo (2004); Obade et al., (2015a, 2015b)
Homa Bay	37.0	1000 ppb	Mutegi et al., (2007)
Makueni	33.5	46,400 ppb	Lewis et al., (2005)
Kitui	47.4	46,400 ppb	Lewis et al., (2005)
Machakos	31.3	160 ppb	Muthomi et al.,(2009)
Embu	23.7	21 ppb	Collins (2010)
Kakamega (Malava)	34.2	5000 ppb (Rotten maize) 1348 ppb (Clean maize)	Alakonya et al., (2009)
Tongaren (Bungoma)	52.1	5000 ppb (Rotten maize) 1348 (Clean maize)	Alakonya et al., (2009)
Kisii South	35.3	3442ppb	Collins (2010)

Appendix 10 WLZ, LAZ, and WAZ among Infants 3 Months Old in Kisumu County.

n=553	**Severe (%)** (Z< - 3)	95%CI	**Moderate (%)** (- 3 < Z- 2)	95%CI	**Normal (%)** (Z ≥ -2)	95%CI
WLZ	2.9	1.6-4.3	4.7	2.9-6.7	92.4	90.2-94.4
HLZ	7.2	5.2-9.6	9.0	6.7-11.6	83.7	80.5-86.8
WAZ	2.0	0.9-3.3	4.7	2.9-6.5	93.3	91.1-95.5

The findings of this study indicated stunting rates among infants at 3 months of age in Kisumu County; 4.7 % of infants had WLZ of -3< Z-2, 9.0% had HLZ of -3< Z-2 and 4.7% had WAZ of -3< Z-2.

Appendix 11 Participants' Characteristics (Infant) at Baseline

Variable Description	% (n=553)
1.Weight of infant in kg at birth	
• 1.5 – 2.9	13.4 (74)
• 3.0 – 3.7	68.2 (337)
• 3.7 – 4.7	18.4 (102)
• Mean (SD)	3.39 ± 0.46
2.Weight of infant in kg at 1 month	
• 2.4 – 3.4	8.5 (47)
• 3.5 – 4.4	33.3 (184)
• 4.5 – 5.4	47.4 (262)
• 5.5 – 6.4	10.8 (60)
• Mean (SD)	4.49 ± 0.75
3. Weight of infant in kg at 2 months .	
• 3.0 – 4.0	5.4 (30)
• 4.1 – 5.0	24.1 (133)
• 5.1 – 6.0	49.2 (272)
• 6.1 – 7.0	18.8 (104)
• 7.1 – 7.9	2.5 (14)
Mean (SD)	5.45 ± 0.82
4.Weight of infant in kg at 3 months	
• 3.8 – 4.8	4.2 (23)
• 4.9 – 5.8	20.2 (112)
• 5.9 – 6.8	48.7 (269)
• 6.9 – 7.8	21.8 (121)
• 7.9 – 8.8	5.1 (28)
Mean (SD)	6.38 ± 0.84
5.Length of infant in cm at birth	
• 30 – 46	13.8 (76)
• 47 – 56	83.2 (460)
• 57 – 64	3 (17)
• Mean (SD)	50.1 ± 3.77
6.Length of infant in cm at 1 month	
• 35 – 51	23
• 52 – 61	75.4
• 62 – 68	1.6
• Mean (SD)	53.8 ± 3.56
7.Length of infant in cm at 2 months	
• 42 – 56	43.6 (241)
• 57 – 66	55 (304)
• 67 – 68	1.4 (8)
• Mean (SD)	57.1 ± 3.6
8.Length of infant in cm at 3 months	
• 49 – 59	30.6 (169)
• 60 – 69	66.1 (366)
• 70 – 76	3.3 (18)

• Mean (SD)	61.55 ± 4.0
9.Amount of porridge (ml) consumed by infant in a day	
• 0	97.6 (540)
• 50 – 300	2.4 (13)
No porridge consumed in a week or month	
10.Amount of cow's milk (ml) consumed in a day	
• 0	93.5 (517)
• 20 – 300	2.9 (16)
None consumed in a week or month	
11.Amount of NAN(g) consumed in a day	
• 0	99.8 (552)
• 30	0.2 (1)
None consumed in a week or month	
12. Amount of water consumed in a day	
• 0	94 (520)
• 5- 300	6 (33)
13.Amount of water consumed in a week	
• 0	99 (547)
• 20 – 140	1(6)
None consumed in a month	
14.Whether infant suffered from malaria or not	
• No	89 (492)
• Yes	11 (61)
No of times infant suffered from malaria	
• 0	89 (492)
• 1-3	11 (61)
15.Whether infant suffered from fever or not	
• No	83.7 (463)
• Yes	16.3 (90)
No of times infant suffered from fever	
• 0	84.3 (466)
• 1-3	20.7 (117)
16.Whether infant suffered from jaundice or not	
• No	99.3 (549)
• Yes	0.7 (4)
No of times infant suffered from jaundice	
• 0	99.3 (549)
• 1-2	0.7 (4)
17.Whether infant suffered from diarrhoea or not	
• No	94.9 (525)
• Yes	5.1 (28)
No of times infant suffered from diarrhoea	
• 0	94.9 (525)
• 1-3	5,1 (28)
18. Whether infant suffered from umbilical cord infection or not	
• No	99.6 (551)
• Yes	0.4 (2)
No. of times infant suffered from umbilical cord infection	
• 0	99.6 (551)
• 1	0.4 (2)

19.Whether infant suffered from common cold or not	
• No	90.2 (499)
• Yes	9.8 (54)
No. of times infant suffered from common cold infection	
• 0	90.2 (499)
• 1-2	9.1 (50)
• 3-4	0.7 (4)
20.Whether infant suffered from cough	
• No	92.6 (512)
• Yes	7.4 (41)
No. of times infant suffered from cough	
• 0	92.6 (512)
• 1-2	7 (39)
• 3-4	0.4 (2)
21.Whether infant suffered from eye infection or not	
• No	99.6 (551)
• Yes	0.4 (2)
No. of times infant suffered from eye infection	
• 0	99.6 (551)
• 1	0.4 (2)
22.Whether infant suffered from pneumonia	
• No	99.1 (548)
• Yes	0.9 (5)
No. of times infant suffered from pneumonia	
• 0	99.1 (548)
• 1-3	1 (5)
23.Whether infant suffered from plastic teeth problem	
• No	96.4 (533)
• Yes	3.6 (20)
No. of times infant suffered from plastic teeth problem	
• 0	96.6 (533)
• 1-3	3.4 (19)
24.Whether infant suffered from neonatal sepsis	
• No	99.5 (550)
• Yes	0.5 (3)
No of times infant suffered from neonatal sepsis	
• 0	99.5 (550)
• 1	0.5 (3)
25.Whether infant suffered from conjunctivitis	
• No	99.6 (551)
• Yes	0.4 (2)
No. of times infant suffered from conjunctivitis	
• 0	99.6 (551)
• 1-2	0.4 (2)

Appendix 12 Ranges of Aflatoxin Levels in Specific Foods

Dagaa: 1.9 ppb to 2.75 ppb for the 14 samples from Ahero market, 0 ppb to 2.76 for the 10 samples from Mamboleo market, 0 ppb to 0.7 ppb for 5 samples from Kibuye wholesale market, 0 ppb to 1.5 ppb for 8 samples from Kibuye open market, and 0 ppb to 0.7 ppb for 13 samples from Oile market.

Rice: 0 ppb to 4.5 ppb for 8 samples from Kibuye open air market, 0 ppb to 9.4 ppb for 13 samples from Ahero Market, 0 ppb to 11.7ppb for 4 samples from Oile market, 0 ppb to 1.2 ppb for 6 samples from Kibuye whole sale market.

Groundnuts: 1.5 to 2.5 ppb for 4 samples from Ahero, 1.5 to 27.6 ppb for 12 samples from Kibuye Open Market, 1.0 to 2.0 ppb B_1 for six samples from Kibuye whole sale market.

Cassava: 0 to 1.5 ppb B_1 for 8 samples from Mamboleo market; 0.5 ppb B_1 for 6 samples from Ahero; 0 to 3.5 ppb $B_1$for 8 samples from Kibuye Wholesale market; 0.5 for 15 samples from Kibuye Open Air Market.

Maize: 0.5 to 1.0 ppb B_1 for the 10 samples from Ahero Market, 0.5 to 35.4 ppb B_1 for 11 samples from Mamboleo market, 0.5 to 2.0 ppb B1 for the 8 samples from Kibuye Wholesale Market, 0.5 to 34.5 ppb $B_1$for the 12 samples from Kibuye Open Air Market.

Sorghum: 5.0 to 23.5ppb B_1 for 7 samples from Ahero Market, 7.3 to 24.5 ppb $B_1$for the 12 samples from Kibuye Open Market, 7.4 to 20.4 ppb B_1 for 5 samples from Kibuye wholesale market, 13.5 to 24.5 ppb B_1 for 4 samples from Mamboleo Market.

Processed milk: Type 1, 0.05 ppb to 0.13 ppb; Type 2, 0.03 ppb to 0.10 ppb; Type 3, 0.02 ppb to 0.09 ppb; Type 4, 0.02 ppb to 0.06 ppb; Type 5, 0.04 ppb to 0.05 ppb

Raw Milk: Mamboleo milk, 0.004 ppb to 0.008 ppb; Guba milk, 0.004 ppb to 0.010 ppb and Ahero; 0.008 ppb to 0.012 ppb.

Appendix 13 Aflatoxin Levels in Foods from Different Markets

The range of aflatoxin levels in the *Dagaa* were; 1.9 to 27 ppb for 14 samples from Ahero market, 0 to 2.75 for fro 20 samples from Mamboleo market, 0 t 0 0.7 ppb for 5 samples from Kibuye wholesale market, 0 to 1.0 for 8 samples from Kibuye open market, and 0 to 0.7 ppb for 13 samples from Oile market.

The levels of aflatoxin in rice samples were; 0 to 4.5 ppb for 8 samples from Kibuye open air market, 0 to 9.4 ppb for 13 samples from Ahero Market, 0 to 11.7ppb for 4 samples from Oile market, 0 to 1.2 for 6 samples from Kibuye whole sale market.

Aflatoxin analysis results for groundnuts were; 1.5 to 2.5 ppb for 4 samples from Ahero, 1.5 to 27.6 ppb for 12 samples from Kibuye Open Market, 1.0 to 2.0 ppb for six samples from Kibuye whole sale market.

Analysis of cassava revealed; 0 to 1.5 ppb B_1 for 8 samples from Mamboleo market; 0.5 ppb B_1 for 6 samples from Ahero; 0 to 3.5 ppb $B_1$for 8 samples from Kibuye Wholesale market; 0.5 for 15 samples from Kibuye Open Air Market.

Analysis of maize samples revealed aflatoxin levels of: 0.5 to 1.0 ppb $B_1$for the 10 samples from Ahero Market, 0.5 t0 35.4 ppb B_1 for 11 samples from Mamboleo market, 0.5 to 2.0 ppb B_1 for the 8 samples from Kibuye Wholesale Market, 0.5 to 34.5 ppb $B_1$for the 12 samples from Kibuye Open Air Market.

Sorghum analysis showed; 5.0 to 23.5ppb B_1 for 7 samples from Ahero Market, 7.3 to 24.5 ppb $B_1$for the 12 samples from Kibuye Open Market, 7.4 to 20.4 ppb B_1 for 5 samples from Kibuye wholesale market, 13.5 to 24.5 ppb B_1 for 4 samples from Mamboleo Market.

Aflatoxin levels ranged from 0.02 to 0.13 ppb M_1 in 11 samples of brand A, 0.03 to 0.10 ppb M_1 in 10 samples of brand B, 0.022 to 0.094 ppb M_1in 10 samples of brand C, 0.01 to 0.030 ppb M_1 in 10 sample of brand D, and 0.04 to 0.046 ppb M_1in 10 samples of brand E, 0.003 to 0.008 ppb M_1 in 10 samples from Mamboleo, 0.0002 to 0.013 ppb M1 for 10 samples from Guba Market, 0.008 to 0.012 ppb $M_1$for 10 samples from Ahero market.

Appendix 14 Weighed Food Records for Selected Participants

		Food 1	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	**TOTAL**
		1	1+2	1+2+3	1+2+3+4	1+2+3+4+5	1+2+3+4+5+6	1+2+3+4+5+6+7	1+2+3+4+ 5+6+7+8	1+2+3+4+5+ 6+7+8+ 9	
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
KDH153	Name	Tea + milk	Bread (41/2 slices) + BB	*Ugali*	Local veges	Tea + milk	Rice	Beans			
	Weight	520mls	660g	1.02kg	1.22kg	1.72kg	2.02kg	2.22kg			9.4 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
KDH157	Name	Mixed flour porridge	Mandazi	Rice	Green gram	*Ugali*	Egg + potatoes fried	Tea + milk	Bread		
	Weight	500mls	540g	840g	1.04kg	1.3kg	1.4kg	1.8kg	1.89kg		9.3 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
KDH215	Name	Tea + Milk	Cake	*Ugali*	Potatoes + meat stew	Kales	Tea + milk	Scrambled egg	Bread and BB		
	Weight	600mls	750g	1.25kg	1.55kg	1.77kg	2.43kg	2.83kg	2.95kg		14.13 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
KDH218	Name	Tea +	Boiled	Soda	Maize	Bread	Avocado	Fried	Tea +	Boiled	

		Milk	s/potatoes		and beans			potatoes	Milk	s/potatoes	
	Weight	340 mls	850g	1.35kg	1.66kg	1.76	1.98	2.17	2.42kg	2.71kg	
		Food 10	**Food 12**								
	Name	Tea + milk	Cooked Bananas								
	Weight	2.85kg	3.35kg								21.44 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
KDH82	Name	Mixed porridge	Tea + milk	Boiled egg	3 slices bread	*Ugali*	Kales	*Dagaa*	Tea + milk	Porridge	
	Weight	500mls	850g	853g	1.013kg	1.51kg	1.61kg	1.71kg	2.19kg	2.49kg	
		Food 10	**Food 11**	**Food12**							
	Name	*Ugali*	Meat	Kales							
	Weight	2.85kg	2.95kg	3.05kg							21.56 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
ADH380	Name	Tea + Milk	Rice	*Ugali*	*Dagaa*	Kales	Ripe bananas	Tea milk	Rice		
		600mls	1kg	1.25kg	1.30kg	1.36kg	1.46kg	2.06kg	2.420kg		11.5 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
ADH65	Name	Tea + milk	Maize & beans	*Ugali*	Rice	Local veges	*Dagaa*	Cooked bananas	Tea + milk	Beans & maize	
	Weight	500mls	850g	1.20kg	1.350kg	1.40kg	1.45kg	1.65kg	2kg	2.250kg	12.7 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	***Food 7***	**Food 8**	**Food 9**	

ADH76	Name	Tea + milk	Mandazi	Porridge	Kales	*Ugali*	Groundnuts	*Dagaa*	Cooked Bananas	Porridge	
	Weight	500mls	530kg	830g	870g	1.07kg	1.47kg	1.52kg	1.86kg	2.36kg	23.7 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
ADH56	Name	Tea + milk	Bread	Porridge	Maize& beans	Porridge	*Ugali*	Local vegetables			
	Weight	400mls	550g	950g	1.45kg	1.85kg	2.20kg	2.45kg			9.5 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
ADH231	Name	Tea & milk	Mandazi	Chapati	Beans	*Ugali*	Kales				
	Weight	500mls	530mls	630g	880g	1.25kg	1.45kg				5.2 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
ADH161	Name	Tea + milk	bread	Tea + milk	*Ugali*	Kales	*Ugali*	Local veges	Tea + milk		
	Weight	370mls	470g	840g	1.32kg	1.42kg	1.880kg	1.900kg	2.30kg		10.5 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
ADH261	Name	Tea + milk	Mandazi	Milk	Rice	Beans	Chapati	Beans	Tea + milk		
	Weight	490mls	530g	975g	1.495kg	1.685kg	1.755kg	2.075kg	2.36kg		11.4 kg
CODE		Food 1	Food 2	Food3	Food 4	Food 5	Food 6	Food 7	Food 8	Food 9	
ADH159	Name	Tea + milk	Bread	*Dagaa*	*Ugali*	Tea	*Ugali*	Kales			
	Weight	660mls	860g	1.01kg	1.30kg	1.9kg	2.28kg	2.31kg			10.3 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	

ADH271	Name	Tea + milk	Mandazi	Tea + milk	*Ugali*	Local veges	2 Ripe bananas	Rice	Green Grams	Tea + milk	
	Weight	600mls	630g	950g	1.41kg	1.56kg	1.720g	1.96kg	2.18kkg	2.48kg	13.5 kg
Code		Food1	Food2	Food3	Food4	Food5	Food6	Food7	Food8	Food9	
ADH317	Name	Tea + Milk	Sweet potatoes	Porridge	*Ugali*	Kales	Rice	Meat	Tea + milk		
	Weight	450mls	750gml	1.05kg	1.450	1.630	1.985	2. 09kg	2.540kg		11.9 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
ADH157	Name	Mixed flour porridge	2 Mandazi	Rice	Green gram	*Ugali*	Egg + potatoes fried	Tea + milk	Bread		
	Weight	500mls	540g	792g	983g	1.3kg	1.45kg	1.9kg	2.2kg		9.7 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
ADH215	Name	Tea + Milk	Cake	*Ugali*	Potatoes + meat stew	Kales	Tea + milk	Scrambled egg	Bread		
	Weight	600mls	750g	1.05kg	1.21kg	1.3kg	1.83kg	1.89kg	2.10kg		10.7 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
ADH218	Name	Tea + Milk	Boiled s/potatoes	Soda	Maize and beans	Bread	avocado	Fried potatoes	Tea + Milk	Boiled s/potatoes	
	Weight	340 mls	850g	1.32kg	1.58kg	1.81kg	1.88kg	2.09kg	2.30kg	2.61kg	12.9 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
ADH353	Name	Tea	Bread	*Ugali*	*Dagaa*	Porridge	Ripe	Avocado	*Ugali*	Fish	

							bananas				
	Weight	600ml	700g	1.05 kg	1.1 kg	1.45 kg	1.57 kg	1.6 kg	1.96kg	2.10 kg	12.1 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
ADH55	Name	Tea + Milk	Mandazi	*Ugali*	Local veges	Ripe banana	Boiled maize	*Ugali*	*Dagaa*	Tea + milk	
	Weight	600g	640g	950g	1.01kg	1.065kg	1.261kg	1.531g	1.64kg	2.25kg	10.9 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
ADH20	Name	Tea	Mandazi	*Ugali*	*Dagaa*	Tea	Mandazi	Rice	Beans		
	Weight	700g	750g	1.11kgg	1.161kg	1. 461kg	1.488kg	1.938kg	2.21kg		12.3 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
ADH107	Name	Tea + milk	Chapati	Tea	Rice	Meat	Kales	*Ugali*	Kales		
	Weight	370g	430g	800g	1.135kg	1.215kg	1.320kg	1.65kg	1.76g		8.7 kg
CODE		**Food 1**	**Food 2**	**Food3**	Food 4	**Food 5**	**Food 6**	**Food 7**	**Food 8**	**Food 9**	
ADH42	Name	Tea	Bread	Porridge	Groundnuts	Beans	Rice	*Ugali*	Kales	Orange	
	Weight	330g	530g	1.06kg	1.1kg	1.12kg	1.453g	1.844g	2. 024g	2.064g	11.5 kg