PREVALENCE OF AFLATOXIN IN MILK SOLD INFORMALLY IN NAIROBI AND THE EFFECT OF BOILING AND FERMENTATION

By

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2020

DECLARATION

I, **Maureen Mijide Kuboka,** hereby declare that this dissertation is my original work and has not been presented for an award in any other institution.

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ACRONYMS

AFB1	Aflatoxin B1
AFM1	Aflatoxin M1
ATM	Automated Machine
BecA-ILRI	Biosciences east and central Africa-International Livestock Research Institute Hub
CAC	Codex Alimentarius Commission
CYP	Cytochrome P450 Enzymes
EAC	East African Community
EC	European Commission
ELISA	Enzyme –linked Immunosorbent Assay
EU	European Union
FDA	Food and Drug Administration of the United States
GAPs	Good Agricultural Practices
GDP	Gross Domestic Product
HCC	Hepato-cellular Carcinoma
HMPA	High and Medium Potential Areas
HPLC	High-Performance Liquid Chromatography
IARC	International Agency for Research in Cancer
ILRI	International Livestock and Research Institute
KDB	Kenya Dairy Board
KEBS	Kenya Bureau of Standards
LAB	Lactic Acid Bacteria
LC	Liquid Chromatography
Kgs	Kilograms

ml	milliliter
MRL	Maximum Residue Limit
parts per trillion	ppt
parts per billion	ppb
SSA	Sub-Saharan Africa
SD	Standard Deviation
UHT	Ultra-high Temperature
UNESCO	United Nations Educational, Scientific and Cultural Organization
USAID	United States Agency for International Development
UV	Ultra-Violet
µl	microliter

DEFINITION OF TERMS

Aflatoxin: a class of mycotoxins naturally produced by *Aspergillus flavus, Aspergillus parasiticus* and other species. Commonly found in maize, peanuts and other important agricultural products and is transferred to milk if consumed by a dairy cow.

Boiling: artisanal heating process for milk where its temperature is raised to its boiling point (above 100 °C), under atmospheric temperature and pressure.

Fermentation: process of breakdown of milk sugars by means of lactic acid bacteria to produce lactic acid.

Informal milk traders: vendors of milk in the informal market, mostly unlicensed.

Lala: cultured milk traditionally made by natural fermentation or commercially made by adding mesophilic culture.

Mycotoxins: naturally occurring secondary metabolites produced by different filamentous fungi which grow on food and feed when there are suitable temperature and moisture conditions.

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ABSTRACT

It has been observed that milk in Kenya is contaminated with aflatoxin M1 which is transferred from the feeds consumed by the cows. This study was designed to assess the prevalence of AFM1 in raw milk informally sold in peri-urban Nairobi, assess knowledge of informal traders on aflatoxins and the effect of boiling and fermentation on the level of AFM1 found in milk.

A baseline survey was carried out in Kasarani sub-County, Nairobi. Simple random sampling procedure was used to select interviewees for the study. A list of informal milk traders operating in Kasarani was established through the help of Sub-County administration, this formed the sampling frame. A sample of 96 milk traders in informal set-ups were randomly selected and interviewed face to face using pre-tested questionnaires. The aim of the interview was to establish socio-demographic, socio-economic, milk-sale characteristics and consumption characteristics, and knowledge on aflatoxins. The traders were also asked to describe how they carried out boiling of milk. Raw milk samples (n = 96) were collected from the interviewed traders and analyzed for AFM1 using ELISA method.

Knowledge score was computed as a percentage of the sum of correct description and positive responses to the questions. Knowledge on aflatoxin was categorized into three; low knowledge (1 - 40%), medium knowledge (41 - 75%) and high knowledge (above 75%).

Boiling and fermentation trials using contaminated milk were carried out in the laboratory at the Department of Food Science, Nutrition and Technology, University of Nairobi.

Boiling process was simulated in the laboratory according to the method described by traders. Fermentation was done by addition of starter culture to standardized milk after pasteurization at 90 °C for five minutes. Samples were taken during and after completing the process of boiling and fermentation. Samples were analyzed for AFM1 using ELISA.

According to the survey, male traders comprised 51.5% while female traders comprised 48.5%. The mean age of the traders was 28.5 ± 14.5 years, (median = 33, range = 54). There was no significant difference in the age of traders between females and males ($p = 0.89$). On average, a trader's household was described to have four members (median = 4, range = 9). A traders' household averagely consumed 1.6 ± 1.4 (median = 1.0, range = 9.75) liters of milk in a day. Majority of the traders (61.5%) earned averagely below Ksh. 50,000 income monthly, while 12.5% earned between Ksh. 50,000 and 100,000 monthly. A small percentage (5%) earned above 100,000 monthly. About 20% of the traders could not tell how much they earned since they did not do frequent computations.

The highest knowledge score among all traders was 65%. Most traders (69.8%) demonstrated low knowledge on aflatoxins while a lower percentage of the traders (30.2%) demonstrated medium knowledge. Knowledge was highly associated with education level and gender; traders that were more educated and female traders were more knowledgeable ($p = 0.015$ and $p = 0.004$ respectively).

Most of the traders (61.5%) obtained milk from distributors coming from counties outside Nairobi. Results showed that all the milk samples (n = 96) were contaminated with AFM1 at a mean level of 290.3 ± 663.4 parts per trillion (ppt). About 66% of samples were above 50 ppt, the limit applied by the European Union (EU), while 7.5% of the samples exceeded 500 ppt limit applied in Kenya.

Boiling trial showed no significant change on levels of aflatoxins ($p = 0.42$). Fermentation significantly reduced AFM1 during *lala* and yoghurt processing ($p < 0.01$). Reduction in AFM1 level was recorded for *lala* after incubation at room temperature for 15 hours was 71.8%; 73.6% reduction was recorded for yoghurt after incubation at 45 °C for four hours. The study concluded that knowledge on aflatoxin by informal milk traders was low and depended on education and gender. Informally marketed milk is contaminated by AFM1 at substantially high levels. Boiling does not reduce the level of contamination but fermentation reduces the level of AFM1 detectable in milk.

CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND INFORMATION

Milk is a nutrient-dense food that makes significant contribution to the nutritional requirements of household members, specifically children (Finnell & John, 2017). The dairy subsector in Kenya contributes to the daily livelihoods of many people involved in the value chain (Muriuki, 2011a). This subsector is also among the largest contributors to the country's economy. Annually, the dairy sub-sector contributes 40% of agricultural GDP (Gross Domestic Product) and 4% of the national GDP, (Ministry of Livestock Development, 2010).

Milk consumption is higher in Kenya compared to other developing countries with an average annual per capita consumption of 100 kilograms (kgs) (Ouma *et al*., 2000). Along with children, individuals involved in milk trading are more likely to consume milk due to ready availability and thus may be at a greater risk of exposure to AFM1 (Kirino,*et al*., 2016). The informal marketing channel for raw milk is growing and reaches a wide population especially due to convenience and cost-effectiveness (Muriuki, 2003, 2011b). Informal milk traders are therefore important stakeholders and play a pivotal role in the dairy value chain. Their knowledge on aflatoxins and use of simple yet practical aflatoxin management strategies is important.

With increased growth in production and consumption of dairy and dairy products, there are associated risks to human and animal health (Skoet, 2013). Occurrence of AFM1 in cow's milk poses a risk to human health, especially among children and immune-compromised individuals (Wu, 2015).

Prolonged exposure to AFM1 leads to chronic aflatoxicosis (Williams *et al*., 2004). Livestock is equally affected by aflatoxin resulting in reduced production, increased susceptibility to disease and death in severe cases (Khlangwiset *et al*., 2011).

The International Agency for Research in Cancer (IARC), has classified aflatoxin M1 (AFM1) as Class One A human carcinogen (International Agency for Research on Cancer, 2002). AFM1 is a hydroxylated metabolite of aflatoxin B1 (AFB1). Cows are exposed when consuming feeds contaminated with AFB1. AFB1 occurs in feed and is translated to AFM1 in the liver of the animal and consequently excreted as AFM1 in milk and urine (Pettersson, 2004).

In Kenya, the limit for total aflatoxin in whole cereals, milled cereals and pulses is 10 parts per billion (ppb) (Kenya Bureau of Standards, 2014). The maximum residue limit (MRL) for AFM1 in milk used in Kenya is 0.5 ppb which is equivalent to 500 parts per trillion (ppt). This limit is adapted for use by the East African Community (EAC) from the Codex Alimentarius Commission (CAC) (Gong *et al*., 2015; Grace *et al*., 2015).

Marketed milk from low and middle/high income areas in Nairobi was sampled and the mean levels were found to be at 61 ppt and 36 ppt respectively (Lindahl, Kagera, & Grace, 2018). Fifty two percent and eighty seven percent of milk samples from Makueni and Nandi respectively were contaminated with AFM1 (Kang'ethe *et al*., 2017). Raw milk from different agro-ecological zones in Kenya had mean levels between 200 and 900 ppt (Senerwa *et al.*, 2016).

Carry-over from feed to milk can be reduced by good agricultural practices (GAPs). Different control and mitigation methods can also be applied to reduce AFB1 level in feed; these include physical methods, treatment with chemicals and application of biological cultures or enzymes (Battacone *et al.*, 2003; Pettersson, 2004). Less has been studied on control of AFM1 already present in milk.

This study was done to assess the occurrence of AFM1 in informally marketed raw milk in a peri-urban area of Nairobi, knowledge of aflatoxin amongst the traders involved, and the effect of fermentation and boiling on concentration of AFM1 present in milk.

1.2 STATEMENT OF PROBLEM

The informal market is the main channel of milk trading in Kenya, yet less is known about characteristics of the trade with regard to aflatoxin as well knowledge of informal milk traders on aflatoxins. Fermentation of milk has been reported to be effective in reducing AFM1 levels in milk. However, fewer studies have been done in Kenya to evaluate the effectiveness of used cultures (*Streptococcus sublactis* and *Leuconostoc mesenteroides)* in reduction of AFM1 in milk. Boiling of milk is widely practiced in Kenyan households to decrease microbial load, but little studied in terms of effectiveness to reduce AFM1.

1.3 JUSTIFICATION

This study was done because milk is important as a food item and source of nutrition in most households in Kenya, more so for children, convalescents and immune-compromised individuals. The informal milk marketing channel is growing and reaches a wider population thus the need to understand the level of aflatoxin in informally sold milk. Informal milk traders are also important stakeholders and their knowledge on aflatoxins is important for improved food safety.

Data generated from this study will help to understand the prevalence of aflatoxin M1 in milk and inform aflatoxin awareness campaigns among all stakeholders in the informal milk market. This will be helpful in development of interventions for reducing exposure to the toxin from milk. Processors and consumers will also understand better fermentation and boiling as possible simple strategies for managing AFM1 level in milk. Fermentation of milk is an important process in providing probiotics which are beneficial to human health and enhances bioavailability of nutrients. Boiling of milk is simple and conveniently practiced at household level in Kenya.

1.4 OBJECTIVES

1.4.1 Main Objective

To evaluate the prevalence of aflatoxin M1 (AFM1) in informally sold milk, assess knowledge on aflatoxin and the effect of boiling and fermentation on level of aflatoxins in the products.

1.4.2 Specific Objectives

1. To determine the socio-demographic, socio-economic, consumption and milk sale characteristics among informal milk traders in Kasarani Sub-County, Nairobi.
2. To determine the knowledge of aflatoxin amongst informal milk traders
3. To determine the level of AFM1 contamination in informally sold milk.
4. To evaluate the effect of boiling and fermentation of milk on concentration of AFM1 in boiled milk, yoghurt and *lala*.

CHAPER TWO: LITERATURE REVIEW

2.1 THE DAIRY SUB-SECTOR IN KENYA

2.1.1 The Smallholder Dairy Industry in Kenya

With a population of approximately 40 million (Kenya National Bureau of Statistics, 2010), 80% of the population in Kenya are said to be living in the rural areas where they rely on crops and livestock as source of their livelihoods. In an average household, dairy plays two major roles; contributing to daily livelihood and nutritional security. The dairy subsector is an important contributor to the country's economy, accounting for approximately 33% of the agricultural GDP and four percent of the national GDP (Muriuki, 2003). The industry is also the most advanced in the livestock subsector in the country and the leading amongst other dairy industries in Sub-Saharan Africa (SSA) (Thorpe *et al.,* 2000).

Majority of the stakeholders in the value chain are small players. They include smallholder farmers who are dominant at the production level and depend on trade as a source of income. Smallholder farmers contribute about 70% of the total milk produced in the country. On average, these farmers have 1.2 to 2.0 hectares of land with 2 to 5 cattle heads each producing 5-10 liters of milk in a day (Muriuki, 2003, 2011a). Climatic conditions, policy and institutional mix seem to favor advancement of smallholder dairy farming (Thorpe *et al.*, 2000).

A significant population of dairy cattle is kept by smallholder farmers in High and Medium Potential Areas (HMPA). These farmers practice grazing and semi-zero grazing. Some smallholder farmers obtain concentrate feeds from agro-chemical shops.

Most times this is supplemented with "cut-and-carry" grass especially Napier grass (*Pennisetum purpurem*) and crop residues such as maize and banana stalks (Baltenweck, *et al.,*1998; Thorpe *et al*., 2000). Use of concentrate feed and stored feed other than fresh green forage is a factor in AFM1 contamination of milk (Senerwa *et al.*, 2016). Challenges faced by smallholder farmers include; animal disease challenges and the high cost of inputs and management required especially by the exotic breeds (Oloo, 2016).

Smallholder dairy farmers mainly sell their milk through the informal marketing channel. Only about 15% is marketed formally mainly through large processing companies (Thorpe *et al.*, 2000) and other licensed processors which include; cooperatives, mini-diaries and cooling plants (Muriuki, 2011b). Informal marketing channel is more predominant and reaches a wider population. This includes farm-gate vending, street traders, itinerant traders and retail outlets. In the urban areas, milk bars licensed by the Kenya Dairy Board (KDB) are common, though some of them operate without the license (Heifer International, 2008). Previous studies have shown the main distribution channel for milk from smallholder farmers to be direct sales to individual consumers and traders which accounted for 42% and 22% respectively. The rest was sold to hotel and restaurants, self-help groups and cooperative societies (Baltenweck *et al.*, 1998; Mbogoh & Okoth, 1995; Stephen Mbogoh, 1995).

Hazards of public health concern that have been associated with smallholder dairy farming and informal milk marketing channel are that of bacterial nature, which include; faecal coliforms including *E. coli,* and others such as *Brucella abortus* and *Mycobacterium bovis.*

These hazards are mainly associated with hygiene and handling practices of the producers, traders and consumers (Muriuki, 2003; Omore *et al*., 2002). Occurrence of aflatoxins in milk from smallholder farmers also has been widely reported in the recent past (Kang'ethe *et al*., 2017; Senerwa *et al*., 2016).

Occurrence of AFM1 in milk from dairy smallholder farming is likely to be as a result of poor knowledge and application of GAPs. Smallholder farmers mainly sell their milk through the informal marketing channel. There is therefore likely occurrence of AFM1 in informally marketed milk, this has been little studied. The informal marketing channel is growing and reaches a wide population. Informal milk traders are therefore important stakeholders and play a pivotal role in the dairy value chain. However, fewer studies have described the characteristics of informal milk trade and knowledge of milk aflatoxins by the traders.

2.1.2 Milk Utilization in Kenyan Households

Dairy cattle provide about 70% of the total milk utilized in Kenya. Camels, goats and sheep are also used in milk production to a small extent especially in arid and semi-arid regions. Milk production in Kenya is estimated at three billion kgs annually. Most of this is consumed by calves and the household (Muriuki, 2011b).

Per capita milk consumption in most developing countries, like Kenya, has increased steadily over the years (Skoet, 2013). Milk consumption is higher in Kenya compared to other developing countries with an average annual per capita consumption of 106 kgs per person and is projected to reach 136kgs per person by the year 2022 (USAID, 2014).

Consumption of dairy is mainly in the form of liquid milk which includes both processed and unprocessed milk. Processed liquid milk products include; ultra-high temperature (UHT) treated milk, pasteurized milk and cultured milk products yoghurt and *lala*. Unprocessed milk products are mainly raw milk and traditionally fermented (sour) milk. Raw milk is often consumed as is, after boiling; or made in tea or gruel. There is more milk utilization in rural areas than urban areas (Muriuki, 2003; Ouma *et al*., 2000).

There is limited consumption of processed milk products especially in the rural areas and some peri-urban areas. The success of the informal marketing system can therefore be attributed to; consumer taste and preferences, price differences between processed milk and raw milk, poor road infrastructure and inaccessibility in the rural areas and liberalization of the milk trade (Muriuki, 2003, 2011b).

Boiling of raw milk for drinking or preparation of tea, just as in pasteurization, has been ascertained to destroy all pathogenic bacteria. Effectiveness of boiling milk in reducing AFM1 level in milk has been little studied. Fermented dairy products are sold in the market, however the effectiveness of fermentation culture that is commercially used in Kenya in reduction of AFM1 has been less described.

2.2 PRESENT STATUS OF AFLATOXIN OCCURRENCE IN KENYAN FOOD AND FEED

2.2.1 Occurrence of Aflatoxin in Food

The major food product implicated in aflatoxin contamination in Kenya are maize, groundnuts, sorghum, millet and milk (Wagacha & Muthomi, 2008). The MRL allowed in the country for total aflatoxins in cereals and pulses is 10 ppb and 5 ppb for AFB1.

A limit of 500 ppt for AFM1 in milk has been adapted by the EAC from the CAC (Gong *et al*., 2015; Grace *et al*., 2015; Kenya Bureau of Standards, 2014; Sirma *et al*., 2018).

A study done by Kang'ethe *et al.* (2017) showed that 25% and 45% of maize sampled from households and market places respectively exceeded 10 ppb limit for total aflatoxin. Thirty percent and thirty seven percent of homegrown sorghum and millet in Makueni and Nandi counties respectively also exceeded the 10 ppb limit. Maize samples drawn from Eastern and South-Western regions of Kenya were found to be contaminated with AFB1 at a mean level of 68 ppb and 22 ppb respectively; which is over and above the 5 ppb limit for AFB1 in cereals (Kenya Bureau of Standards, 2014; Mahuku *et al*., 2019). *Aspergillus* species were isolated from groundnut samples obtained from Nairobi and Nyanza, while peanut butter was found to be contaminated with aflatoxin ranging from 0 to 2377 ppb (Ndung *et al*., 2013).

Marketed milk from low and middle/high income areas in Nairobi was also sampled and the mean levels were found to be at 61 ppt and 36 ppt respectively (Lindahl *et al*., 2018). Fifty two percent and eighty seven percent of milk samples from Makueni and Nandi respectively were contaminated with AFM1, out of which eight percent exceeded the EU limit of 50 ppt (Kang'ethe *et al*., 2017). Milk from different agro-ecological zones in Kenya had mean levels between 200 and 900 ppt and the levels varied between the dry and wet season (Senerwa *et al*., 2016).

Acute aflatoxicosis led to the death of 125 individuals in Kenya in 2004. Some of the samples obtained from the region were contaminated to levels above 1000 ppb (Lewis *et al*., 2005).

Aside from being a food safety risk, aflatoxin contamination is an economic problem and a potential barrier to trade (Wu, 2015). There are additional costs imposed on producers, traders as well as consumers in a bid to meet the regulatory standards set for aflatoxin. Stringent maximum limits that have been set especially in the EU may be unattainable by developing countries thus a barrier to trade (Wu & Guclu, 2012).

Mitigation strategies that have been applied have focused on reducing field infestation (Mahuku *et al*., 2019; Mutegi *et al.*, 2018). Less has been documented on the simple, practical and effective post-harvest decontamination strategies that are applicable in the country.

2.2.2 Occurrence of Aflatoxin in Feed

In Kenya, feed is either domestically formulated or obtained from manufacturers through agro-chemical outlets. Smallholder farmers supplement dairy diets with feed concentrate to boost production (Thorpe *et al.*, 2000). Feed concentrates are made up of cereal grains, and press cakes from oilseed plants such as peanut, cotton seeds and sunflower seeds which are highly prone to aflatoxin contamination.

Senerwa *et al*. (2016) studied feed from different agro-ecological zones in Kenya and found that the level of contamination ranged from 1 to 9661 ppb. Kang'ethe and Lang'a, (2009) found that 86% of feed samples from different urban centers in Kenya were contaminated with AFB1, while 67% of these exceeded 5 ppb limit. The European Commission (EC) has set a maximum level for AFB1 at 20 ppb for all feed materials and 5 ppb for feed materials specifically meant for dairy animals (EC, 2000).

2.3 OCCURRENCE OF AFLATOXINS IN FOOD AND FEED

Mycotoxins are naturally occurring secondary metabolites produced by different filamenteous fungi of the genera; *Aspergillus, Fusarium, Penicillium, Alternaria* and *Claviceps* (Huwig *et al*.,2001 ; Kosicki *et al.*, 2016). These fungi grow on food and feed when there is suitable temperature and moisture conditions (Bennett & Klich, 2003). Over 300 different mycotoxins have been discovered, some are toxigenic while others are atoxigenic. Mycotoxins have no reported significant biochemical benefits to the growth and development of the fungi, however some are of significance to public health and economic wellbeing (Zain, 2011). These are mainly produced by toxigenic molds and include; Aflatoxins and Fusarium toxins such as, Fumonisins, Zearalenone, Deoxynevalenone and Trichothecenes (Binder, 2007; Bryden, 2012; Huwig *et al*., 2001; Richard & Payne,2003).

Aflatoxins are the most common and dangerous of mycotoxins and are produced by *Aspergillus flavus*, *Aspergillus parasiticus* and other rare species (Prandini *et al*., 2009). Aflatoxins are categorized as Class One A human carcinogen by the IARC (International Agency for Research on Cancer, 2012). The main classes of aflatoxins are B1, B2, G1, G2.

These two groups differ in their molecular structures; B group contains a cyclopentanone ring while the G group contains a lactone ring. M1 and M2 are hydroxylated metabolites of B1 and B2 respectively (International Agency for Research on Cancer, 2002).

Aflatoxins affect foods that are considered as major cereal staples in the developing countries. These include; maize, sorghum and millet. They also occur in groundnuts, tree nuts, dried fruits, chilies and spices. When animals are infected with aflatoxins through feed, this is transferred to animal products, mainly milk and eggs which are used by humans as food (Iqbal *et al*, 2015; Prandini *et al*., 2009;Sirma *et al.*, 2016).

Aspergillus parasiticus and *Aspergillus flavus* are ubiquitous fungi capable of growing in plant and plant products leading to production of toxic secondary metabolites (Gacem & Ould El Hadj-Khelil, 2016; Prandini *et al*., 2009). Some aflatoxigenic strains are said to produce up to 106 ppb of aflatoxins (Edite Bezerra da Rocha *et al*., 2014). They can remain dormant in the soil and crop residues over a long period of time and produce spores when conditions become favorable. *A. flavus* is of more concern in maize than *A. parasiticus.* This is because it colonizes the grain, producing hydrolytic enzymes which allows it to access the internal parts of undamaged grains (Prandini *et al*., 2009).

In SSA, aflatoxin contamination is widespread due to hot and humid climatic conditions that favor growth and proliferation of aflatoxigenic fungal species (Wagacha & Muthomi, 2008). Field infestation of crops is promoted by drought stress, wet climatic conditions and high temperature during harvest (Kebede *et al*., 2012). Insect, bird and mechanical damages also present opportunities for fungal attack.

In storage, high moisture content of the products, high temperatures and poor aeration are the main factors that promote formation of aflatoxins (Kosicki *et al.*, 2016; Wagacha & Muthomi, 2008).

2.3.1 Aflatoxin M1 in Milk

AFM1 is a hydroxylated metabolite of AFB1. When a dairy animal consumes feed contaminated by AFB1, part of it is degraded in the rumen and the other part is rapidly absorbed and metabolized into AFM1 in the liver. AFM1 is adsorbed into the blood and secreted in milk, urine and bile or further metabolized (Pettersson, 2004; Prandini *et al*., 2009). AFM1 is used as one of the linear biomarkers used to predict intake and assess exposure to AFB1 (Mitchell *et al*., 2013).

AFM1 is a product of hydroxylation of the terminal furan ring of AFB1, that results from a series of oxidative reactions catalyzed by Cytochrome P450 enzymes (CYP) (Williams *et al*., 2004). AFM1 represents 95% of the metabolites formed from AFB1, others being aflatoxin M2, aflatoxicol, aflatoxin M4 and aflatoxin Q1 which are produced in trace amounts and are of less public health concern (Giovati *et al.*, 2015). Once formed, AFM1 is either conjugated to glucuronic acid and excreted via bile or enters blood circulation. Circulating AFM1 is excreted either in urine or milk. AFM1 is detected in blood 15 minutes after ingestion of contaminated feed and in milk after 12 hours (Fink-Gremmels, 2008).

Carry-over of aflatoxin in milk varies between 0.3 and 6.2%. The mean value calculated from various studies is 1.81% with a standard deviation (SD) of 1.22 (Pettersson, 2004). In dairy cows, the degree of carry-over is influenced by a number of factors including; breed, health status of the animal, stage of lactation, biotransformation capacity in the liver and milk yield (Giovati *et al.*, 2015). Cows with a higher milk producing capacity have higher excretion of AFM1. Cows affected by mastitis also have a higher excretion that can be attributed to increased permeability of cell membranes (Pettersson, 2004; Veldman *et al.*, 1992).

Continuous exposure of an animal to AFB1 up to a constant level increases the concentration of AFM1 in milk in a linear fashion before a steady state is reached where there is an equilibrium between AFB1 intake and AFM1 excretion (Battacone *et al*., 2003; Giovati *et al*., 2015; Pettersson, 2004). After withdrawal of contaminated feed, AFM1 gradually disappears in milk reaching undetectable levels after three to five days (Giovati *et al*., 2015; Prandini *et al*., 2009).

2.4 AFLATOXICOSIS

2.4.1 Aflatoxicosis in Humans

Exposure to AFM1 in humans occurs by ingestion of contaminated milk (Giovati *et al*., 2015). Susceptibility to aflatoxicosis is high in children than in adults (Williams *et al*., 2004). The use of milk and its products in weaning/supplementary feeding in children is therefore worrying (Kang'ethe *et al.*, 2017; Kiarie *et al*., 2016).

AFM1 has been found to be cytotoxic to human liver cells *in vitro* and has shown acute toxicity similar to that caused by AFB1 in several species. AFM1 can also cause gene mutation, chromosomal abnormalities and cell transformation in mammalian cells *in vitro* (Prandini *et al*., 2009). Acute aflatoxicosis in humans may lead to death. Symptoms include those of acute liver failure accompanied by edema and lethargy. Chronic exposure results in hepatic cancer which is among the common causes of death from cancers. Workers that are exposed to contaminated products are also at risk of lung cancer through inhalation (Williams *et al*., 2004).

Both AFB1 and AFM1 are Class One A human carcinogen (International Agency for Research on Cancer, 2012). However, AFM1 has been found to be less genotoxic, mutagenic and carcinogenic compared to AFB1 (Prandini *et al*., 2009). The potential of AFM1 to cause cancerous tumors in sensitive species was found to be one magnitude level less than that of AFB1(International Agency for Research on Cancer, 2002). Other toxicological properties of AFM1 are comparable to AFB1 (Fink-Gremmels, 2008).

In children, chronic exposure to aflatoxin could potentially result in growth retardation (Khlangwiset *et al*., 2011; Kiarie *et al*.,2016). Immune suppression is also experienced thus children become more susceptible to other diseases. Growth impairment especially stunting and cognitive impairments which last beyond childhood have been associated with exposure to aflatoxin (Khlangwiset *et al*., 2011).

The potential mechanism for this is the binding of aflatoxin to protein molecules reducing their bioavailabilty thus resulting in protein energy malnutrition and recovery from this is delayed with continued exposure (Williams *et al.*, 2004; Wu *et al*., 2011).

The highest risk of chronic exposure to aflatoxins in humans is hepatic cancers, specifically hepato-cellular carcinoma (HCC) in humans. Many studies have established a correlation between aflatoxin exposure through the diet and incidence of HCC in different populations (Wang & Tang, 2004). In Africa, the mortality rate of hepatic cancers is 8.19 per 100,000 population (Williams *et al*., 2004). Exposure to aflatoxin coupled with Hepatitis-B Virus infection, has been found to have synergistic effect resulting in occurrence of HCC in humans. There has also been found to be an interaction between exposure to aflatoxin and HIV/AIDS where exposure to aflatoxin further exacerbates effects of decreased immunity (International Agency for Research on Cancer, 2012).

2.4.2 Aflatoxicosis in Animals

A typical case of chronic aflatoxicosis in animals is marked by decline in productivity with no display of clinical signs. Majority of the effects of chronic aflatoxicosis are subtle and may go unnoticed due to lack of clinical signs. These effects include; reduced growth rate, reduced feed conversion efficiency, infertility syndrome in pigs and cattle and loss of quality in animal products. Other effects of chronic exposure are reduced weight gain, decreased productivity, jaundice, anemia and decreased immunity leading to increased susceptibility to other diseases and increased mortality rates (Wu *et al*., 2011).

Acute aflatoxicosis results in anorexia, depression, gastrointestinal bleeding, pulmonary edema and liver damage (Wu *et al*., 2011). The liver is the target organ for AFB1 toxicity. AFB1 has been reported to cause liver damage and HCC in rodents, poultry, fish, pigs, lambs and cows (Bennett & Klich, 2003). Liver damage is caused by congestion, oxidation of liver cells and haemorrhage. Death of the animal may occur within hours or few days (Lizárraga-Paulín *et al.*, 2011). Severity of the effects depends on the dose of aflatoxin, the length of period of exposure and the age of the animal (Lubulwa & Davis, 1995).

Different species show varied levels of sensitivity to aflatoxin toxicity. AFB1 has more severe effects in poultry, fish, swine and other monogastric animals compared to ruminants (Rawal, Kim, & Coulombe, 2010). Clinical signs are seen in some monogastric animals when aflatoxin concentration in the feed is above 50 ppb and above 150 ppb in cows (Lizárraga-Paulín *et al.*, 2011). In ruminants, microbial organisms in the rumen play the role of a barrier by degrading mycotoxins thus hinder assimilation into the blood. Other toxins however such as AFB1 may pass through the rumen unchanged and assimilated into the blood where they are converted into active metabolites (Fink-Gremmels, 2008).

Applebaum & Marth (1983) studied dairy cows under diet contaminated with impure aflatoxin. There was reduced serum-glucose level accompanied by decrease in milk yield with no apparent clinical signs of illness. These findings may link exposure to aflatoxin and reduced feed conversion rate in animals.

AFB1 contamination also results in reduced quality of milk by transfer of AFM1 to the milk. This has been extensively studied and reported (Battacone *et al*., 2003; Kang'ethe & Lang'a, 2009; Laura Anfossi & Giraudi, 2008; Maki *et al*., 2016;).

Immune suppression through aflatoxin exposure has been observed, however it has not been adequately explained (Kumar *et al*., 2017). Decreased immunity increases chances of other infections caused by parasites, bacteria and fungi, thus in many occasions aflatoxicosis may be overlooked in final diagnosis (Eaton & Groopman, 1993). The magnitude of the effects of AFB1 are determined by; the weight of the animal, sex, nutrition status, age individual variations and co-contamination by other mycotoxins and pharmacologically active substances (Wu *et al*., 2011).

2.5 METHODS FOR CONTROL OF AFLATOXINS IN FOOD AND FEED

The primary strategy for managing mycotoxins is minimizing their production by controlling field infection, harvesting grains at optimum maturity and storage of products in cool and dry conditions (Huwig *et al*., 2001). Where contamination has already occurred, decontamination strategies are required before products can be used for food or feed (Lásztity & Bara, 1999). Mycotoxin control strategies in feed are divided into three; biological, chemical and physical (Huwig *et al*., 2001).

Strategies employed should be able to destroy the mycotoxin and the fungal spores optimally to ensure that new toxins are not produced. The method should not significantly alter the physical and nutritional qualities as well as acceptability of the feed. Above all, the method should be cost-effective (Ji, Fan, & Zhao, 2016; Lásztity & Bara, 1999).

2.5.1 Physical Methods

Some physical methods of decontamination can be simple and easy to apply (Lásztity & Bara, 1999). Sorting is one such simple method. This is applied by differentiation of grains based on color, density, size, physical and insect damage and visible fungal growth. While manual sorting is labor-intensive, color and fluorescence-sorting is more efficient.

Other more advanced image-based technologies can be applied in sorting of AF-contaminated grains. These include; Near Infrared Spectroscopy (NIRS), Hyperspectral Imaging (HIS), Ultra-Violet (UV) light system coupled with color detection. These technologies are fast, non-destructive, effective and can be used on large scale (Udomkun *et al*., 2017).

There exist other physical methods that can be applied and have been proved to be important in reducing aflatoxin contamination in grains. These methods included; washing, winnowing, crushing combined with dehulling (Fandohan *et al*., 2005; Karlovsky *et al*., 2016).

To reduce aflatoxin to less toxic or non-toxic products, energy is required to excite the aflatoxin molecules causing biotransformation. Energy can be provided in the form of irradiation, heat, UV or visible light (Udomkun *et al*., 2017). Exposure to sunlight also decreases the levels of aflatoxin contamination. Herzallah, Alshawabkeh, & Al Fataftah (2008) showed that exposure time was directly related to level of decontamination.

Ionizing radiation with Co-60 enables decontamination with minimal handling (Calado, Venâncio, & Abrunhosa, 2014). Doses between five and seven kilo-Gray of gamma radiation are used in mycotoxin decontamination. Higher doses above 10 kilo-Gray cannot be used especially on grains as they reduce germination capacity (Jouany, 2007).

Environmental conditions determine the susceptibility of aflatoxin to destruction by heat (Kumar *et al*., 2017). Presence of moisture may enhance hydroxylation of the lactone ring on aflatoxin molecule. Some food constituents also may act as cushion to shield aflatoxin from destruction (Waliyar *et al*., 2015). Proteins molecules bind to aflatoxin thus shielding them from destruction. Destruction of AFB1 in oily food/feedstuff, may require higher temperatures above 200 °C since oil is a protective factor (Samarajeewa *et al.*, 1990). However, AFM1 is relatively heat-resistant and has been detected in processed milk products such as pasteurized and UHT treated milk, infant formulas and cheeses (Martins & Martins, 2000; Lindahl *et al*., 2018).

2.5.2 Treatment with Chemicals

Chemicals combine with aflatoxin forming other compounds. Toxicity of the resulting compounds should be evaluated to ensure that there are no negative health effects to animal and human health. Chemicals should also not diminish nutritional, organoleptic and other quality characteristics of the food/feed (Karlovsky *et al*., 2016). The EC provides criteria to ensure proper application of detoxification procedures for feed (European Commission, 2015).

Some of the chemicals agents used in aflatoxin detoxification include: oxidizing agents (hydrogen peroxide, ozone); reducing agents (sodium bisulphite); chlorinating agents (gaseous chlorine, chlorine dioxide, sodium hypochlorite); hydrolytic agents (acids and alkalis) and some food ingredients and medicinal plants (Karlovsky *et al*., 2016; Samarajeewa *et al*., 1990; Udomkun *et al*., 2017).

Organic acids convert AFB1 to β-keto acid which in turn is converted to lesser toxic compound (Udomkun *et al*., 2017). Ammonization has been proved to be an effective and practical detoxification strategy for aflatoxin both in laboratory experiments and field trials. The efficacy level reported in feedstuff has been between 75 to 100%. Ammonization however has been little applied across the world (Karlovsky *et al*., 2016).

Ozone is a strong oxidizing agent that cleaves the double-bond at position 8, 9-of the furan ring in AFB1 molecule by electrophilic reaction resulting in compounds that are non-toxic. However with ozone, longer exposure periods are required which lead to reduced protein efficiency ratio and availability of essential amino acids especially in protein-based feeds (Samarajeewa *et al*., 1990).

Sodium bisulphite was found to be not only beneficial in detoxification, but also improved feed color, palatability and the bisulphite molecule also acts as a preservative (Karlovsky *et al.*, 2016). Phytochemicals have also been found important in decreasing aflatoxin biosynthesis. These include; phenolic compounds such as tannins, ascorbic acid (Gacem & Ould El Hadj-Khelil, 2016).

2.5.3 Biological Methods

Biological control methods include using microbial organisms and/or enzymes have been used to bio-transform the toxins in into lesser toxic or non-toxic compounds (Ji *et al*., 2016). Fungal species have been used in aflatoxin decontamination. These are mainly non-toxigenic *Aspergillus* species including *A*. *parasiticus, A.flavus, A. niger* and *A.white* (Dorner, 2004; Ji *et al*., 2016). These are applied pre-harvest in crops and the approach is to out-compete toxigenic strains (Kabak & Dobson, 2009). Several bacterial species, such as *Bacillus*, *Pseudomonas, Corynebacterium, Mycobacterium* have also been shown to inhibit growth of fungi and production of aflatoxin (Dorner, 2004; Ji *et al*., 2016).

2.5.4 Effect of Fermentation on Aflatoxin in Milk

Lactic acid bacteria (LAB) have been found to have the ability to degrade or bind aflatoxins (Ahlberg *et al*., 2015; Karlovsky *et al*., 2016). In addition, when used in milk, LAB provides probiotics. LAB cultures are generally regarded as safe by Food and Drug Administration of the United States (FDA) (Adibpour *et al,* 2016). LAB culture is majorly used in yoghurt making and has been found to be effective in absorbing M1 in milk, although different studies show different effectiveness (El Khoury *et al*., 2011; Elsanhoty *et al*., 2014; Ahlberg *et al*., 2019).

Lactobacillus species and *Streptococcus bulgaricus* have been found to have the capacity to bind and release AFB1, (Gacem & Ould El Hadj-Khelil, 2016). LAB culture used in *ergo* production, a local fermented milk product in Ethiopia, led to 54% decrease in AFM1 concentration (Shigute & Washe, 2018).

A combination of probiotic LAB and yeast cultures were evaluated for reduction of AFM1 in milk. Significant reduction, up to 90% was noted (Abdelmotilib *et al.,* 2018). Fermentation coupled with incubation over specified period has been seen to be even more effective in reduction of AFM1. The longer the incubation period, the more the reduction of AFM1 concentration (Shigute & Washe, 2018).

In Kenya, fermentation of milk is widely done using LAB cultures as well as natural fermentation, some of the products are commercialized. *Lala* is a fermented milk product in Kenya, made using *Streptococcus sublactis* and *Leuconostoc mesenteroides.* Yoghurt is made using *Lactobacillus bulgaricus* and *Streptococcus thermophilus.* The effectiveness of these in reducing AFM1 concentration in Kenya has been little studied.

2.5.5 Effect of Heat Treatment on Aflatoxin in Milk

AFM1 has been found to be highly stable and resistant to process conditions such as pasteurization and mild acidification. AFM1 therefore persists in milk and is found present in milk products including pasteurized milk, UHT treated milk, ice cream, butter, and yoghurt (Giovati *et al*.,2015; Laura Anfossi & Giraudi, 2008; Tekinşen & Eken, 2008).

There are fewer studies to show the effect of boiling on AFM1. Boiling is a common practice in many households in Kenya who consume raw milk after boiling or used in tea or gruel.

2.6 METHODS OF ANALYSIS OF AFLATOXIN IN FOOD AND FEED

There are three broad categories of methods used in detection of aflatoxin in food and feed, these are; Chromatography, Spectroscopy and Immunochemical methods (Wacoo *et al.*, 2014).

The choice of method used depends on chemical features of the analyte, complexity of the food/feed matrix, duration of sample preparation and testing, limits of detection and quantification required, precision and cost. Detection of AFM1 in milk has been done mainly using Chromatography and Immunochemical methods (Bellio *et al*., 2016).

2.6.1 Chromatographic Methods

There are different chromatographic techniques depending on interaction between the mobile phase and the stationary phase. These include; Gas Chromatography (GC), Liquid Chromatography (LC), Thin-Layer Chromatography (TLC) and High-Performance Liquid Chromatography (HPLC). High-Performance Liquid Chromatography and TLC are widely used in analyzing aflatoxins while LC coupled with mass spectrometry (LC-MS) is an emerging technology (Wacoo *et al*., 2014).

a) High Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography is commonly used in quantitative analysis of mycotoxin in foods and feeds, often coupled with Ultra-Violet (UV) detectors, florescence detectors or mass spectroscopy. HPLC can be used in identification as well as determining the concentration of toxin (Espinosa-calderón *et al.*, 2011; Wacoo *et al.*, 2014).

This method is suited for online clean-up of the extracted sample. The sample to be analyzed is distributed between the mobile and stationary phase depending on the interaction of the sample components to the two different phases. Different fractions of the sample emerge from the mobile phase. The detector records elution time for each analyte which is used for identification. Normal phase and reverse-phase HPLC techniques have been used in analysis of aflatoxins (Rahmani, Jinap, & Soleimany, 2009; Wacoo *et al*., 2014).

High-Performance Liquid Chromatography is used because of its high sensitivity and selectivity thus gives accurate detection of aflatoxins. This method is also fast, thus results are obtained within a short time. However, this method requires sample purification which is a rigorous process. In addition, pre-column and post-column derivatization done to improve detection limits makes HPLC a tedious method of analysis. Mass spectrometry which is often coupled with HPLC does not require derivatization, however high-level training and skill is needed to operate. HPLC equipment is also quite costly (Wacoo *et al*., 2014).

b) Thin-Layer Chromatography

The stationary phase is composed of silica, alumina or cellulose in an inert material like plastic or glass. The mobile phase is a mixture of water, methanol and acetonitrile. The difference in solubility of the analyte in the two phases brings about the distribution of the analyte, such as aflatoxins. The molecular structure of the analyte determines the interaction with either the mobile or stationary phase, thus quick separation (Wacoo *et al*., 2014).

Thin layer chromatography has high sensitivity and selectivity in detection and quantification and can be used in multiple analyses of mycotoxins. However, it requires highly skilled operation, sample preparation and pre-treatment and costly equipment.

The technique has poor precision which is brought about by errors accumulated during sample application, palate development and interpretation (Espinosa-calderón *et al.*, 2011; Wacoo *et al.,* 2014).

c) Liquid Chromatography- Tandem Mass Spectrometry (LC-MS/MS)

This is a modification to the normal LC technique. This is a multi-analyte technique used to detect and quantify a broad range of mycotoxins. Tandem mass spectrometry is used in the place of fluorescence or mass spectrometry detectors. The derivatization and clean-up steps which take longer time and make the method highly prone to errors are eliminated in LC-MS/MS. It is therefore fast, highly sensitive and selective in detection (Rahmani *et al*., 2009). It is also more precise and accurate compared to other LC techniques.

Liquid Chromatography- tandem Mass Spectrometry technique uses expensive high technology equipment which requires high level training and skills to operate. In addition, internal standards are required for matrix matching and calibration of curves. Standards are costly too and some mycotoxins have no standards (Lauwers *et al*., 2019).

2.6.2 Spectroscopic Methods

Spectroscopic methods are non-destructive and can be used for both qualitative and quantitative analysis of aflatoxins. The principle of spectroscopic methods is absorption and emission of light over a wide range of wavelength (Min & Cho, 2015). Optical spectroscopic techniques such as Fluorescence Spectroscopy (Smeesters *et al*., 2015) and Near-Infrared Spectroscopy (NIR) (Wang *et al*., 2015) have been used in aflatoxin assay.

These methods however have the limitation of being time-consuming , expensive, complex and are affected by extraneous factors such as light and temperature (Min & Cho, 2015).

a) Fluorescence Spectroscopy

Fluorescence spectroscopy is a rapid and non-destructive technique used in detection, characterization and quantification of aflatoxins. Aflatoxins molecules have the ability to fluoresce under UV light. Ultra-violet light is absorbed in the visible region of the molecules thus exposing their structure. The molecules emit the absorbed energy at different wavelength; this principle is used in quantification of aflatoxins. Derivatization is done for better analysis (Wacoo *et al*., 2014). Florescence has been used in maize and peanuts (Yao *et al.*, 2015). More studies are required to show its application in other foods/feedstuff.

Other fluorescent molecules present in the food/feed apart from aflatoxin molecules are detected thus this may not be very accurate in quantification of aflatoxins. Fluorescence of the molecules fades over time, thus fresh samples should be used. Fluorescence techniques also uses high-powered lasers and dyes which are costly (Espinosa-calderón *et al*., 2011).

b) Near-Infrared Spectroscopy (NIR)

This is a rapid, non-invasive technique used in quantitative and qualitative analysis of aflatoxins. Compared to chromatographic methods, it is easier and faster since it does not require sample extraction or clean-up of columns.

Near-Infrared Spectroscopy uses simple instrumentation and data acquisition is faster. It uses both reflectance and transmission spectra and has been found to have better accuracy in detection, up to 99%. Near-Infrared Spectroscopy has been used in detection of fungi on maize and also aflatoxins on single maize kernels (Yao *et al*., 2015).

Use of NIR is limited because of the cost of equipment, knowledge of mathematical and statistical methods used in measuring the transmission and reflection spectra, poor sensitivity and thus a high limit of detection and lack of calibration models (Krska *et al.*, 2008).

2.6.3 Immunochemical Methods

Immunochemical methods are based on affinity and formation of selective complexes between antigens and antibodies (Leszczynska *et al*., 2001). Immunochemical methods are often preferred to chromatographic or spectrometric methods since they require less expertise, they are less costly and take a shorter time. In order to enhance signaling of antigen-antibody complexes, labels such as enzymes, radioisotopes and others are used (Espinosa-calderón *et al*., 2011; Leszczynska *et al.*, 2001; Wacoo *et al.*, 2014).

a) Radio-Immunoassays (RIAs)

Radioisotopes are used in Radio-immunoassays (RIAs), enabling multiple analyses simultaneously with high specificity and selectivity. The principle of RIA is competitive binding between a labeled radioactive antigen and a non-radioactive antigen on binding sites an antibody. Radio-immunoassay has been used in detection and quantification of AFB1 and AFM1.

This method however, is limited by the use of radioisotope label which has a short half-life, posing environmental challenges in its disposal as well as being a threat to the human body after prolonged exposure (Wacoo *et al*., 2014).

b) Biosensors

Biosensors are small analytical devices that are portable and used in rapid detection of aflatoxins with high specificity and selectivity. Biosensors are divided into different categories depending on the signal of transduction; electrochemical, optical, thermometric, piezo-electric and magnetic. Electrochemical and optical biosensors are used in aflatoxin analysis. The principle of detection of biosensors is change in physico-chemical properties of the medium when an analyte (antibody) binds with a bio-recognition element (antigen), these properties are such as pH, electron transfer, heat transfer or refractive index (Espinosa-calderón *et al*., 2011).

Solid samples such as grains and feed materials require extraction and preparation, which prolong the time required for analysis. A clean-up step is also mandatory to improve sensitivity. Cross-reaction between related mycotoxins may bring about errors in detection.

This technique is still in development and has not been widely used; improvement on reproducibility and repeatability by use of nanotechnology materials is on-going (Yao *et al*., 2015).

c) Enzyme-Linked Immunoassay (ELISA)

Enzyme-Linked Immunoassay (ELISA) is used as an alternative to RIA because enzymes labels do not pose a threat to health. Enzymes used as labels in ELISA kits are alkaline phosphatase (AP) and Horseradish Peroxidase (HRP).

This technique uses the principle of competition for binding site between antibody specific to the toxin or a labeled toxin-enzyme conjugate and toxin in the sample. The resulting complex is quantified using a plate reader (Wacoo *et al*., 2014).

Enzyme-Linked Immunoassay has been a preferred method because it is simple, cheap, does not require extensive preparation of the sample or clean-up steps. Enzyme-Linked Immunoassay technique is highly sensitive, selective, has a low limit of detection and is rapid in operation. There is also limited use of organic solvents. Enzyme-Linked Immunoassay kits are commercially and readily available (Yao *et al*., 2015). However, matrix effect can be encountered. This is where components of the sample interfere with enzyme activity hindering effective formation of antigen-antibody complexes. This can be reduced by using low-matrix ELISA kits. There are also various washing steps involved thus lengthening the process and making it labor-intensive (Hyunh *et al*., 2012; Wacoo *et al*., 2014).

ELISA has been used in quantification of AFB1 in feed and AFM1 in milk and dairy products and has been proved to be effective (Bellio *et al*., 2016; Kang'ethe & Lang'a, 2009; Kirino *et al*., 2016; Leszczynska *et al*., 2001; Lindahl *et al.*, 2018; Senerwa *et al*., 2016).

ELISA method was used in this study because it is cost-effective, faster and requires simpler technology.

CHAPTER THREE: STUDY DESIGN AND METHODOLOGY

3.1 STUDY DESIGN

The study incorporated cross-sectional and experimental study designs with analytical component. The study was divided into three phases as follows**:**

Phase 1: Survey of informal milk traders

This was carried out amongst informal milk traders to establish socio-demographic, socio-economic, consumption and milk sale characteristics as well as knowledge on aflatoxin. A pre-tested, semi-structured questionnaire was used.

Phase 2: Prevalence of aflatoxin M1 contamination

Milk samples were collected from the interviewed traders during the survey. Laboratory analysis was done to assess prevalence of AFM1 in raw milk marketed informally in Kasarani sub-county, a peri-urban area in Nairobi.

Phase 3: The effect of boiling and fermentation trials

These trials were designed to evaluate effectiveness of fermentation of milk (to yoghurt and *lala)* as well as boiling in reduction of AFM1. *Lala* is sour milk, made either traditionally by natural fermentation process, or commercially made by adding mesophilic culture. In this experiment, mesophilic culture was used.

3.2 METHODOLOGY

Methodology is divided in three phases as follows; Phase 1- Survey of informal milk traders, Phase 2- Boiling and fermentation trials and Phase 3- Laboratory analysis of AFM1.

3.2.1 Phase 1: Survey of informal milk traders

3.2.1.1 Study setting

A cross-sectional study was conducted in Kasarani sub-County, Nairobi, in June 2018.

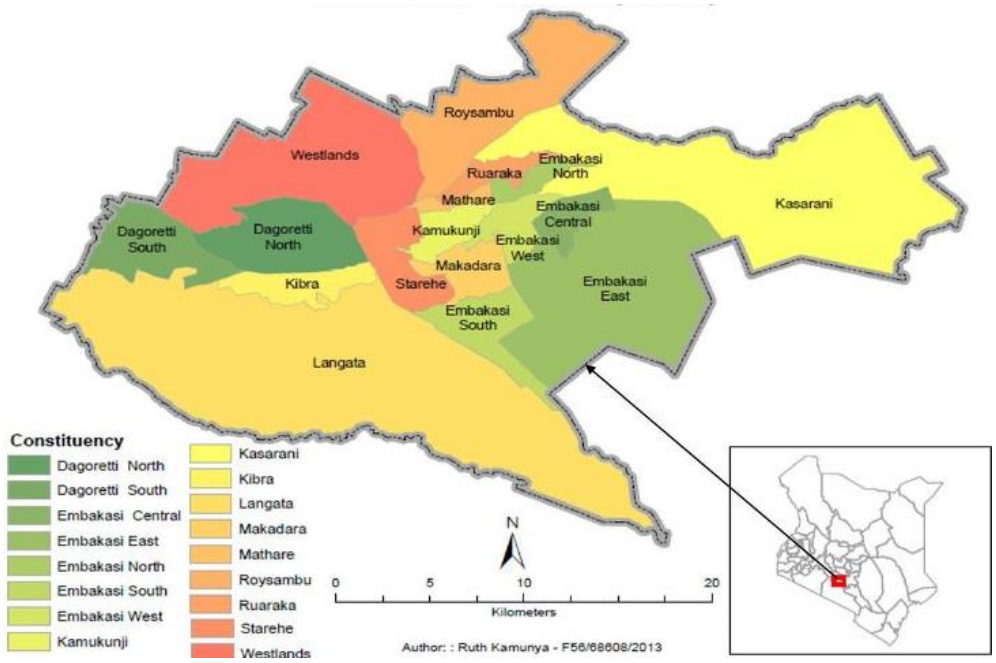

Figure 1: Map showing positioning of the study area, Kasarani sub-County, as part of Nairobi County

Source: (Kamunya, 2015)

Kasarani is a peri-urban sub-County that covers approximately 86 square kilometers. It has an estimated population of 525,624 people and is divided into five wards (Kenya National Bureau of Statistics, 2010). Kasarani, as a peri-urban center in Nairobi, was chosen based on intensive small-scale dairy farming activities that take place in the area, according to the Ministry of Agriculture Livestock and Fisheries.

3.2.1.2 Population of Study

The population of study included milk traders in the informal sector. This comprised traders in different milk vending set-ups such as; on-farm milk kiosks (milk sold at the farm-gate), dairy shops (shops either selling milk exclusively or other goods together with milk), milk ATM (automated milk dispensing machines) and street traders (milk stands along streets). Milk ATM is a Kenyan term for automated milk dispensing machines which allows consumers to purchase milk in quantities they can afford. Milk is dispensed through a nozzle in units as small as 100 milliliters (ml). The machine should have a built-in refrigeration system maintained between 5 to 10 ^{o}C and fed only with pasteurized milk, although this is not always the case.

3.2.1.3 Sampling Procedure and Sample Size Calculation

Simple random sampling procedure was used to select informal milk traders as interviewees for the study. A list of informal milk traders operating in Kasarani ward was established through the help of sub-County administration, this formed the sampling frame. By randomization in Microsoft Excel, a sample of 96 milk traders were randomly selected from the list and interviewed in this study. The sample size was calculated based on expected prevalence (p) of aflatoxin M1 (AFM1) of 50% with a desired normal deviation of 1.96 which corresponds to 95% and a 10% degree of precision. Fischer's formula for a population below 10,000 was used as follows (Naing, Winn, & Rusli,2006):

$$n = \frac{z^2 pq}{d^2} \qquad \frac{1.96^2 \times 0.5 \times 0.5}{0.1^2} = 96.04 \sim 96$$

n = desired sample size

Z = desired normal deviation set at 1.96, which corresponds to 95%

p = prevalence (proportion of population with characteristics of interest)

q = proportion of population without characteristics of interest

d = degree of precision, set at 10%

3.2.1.4 Data Collection Methods

Traders were interviewed face-to-face using pre-tested, semi-structured questionnaires. Informed consent was obtained prior to the interview. The aim of the interview was to establish socio-demographic characteristics, socio-economic characteristics, milk-sale characteristics, milk consumption practice and knowledge on aflatoxins. Traders were also asked to describe how they carried out the process of boiling. Questions asked were therefore related to socio-demographic characteristics (gender, age, level of education), business characteristics of the trader (role in the business, sources of marketed milk, volume sold, price of buying and selling, income from milk sale), milk consumption practices (consumption of milk in the trader's household, consumption of milk by children between six months and three years in the household), knowledge of aflatoxins and willingness to pay for milk with reduced aflatoxins, and description of boiling practice.

Milk samples were collected from the interviewed traders, but where milk was not present at the time of the interview, the team recorded details of the outlet and requested that a sample be put aside from the next batch to be received and the sample was collected the following day.

Milk samples were collected in sterile 50ml-tubes and transported in cool boxes to Biosciences for east and central Africa-International Livestock Research Institute (BecA-ILRI) labs within eight hours where they were stored at -20 °C before analysis.

3.2.2 Phase 2: Boiling and Fermentation Trials in the Laboratory

Boiling and fermentation trials were carried out at Food Science Pilot Plant, College of Agriculture and Veterinary Sciences, University of Nairobi. Milk used in the trials was obtained from the University of Nairobi veterinary farm in Kanyariri, Lower Kabete, Nairobi. Two different raw-bulk samples were obtained, one for fermentation and one for boiling. Samples of the raw milk were drawn in sterilized 50ml tubes and initially analyzed for AFM1 using ELISA technique.

Boiling Trial

Boiling of milk was simulated as according to the description given by the traders. Below is the summary: One liter of milk was heated in a *sufuria* (boiling pot) until it started to bubble, heat was turned down so that the milk was boiling at lower heat until steady bubbles and foam formed and the milk began to rise. A stirrer was used to break up the foam and create a gap for steam to escape. After this, milk was boiled for two minutes while constantly stirring. At this point, milk was considered to have boiled completely; boiling temperature was taken at this point which was 94 °C. Samples were taken before boiling, during boiling process (after three minutes and six minutes), immediately after complete boiling and after cooling to room temperature (23 °C). Samples were stored at -20 °C until the day of analysis. Samples were analyzed in triplicate.

Fermentation Trial

Milk was pasteurized at 90 °C for 30 minutes then cooled to 43 °C. Two percent of yoghurt starter culture (*Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus salivarius ssp. thermophilus*) was added (YF-L903, Thermophilic Yoghurt Culture -Yo Flex®, Batch No. 2402687). Inoculated milk was incubated at 45 °C for four hours.

After incubation, the milk was cooled overnight to 3 to 4 °C and then stored at 4 °C for seven days. The pH was measured using MetroHM 632-pH Meter at the end of incubation and during sampling on day one, two, and seven while storing at 4 °C. Samples were drawn after pasteurization, after cooling to 43 °C, after incubation, after overnight cooling and after storage on day one, two, and seven. Samples were stored at -20 °C until the day of analysis. Samples were analyzed in triplicate.

Lala is sour milk, traditionally made by natural fermentation but commercially made by adding mesophilic culture. In this experiment, mesophilic culture was used. Milk was pasteurized at 90 °C for 30 minutes then cooled to 23 °C. Two percent *lala* starter culture (*Streptococcus lactis* and *Leuconostoc mesenteroide ssp. mesenteroides*) was added, (CHN-22, Mesophilic Aromatic Culture -Yo Flex®, Batch No. 3399963). Inoculated milk was incubated at room temperature for 15 hours. The pH was measured after incubation and after storage day one, two, and seven at 4 °C. Samples were drawn after pasteurization, after cooling to 23 °C, after incubation, and after storage at 4 °C on day one, two, and seven. Samples were stored at -20 °C until the day of analysis. Samples were analyzed in triplicate.

3.2.3 Phase 3: Laboratory Analysis for AFM1

Laboratory analysis of AFM1 in milk and dairy products was carried out at BecA-ILRI labs. Quantitative detection of AFM1 in raw milk, boiled milk and fermented milk samples and was done using competitive ELISA kit (Helica Biosystems Inc., Santa Ana, CA, USA) based on guidelines provided under ISO 14675/186:2002. The ELISA kit had a lower limit of detection of 5 ppt and the highest standard was 100 ppt for AFM1.

3.2.3.1 Materials and Reagents

ELISA full kit with antibody coated micro-well plate, AFM1 standards, aflatoxin HRP-conjugate (green cap), substrate reagent (blue cap), stop solution- (red cap) and washing buffer (PBS).

Single and multichannel pipettor with 100 and 200 micro-liters (µl) tips

Timer

Absorbent paper towels

Centrifuge

Microplate reader with 450nm filter

3.2.3.2 Sample Preparation and Procedure for AFM1 Analysis

Sample Preparation

Before the start of analysis, frozen raw, boiled and fermented milk samples were transferred from the freezer (-20 °C) to the refrigerator (4 °C) for thawing overnight. In the morning the samples were retrieved from the refrigerator, vortexed with a Vortex-Genie 2T mixer and centrifuged at 2000 revolutions per minute (rpm) for five minutes with Eppendorf 5418 centrifuge to induce separation of the upper fatty layer.

ELISA kits used for analysis were transferred from refrigerator to the working surface to raise the temperature of the wells to at least 23 °C.

Procedure for AFM1 Analysis

Two hundred micro-liters aliquots of the sample and standards provided in the kit were pipetted carefully into the anti-body coated wells on a plate. The plate was sealed with an aluminum cover to protect from excess UV light and incubated at room temperature (23 °C) for two hours. After incubation, the contents of the micro-wells were discarded and the empty wells were washed by washing buffer. Washing procedure was repeated three times.

Hundred micro-liters of conjugate provided in the kit was pipetted into the wells. The plate was sealed as before and incubated at room temperature for 15 minutes. After incubation, the contents were discarded and the walls were washed with washing buffer, three times as before. Hundred micro-liters of enzyme substrate provided in the kit was pipetted into the wells, sealed and incubated at room temperature for 15 minutes.

After this, 100 µl of stop solution (provided in the kit) was added to stop the reaction. Color changed from blue to yellow. Optical density of the wells was read at 450 nm using a micro-plate reader. Levels of aflatoxin were quantified using logarithmic standard curve made out of the optical densities of the standards with R^2 of above 97%. Milk samples that exceeded the upper limit (100 ppt) were diluted with skim milk provided with the kit and re-tested following the above procedure.

3.3 STATISTICAL DATA ANALYSIS

Data obtained will be entered into Microsoft Excel® 2010 and analyzed using Genstat® 15th Edition and IBM SPSS version 20.

Descriptive statistics (arithmetic means and standard deviations (SD), median, minimum and maximum values, range and frequencies) were used to explain data on socio-demographic characteristics, consumption of milk, milk-sale characteristics, level of AFM1 in milk, knowledge of aflatoxin and effect of the treatments on AFM1 concentration.

A set of 10 questions was used in assessing knowledge on aflatoxins. Knowledge score was computed as a percentage of the sum of correct description and positive responses to the questions. Respondents with absolutely no information on aflatoxin got a score of zero which was up-scaled to one to avoid corner point solution. Knowledge on aflatoxin was categorized into three; high knowledge, medium knowledge and low knowledge. Based on the percentage scores, low knowledge was computed to be between 1 and 40%, medium knowledge was 41 and 75%, while high knowledge was scores above 75%, (Ilesanmi & Ilesanmi, 2011; Matumba *et al*., 2016).

Chi square test was used to test association between categorical data. Independent T-Test was used to compare the means of two sets of continuous data. One–way Analysis of Variance (ANOVA) and Least Squares Difference (LSD) tests were used in comparison of multiple means at ($p \leq 0.05$). Data was presented in narrative, tables and graphs.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 SOCIO-DEMOGRAPHIC, SOCIOECONOMIC, CONSUMPTION AND MILK SALE CHARACTERISTICS OF INFORMAL MILK TRADERS

4.1.1 Socio-demographic Characteristics

A total of 96 traders were interviewed. Male traders comprised 51.5% while female traders comprised 48.5%. There was no significant difference in the age of traders between females and males ($p = 0.89$).

The table below shows the distribution of the informal milk traders by age. The mean age of the traders was 28.5 ± 14.5 years, (median = 33, range = 54).

Table 1: Distribution of informal milk traders by age-groups

Age group	Percentage (%)
Below 20	1.0
20-30	40.0
31-40	30.2
41-50	15.5
51-60	5.2
61-70	3.2
Above 70	1.0

The table below shows the distribution of the traders by the level of education.

Table 2: Distribution of informal milk traders by the level of education

Level of education	Percentage (%)
Never attended formal school	6.1
Primary school (lower)	5.1
Primary school (upper)	9.2
Secondary school (not completed)	13.3
Secondary school (completed)	35.7
Tertiary education	30.6

Both females and male were found to be participating in milk sale business, almost at equal frequency. This is contrary to a study by Thorpe *et al.* (2000) where the dairy business was largely considered to be a male-dominated venture. However, Tavenner, Saxena, & Crane (2018) later found that the informal milk market in Kenya was largely made up of more women than men. This shows that more women may be currently involved in milk selling. Kiama *et al*. (2016) also showed participation of both women and men in dairy production.

There has been increasing rate of enrolment into secondary schools between the year 2002 and 2009. This may explain why most informal milk traders in this study had attained post-primary education. There was no significant association between gender and the highest level of education attained by the traders ($p = 0.91$). However, data from United Nations Educational, Scientific and Cultural Organization (UNESCO) shows that there was a difference in literacy rate between males and females.

Literacy rate for individuals between 15 years of age and older in 2014 in Kenya was at 83.78% and 74.01% among males and females respectively (UNESCO, 2013).

4.1.2 Socioeconomic Characteristics

Some of the traders interviewed were casuals (12.2%) who were employed to sell milk, as seen in table three below. Majority of the traders (61.5%) earned averagely below Ksh. 50,000 income monthly, while 12.5% earned between Ksh. 50,000 and 100,000 monthly. A small percentage (5%) earned above 100,000 monthly. About twenty percent of the traders could not tell how much they earned since they did not do frequent computations.

Table three below shows major socio-economic activities carried out by informal milk traders.

Table 3: Source of income for informal milk traders

Source of income	Percentage (%)
Milk business only	65.3
Milk business and crop farming	8.2
Milk vending and other businesses (shop/grocery)	14.5
Informal employment	12.2

Dairy business has been described as a profitable venture, hence participation of more individuals (Muriuki, 2011a). Margin analysis has shown up to three times increase in value of milk from the primary producer to the consumer, fetching income of up to Ksh. 172,000 per year (USAID, 2014).

These studies show profits from the production level, fewer studies have been done to show profits earned from milk traders who are up the value chain.

4.1.3 Consumption Characteristics

On average, a trader's household was described to have four members (median = 4, range = 9). A trader's household averagely consumed 1.6 ± 1.4 (median = 1.0, range = 9.75) liters of milk in a day. Traders with children between six months and three years reported that the children mostly consumed boiled milk and tea (33%) or boiled milk alone (24.2%).

Table four below shows different forms of milk consumed in the informal milk trader's household.

Table 4: Dairy consumption by traders' households

Form of milk consumed	Percentage (%)
Raw milk only	2.1
Boiled milk only	4.2
Milk made in tea only	42.2
Both boiled milk and made in tea	35.8
Made in porridge only	10.6
Fermented only	4.3

Muriuki (2003) reported consumption of raw milk, after boiling or made in tea or gruel. This is common because of consumer tastes and preferences and also pricing. Most consumers consider raw milk to be richer and creamier, with a fuller mouth-feel and cheaper compared to pasteurized milk (Heifer International, 2008).

Due to public health risks associated with consumption of raw milk directly from the animal, reduced consumption of raw milk has been reported, similar to what was observed in this study (Omore *et al*., 2002). For both adults and children, forms of milk mostly utilized included boiled milk, milk in tea or porridge and fermented milk (Kirino *et al.*, 2016; Muriuki, 2003).

4.1.4 Milk-sale characteristics

Table five below shows different forms of informal-milk business set-ups identified during the study. Majority of the businesses identified in our study were farm shops or kiosks, (37 out of 96).

Table 5: Distribution of informal milk traders in different business set-ups

Type of Business	Percentage (%)
Farm shops or kiosks	42
Dairy shops	38.6
Milk Automated Machines (ATMs)	14.8
Street-vended milk	4.5

Most traders (61.5%) sourced milk through distributors from distant areas including Embu, Murang'a, Mount Kenya, Limuru, Kinangop, Kerugoya and Nyeri. Other traders obtained milk directly from farms (26%). There was no significant difference in buying price of milk by the traders from the different sources; (farms, distributors, dairy shops, aggregation centers, farm and/or distributors) where ($p = 0.725$).

However the highest buying price for milk was KES 52.2 ± 7.6, sourced from distributors and the lowest was at KES 43.5 ± 19.2 obtained from milk farms. On average, the traders sold 78.86 ± 104.99 liters of milk in a day (median = 50, range = 797), at KES 62.7 (±5.2) per liter, (median = 65, range = 30).

Most traders (76.4%) stored their milk at refrigeration temperatures of between 5-10º C prior to selling, mainly in milk dispensers (which has an in-built refrigeration system) or normal refrigerators. A number of the traders (16.9%) stored milk in clear plastic buckets at an open area for visibility while others used aluminum cans (5.6%).

About a half (52.6%) of the traders believed the safety of milk could be judged by senses, mainly by sight and taste. A small percentage (3.1%) thought milk safety could easily be determined using quick tests such as lactometer test. Some traders (42.6%) confessed that their milk got spoilt, from time to time. Of these, 42.6% discarded the milk, 16.7 % gave the milk to animals such as calves, pigs and dogs, while 19% made *lala* out of the milk. Traders especially those with direct connection with the suppliers (21.4%) were able to alert the supplier, where possible the product was recalled.

Informal markets are often preferred to formal markets because of quick sales and better earnings, unlike contractual sales to cooperatives and large processing plants where payments are done monthly (Heifer International, 2008). The main informal milk outlets described in the past include; itinerant traders, street traders, on-farm sales, milk bars and shops, and sales to hotels and restaurants (Baltenweck *et al*., 1998; Thorpe *et al*., 2000).

In Kenya, the informal dairy sector is dominant with about 80% of milk marketed through this channel, however, due to poor documentation, there lacks sufficient data on informal milk traders and the operations through this channel (Muriuki, 2011b).

On-farm shops or kiosks were found to be popular because they allow personal interaction between the producer and consumer, thereby establishing trust (Mbogoh, 1995). A number of street traders declined to participate in the study in fear of any possible legal consequences since they are unlicensed. Because of this, they are under-represented in this study.

Dairy actors in Kenya include dairy cooperatives, wholesale and retail traders in dairy shops and itinerant traders such as hawkers. Cooperative societies are an integral part of the milk marketing system in Kenya. Smallholder farmers organize themselves in groups where their milk is collected, bulked and distributed to the bigger processors and high potential markets such as Nairobi (Mbogoh & Okoth, 1995). This explains why distributors have become a chief source of milk in peri-urban centers such as Kasarani.

The informal milk marketing channel has been described by sale of raw milk, lack of cold chain and minimum regulatory control (Muriuki, 2011b; Skoet, 2013). Sale of raw milk is discouraged by the KDB. The practice of milk sale through plastic buckets has been observed before by Kirino *et al.* (2016). This is discouraged under the code of hygienic practice for milk and milk products, instead aluminum or steel cans are recommended (Kenya Bureau of Standards, 2004).

Cold storage of milk is recommended and majority of traders in this study were seen to comply, unlike in previous studies, where only 17% (Omore *et al*., 2002) and 21% (Kirino *et al*., 2016) of the milk outlets practiced cold storage. Omore *et al*. (2002) noted that though some traders carried out quality checks before receiving and selling the milk, these checks were however ineffective in correctly indicating the safety of the milk.

Milk automated machine (ATMs) is a fairly new technology for dispensing milk. These machines are required to operate at chilling temperatures to prevent spoilage of milk as it waits selling. The machines are required by KEBS to sell pasteurized milk only (Kenya Bureau of Standards, 2004), however, this is not always the case as seen in this study. Milk ATMs are becoming popular in the peri-urban centers because of convenience, as consumers are able to access milk in economic packages depending on their purchasing power (Galičič, *et al*., 2015), less studies have been done on use of milk ATMs in Kenya.

4.2 LEVEL OF KNOWLEDGE OF AFLATOXINS BY INFORMAL MILK TRADERS

Table six below displays the responses by traders to knowledge questions on aflatoxins. Though most of the traders (68.4%) had heard about aflatoxins, only a few (26.8%) could correctly describe the toxin and its dangers in the human body (11.5%).

Table 6: Responses to questions on aflatoxin by informal milk traders

Aflatoxin Knowledge Items	With Knowledge (%)	Without Knowledge (%)
1. Have you heard of aflatoxins	68.4	31.6
2. What are aflatoxins	26.8	73.2
3. Do you think aflatoxin present in milk can be seen with the naked eye	42.2	57.8
4. Which products (food types) would you expect to be easily contaminated with aflatoxins	37.6	62.4
5. Do you think the presence of aflatoxins in these foods pose any danger to humans	65.3	34.7
6. Which danger(s)	11.5	88.5
7. Do you think aflatoxins can be present in animal feed	12.9	87.1
8. Do you think that aflatoxins present in the feed can be expressed by the cow through milk	49.5	50.5
9. Do you think boiling of milk reduces aflatoxin contamination	31.3	68.7
10. Do you think fermentation of milk to yoghurt and *lala* reduces aflatoxin contamination	20.8	79.2

Table seven shows the distribution of traders according to aflatoxin knowledge scores attained.

The mean knowledge score was 27.8% ± 16.2, while the minimum and maximum scores attained were 10% and 65% respectively. None of the traders had high knowledge on aflatoxins.

Table 7: Knowledge of aflatoxin by traders

Level of Knowledge (Score)	Percentage (%)
Low (1-39%)	69.8
Medium (40-74%)	30.2
High (75-100%)	Nil

4.2.3 Level of knowledge on aflatoxins and level of education

Chi-square test showed significant association between level of knowledge on aflatoxins and level of education of the traders ($p = 0.015$). Individuals that had attained secondary and college level education were more aware of aflatoxins than individuals with primary level education and those with no education at all. This is demonstrated in Figure two.

This study found low knowledge of aflatoxins amongst milk traders. Similar results were found amongst dairy farmers by Kiama *et al*. (2016). Education level of traders was significantly associated with knowledge on aflatoxins. This confirms a previous study by Matumba *et al*. (2016). Education has been associated with knowledge on food safety. More educated individuals have been seen to be more aware of food safety risks brought about by chemical hazards such as aflatoxin (Dosman, Adamowicz, & Hrudey, 2001). Poor knowledge on aflatoxins affects the adoption of technologies for management of aflatoxins (Kumar & Popat, 2007).

There was significant association between gender and aflatoxin knowledge at (p = 0.04) with female traders demonstrating more knowledge than the male ones. Higher knowledge on aflatoxins among women as compared to men can be associated with their key role in food production and household food security (Quisumbing *et al*., 1995).

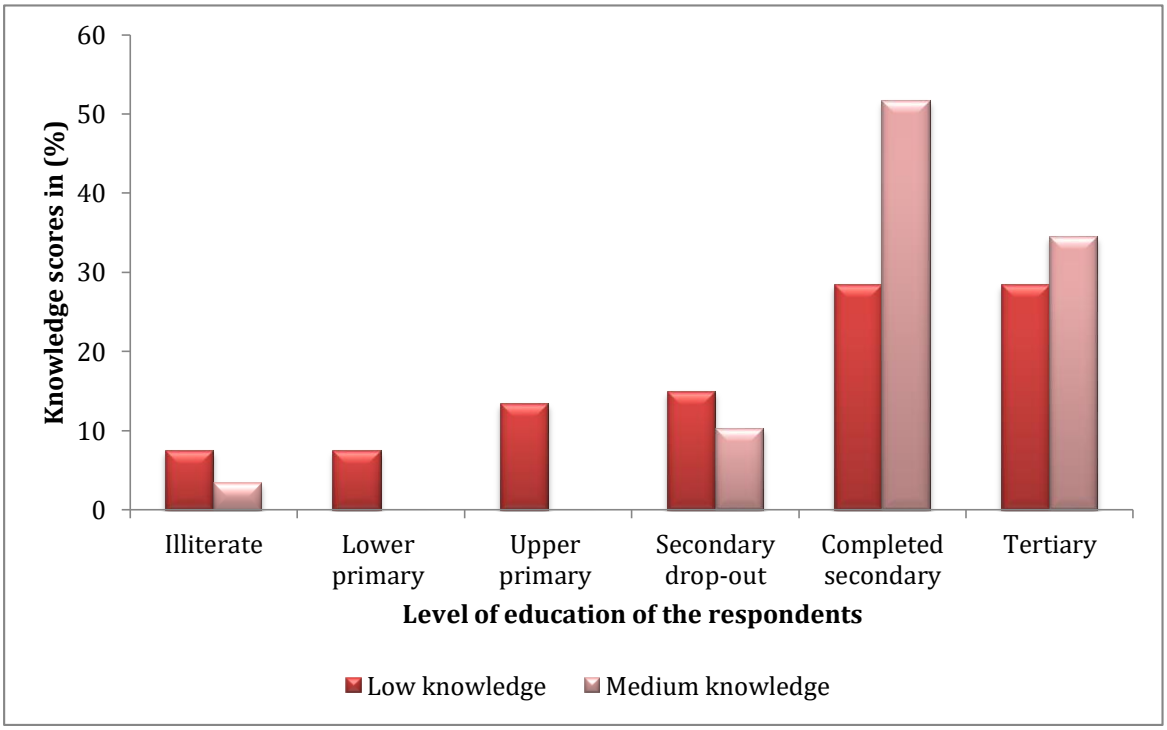

Figure 2: Association between level of education and knowledge on aflatoxin for informal milk traders

4.3 LEVEL OF CONTAMINATION OF MILK WITH AFM1

In total, 96 raw milk samples were collected and analyzed for AFM1. All milk samples (n = 96) were contaminated with aflatoxins above the lower limit of detection, 5 ppt. The mean level of AFM1 in milk was 290.3 ± 663.4 ppt. The minimum level detected was 15.4 ppt and the maximum was 4563 ppt.

Of the samples analyzed, 66.4% were above 50 ppt which is the legal limit allowed by EU, while 7.5% of the samples exceeded 500 ppt, the legal limit allowed by the FDA and is also adapted by the EAC. Milk samples obtained from on-farm kiosks had significantly higher levels of AFM1. The means of AFM1 in the milk from different business set-ups are summarized in Table eight below.

Table 8: Level of AFM1 in milk from different milk-vending set-ups

Type of Business	Mean ± SD
On-farm kiosk (37)	269.5 ± 413.6[a]
Dairy shop (34)	139.9 ± 187.8[b]
Milk ATM (13)	175.3 ± 46.98[b]
Street trader (4)	246.9 ± 204.6[a]

*Mean ± SD, Values with different superscript show significant difference while same superscript show no significant difference at $p < 0.05$.

The level of contamination of milk observed in this study was higher than that previously reported in Kenya. Kang'ethe *et al.* (2017) reported 70% while Kang'ethe & Lang'a (2009) reported 72% rate of contamination. The mean level of AFM1 in the milk was also higher than those reported in previous studies in Nairobi (Kirino *et al.*, 2016; Lindahl, Kagera, & Grace, 2018; Kiarie *et al*., 2016). This level of aflatoxin M1 contamination in milk points to possible risk of aflatoxicosis and other health implications among consumers (Sirma *et a*l., 2018; Ahlberg *et al.*, 2018). The mean level of AFM1 was however lower compared to those observed by Senerwa *et al*., (2016) who sampled milk from different agro-ecological zones in Kenya.

Variation in AFM1 can be attributed to the different regions where the milk came from, mainly HMPA which experience medium to high rainfall resulting in high relative humidity. Conditions of high humidity provide a good environment for growth of fungi and production of mycotoxins in feed and feed ingredients (Senerwa *et al*., 2016 ; Koteswara Rao *et al*., 2016). Accessibility to feed in different regions and/or seasons is a factor that could explain the different AFM1 contamination levels observed in milk (Lindahl *et al*., 2018). Lack of green forage in some regions and /or seasons results in farmers using feed concentrate and feed stored for a prolonged period which may be contaminated by aflatoxin (Kang'ethe & Lang'a, 2009).

4.4 EFFECT OF BOILING AND FERMENTATION ON AFM1 LEVELS IN MILK

4.4.1 Effect of Boiling on AFM1 in Milk

There was no significant change in AFM1 levels during the process of boiling and after cooling to room temperature, 23 °C ($p = 0.42$). The mean levels of AFM1 obtained during the process are presented on Table nine.

Table 9: Effect of boiling on AFM1 in milk

Product	Time of boiling	Mean Level of aflatoxin ± SD	(Max-Min)
Boiled milk	Raw milk	188.7 ± 4.3[a]	193.1 - 184.5
	After 3 minutes	190.2 ± 5.5[a]	195.7 - 183.5
	After 6 minutes	187.5 ± 8.2[a]	195.6 - 180.4
	Complete boiling (8minutes)	190.4 ± 7.3[a]	197.3 - 185.2
	Cooled to 23 °C	179.3 ± 17.1[a]	187.9 - 169.4

*Mean ± SD, Values with same superscript show no significant difference at $p < 0.05$.

4.4.2 Effect of Fermentation of milk to yoghurt and *lala* on AFM1

The pH of yoghurt was 4.43 after incubation and reduced to 4.02 after day seven at 4 °C. The pH of *lala* was 4.6 after incubation and reduced to 4.2 after day seven at 4 °C. There was significant reduction in AFM1 during *lala* and yoghurt processing ($p < 0.01$). After incubation, 71.8% reduction in AFM1 level was recorded for *lala* as compared to 73.6% reduction for yoghurt using LAB cultures (*Streptococcus lactis* and *Leuconostoc mesenteroide ssp. mesenteroides*) and (*Lactobacillus delbrueckii ssp. bulgaricus* and *Streptococcus salivarius ssp. thermophilus*) for *lala* and yoghurt respectively. There was no significant difference in reduction of AFM1 in both *lala* and yoghurt during storage. The mean levels of AFM1 during the processes of production are presented in Table 10 below.

Table 10: Effect of fermentation on AFM1 in mala and yoghurt

Product	Stage of sampling	Mean aflatoxin levels ± SD	(Max-Min)
Lala	Raw	307.4 ± 22.9[b]	329.2 -308.5
	Pasteurized	379.3 ± 20.2[c]	398.7- 353.6
	Cooled to 23 °C	343.0 ± 7.5[b]	351.7 - 332.2
	After incubation	86.7 ± 4.6[a]	92.0 - 84.1
	After day one at 4 °C	89.3 ± 1.5[a]	91.1 - 88.4
	After day two at 4 °C	83.9 ± 1.4[a]	85.5 - 82.9
	After day seven at 4 °C	92.2 ± 6.9[a]	98.3 - 86.2
Yoghurt	Raw	307.4 ± 22.9[b]	329.2 - 308.5
	Pasteurized	379.3 ± 20.2[c]	398.7 - 353.6
	Cooled to 43 °C	334.0 ± 4.2[b]	338.9 - 331.6
	After incubation	90.8 ± 2.0[a]	91.9 - 88.4
	After overnight cooling	96.1 ± 2.2[a]	98.7 - 94.8
	After day one at 4 °C	93.9 ± 0.0[a]	98.7 - 94.8
	After day two at 4 °C	79.4 ± 2.4[a]	89.5 - 78.1
	After day seven at 4 °C	81.0 ± 2.1[a]	84.4 - 79.8

*Mean ± SD, Values with different superscript show significant difference while same superscript show no significant difference at $p < 0.05$

While boiling has been little studied before, pasteurization has been seen to bring about no significant change in AFM1 level (Galvano, Galofaro, & Galvano, 1996; Laura Anfossi & Giraudi, 2008). Likewise, in this study, boiling of milk did not reduce the levels of aflatoxin in milk.

Aflatoxins have been described to be heat stable, thus explaining no change after boiling of milk (Giovati *et al*., 2015). During the process of fermentation and boiling, elevated levels of aflatoxin in milk were observed after pasteurization (90 °C) and complete boiling of milk (94 °C), but the level detected after cooling to 45 °C and 23 °C respectively was lower. Fat globules in milk encapsulate aflatoxin molecules (Samarajeewa *et al*., 1990) , thus may not detected at lower temperatures, when the globules link up and solidify, unlike at high temperatures when the fat globules melts and free aflatoxin molecules. This may explain the different aflatoxin level at boiling and pasteurization temperatures and after cooling.

LAB has been shown before to have ability to bind aflatoxins (El Khoury *et al*., 2011; Giovati *et al*., 2015). Thermophilic (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) and mesophilic (*Streptococcus lactis* and *Leuconostoc mesenteroides*) culture for yoghurt and *lala* respectively demonstrated ability to bind AFM1. El Khoury recorded 87.6% binding in (Phosphate-buffer Salt)PBS medium after 14 hours and 46.7% binding in milk after six hours at 42 °C (El Khoury *et al*., 2011). Shigute and Washe (2018) noted 54% decrease when a stock of LAB culture was used, including *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Leuconostoc mesenteroides* after incubation for five days at 20 to 30 °C. Gradual decrease in pH was noted. In their study Adibpour *et al.* (2016) noted that with increase in storage time at 4 °C, AFM1 binding increased which was not the case in this study.

There are various factors that determine the activity of LAB culture, among them are; incubation period, temperature of incubation and pH of the medium (Dalié *et al*., 2010). At 4 °C, though the cells remain viable, their activity is reduced since the temperature is below the minimum required for growth (Hamann & Marth, 1984; Vaningelgem *et al*.,2004). This possibly reduces the ability of the cells to efficiently bind the toxin. The mechanism for decontamination of milk by LAB has not been fully understood. However, some studies have suggested the ability of the toxin to bind to the cell wall of the bacteria. Polysaccharide and peptidoglycan components of the cell wall play a major role in this (Kabak & Dobson, 2009).

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 CONCLUSIONS

In Kasarani sub-county, both men and women are engaged in informal milk sale almost at equal frequency. The highest level of education of the traders was tertiary/college level, however majority of them were those who had completed secondary school education. There was no significant association between gender and the level of education attained by the traders. On average, a trader's household had four members who averagely consumed 1.6 liters of milk in a day. Members of the household consumed milk as boiled milk only, made in tea only, both boiled milk and included in tea/porridge, or tea together with fermented milk products. Traders with children between six months and three years reported that the children mostly consumed boiled milk and tea or boiled milk alone. Characteristics of informal milk sale identified in this study was sale of raw milk, cold storage, use of both plastic and aluminum cans for storage, use of senses to judge safety and minimal use of milk tests such as lactometer test.

Traders had low knowledge of aflatoxins, the highest score was 65%. Most of the traders had low knowledge on aflatoxins. There was an association between the level of education and knowledge of aflatoxins, traders who were more educated were more knowledgeable on aflatoxins. There was also association between gender and aflatoxins, female traders were more knowledgeable on aflatoxins than male traders.

The mean level of AFM1 in milk sampled from the traders was 290 ppt. The minimum level detected was 15.4 ppt and the maximum was 4563 ppt. Boiling of milk, which is a common practice amongst households, had no effect on the level of aflatoxins in the milk.

Thermophilic and mesophilic cultures used in milk fermentation (yoghurt and *lala*) have demonstrated the ability to bind AFM1 and reduce milk contamination. Incubation temperature and time have been seen to be important in reduction of AFM1.

5.2 RECOMMENDATION

It is important to raise awareness and education on aflatoxins not only among farmers but also among informal milk traders since they are part of the stakeholders along the dairy value chain. The general public too should be educated on the effects of aflatoxins and practical methods to reduce contamination of milk. GAPs and good feeding practices should be encouraged among smallholder farmers to reduce contamination in milk.

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APPENDICES

Appendix 1: Informed consent form

INTRODUCTION

Good morning / Good afternoon. My name is _____ and I work for the International Livestock Research Institute (ILRI) which is based in Nairobi. We are conducting a study on health impacts of aflatoxins in Urban and peri-urban areas of Nairobi County. Our visit today is a follow up to research activities that have been conducted in this area before; namely 1) an initial baseline survey that helped us understand smallholder milk production systems in your area and 2) a field trial with a few of the farmers to analyze the effect of selected interventions on milk quality and safety and 3) a follow up survey to help analyze the impact of what was done in the previous visits, with regards to improving milk quality and safety. We are visiting you because you are a key stakeholder in the dairy value chain. If you accept to participate in the baseline study, we will ask you a few questions related to trading of milk, and knowledge of hazards in milk. We will also request you to provide us with a sample of milk for further laboratory testing, to assess contamination. We promise to respect privacy and confidentiality of what you tell us and wish to assure you that the information you give us will only be shared with our research team members, in the sharing, all names will be removed so that no one can be able to trace back the information to you.

If you have any questions now or later you are welcome to call the researchers:

Dr. Johanna Lindahl 0718-929937
Dr. Florence Mutua 0733-546859

INFORMED CONSENT FORM

Do you have any questions about the research we wish to conduct? Once again, we thank you for accepting in your business place and now wish to ask for your availability to participate in the study. Please note that your participation in the study is voluntary and that you can withdraw your participation at any time. We assure you that whatever information you share with the research team is confidential.

Are you willing to be part of this study?

We respect your choice and do appreciate your participation			
		Trader`s Initials	signature
YES	Verbal		
	Written		
NO			

Appendix 2: Questionnaire on assessing the levels of aflatoxins in informally marketed milk

NAME OF THE ENUMERATOR _____

CODE:

DATE: ___/___/2018

CHECK IF: Adequate Trader introduction has been done ___√ and Consent is granted ____√

1.1 Location of the business

Sub-County:	**Ward:**	**Town**

1.2 Respondent details

a. Respondent details	Gender:	Age:
	Highest level of education: [ ]	Sources of income: [ ] [ ] [ ]
	1=never been to school 2= primary (lower) 3=primary (upper) 4=secondary school (not completed) 5= secondary school (completed) 6= college / university	0=employed casual 1=employed full time 2= farming 3=other
b. Role in the business	What is your position in the business? [ ] [ ] [ ]	Role in the milk business [ ] [ ] [ ]
	1=Owner 2=Employee 3= Owner'sFamily 4=other – specify	1= I stay in the shop to sell milk 2= I collect milk from farmers 3= I go out to sell the milk 4=Control money from sale 5= Other

1.3 Milk Trading

1.3.1 How many liters of milk do you sell in a day?----------

1.3.2 Where do you source your milk from? ( ) ( ) ( )

Source:	How many	Purchase price yesterday Ksh./litre
1=Farm		
2=Dairy Shop		
3=Distributor		
4=Farm and/or Distributor		
5=Milk Aggregation Centre		

1.3.3 Description of sold milk

What is the selling price of milk per liter	
How long is the milk stored before being sold (hrs)	
Describe the milk products you sell ( ) ( ) ( )	1=Raw milk only 2=Boiled milk 3=Fermented milk products only 4=Raw and Boiled milk 5=Boiled and fermented products 6=Raw and fermented products
If boiled, describe how this is done	

1.3.4 Describe how you store fresh milk prior to selling

1.3.5 Can the safety of milk be judged solely by sight?

_____ [yes] [no]

1.3.6 Does your milk ever get spoilt? If yes, what do you do to the spoilt milk?

1.37 How much income do you get from the milk business monthly----------

1= below Ksh 50,000 2= between Ksh. 50,000 and 100,000 3= Above Ksh. 100,000 4= I don't know

1.4 Consumption Patterns

1.4.1 Household Description

What is your household size?	
What is your position in the household?	1= 2= 3= 4=
Do you have a child below between six months and 3 years in the household?	Yes ( ) No ( )

1.4.2 Milk consumption in the household

How much milk do you consume in your household per day (in liters)	
In what form do you consume milk ( ) ( ) ( )	1=Raw 2= Boiled 3=Fermented milk 4=Made in tea/porridge 5=Other form
In what form do the children below 5 years in your household consume milk?	1=Raw 2= Boiled 3=Fermented milk 4=Made in tea/porridge 5=Other form

1.5 Aflatoxin knowledge

1.5.1 Have you heard of aflatoxins _____ [yes] [no]

1.5.2 If yes, what are they?

1.5.3 If yes to 1.5.1 above, which products (food types) would you expect to be easily contaminated with aflatoxins? _____

1.5.4 Do you think the presence of aflatoxins in these foods pose any danger to humans?

_____ [yes] [no]

1.5.5 Which danger(s)?

_____

1.5.6 Do you think that Aflatoxins present in the feed can be expressed by the cow through milk?

_____[yes] [no] (Don't know)

1.5.7 Do you think boiling of milk reduces aflatoxin contamination?

_____[yes] [no] (Don't know)

1.5.8 Do you think fermentation of milk to yoghurt and *lala* reduces aflatoxin contamination?

_____[yes] [no] (Don't know)

1.6 Willingness to pay

1.6.1 As a trader would you be willing to pay more for milk with reduced aflatoxin contamination? _____[yes] [no]

a. If there is reduced aflatoxin contamination and I can sell milk at a higher price, I would be willing to pay _____ KES per day
b. If there is reduced aflatoxin but I cannot sell to a higher price, I would be willing to pay _____ KES per day

SAMPLING OF MILK

Collect 2 x 40 ml in sterile falcon tubes from milk that is meant for household consumption or for sale	
Indicate the approximate time the sampled milk was milked	
Indicate if the sampled milk has been treated in any way, e.g. by boiling, fermented, chilling	
Indicate the approximate date and time when the sample is collected	

Would you be willing to participate in a future program to make your milk safer? If yes, please give us your name and phone number. Note that you can change your mind and say no when invited to participate.

Name: _____

Phone Number: _____

….THANK YOU VERY MUCH FOR YOUR TIME, WE VALUE YOUR INPUTS….''