EFFECT OF FARMERS TRAINING AND USE OF NOVASIL BINDERS ON AFLATOXIN M1 IN MILK FROM SMALLHOLDER FARMS IN KASARANI, KENYA

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Effect of Farmers Training and Use of Novasil Binders on Aflatoxin M1 in Milk from Smallholder Farms in Kasarani, Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university

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DEDICATION

I dedicate this work to my parents, Mr John Kagera and Mrs Evelyn Muthoni, my children John Vincent Junior, Victoria Vincentia Muthoni & Matteo Ng'ang'a. To my grandparents Mr. Kiiru Ndegwa and Mrs. Wanjiku Kiiru, Kiiru's and Gathuru's family.

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ABBREVIATIONS AND ACRONYMS

AF	Aflatoxin
AFB1	Aflatoxin B1
AFB2	Aflatoxin B1
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AFM1	Aflatoxin M1
AFM2	Aflatoxin M2
ANOVA	Analysis Of Variance
CAC	Codex Alimentarius Commission
Don	Deoxynivalenol
EAC	East African Community
ELISA	Enzyme-Linked Immuno Assay
EU	Eurpoean Union
FAO	Food and Agricultural Organization
Fum	Fumonisins
IEBC	Independent Electoral And Boundaries Commission
ILRI	International Livestock Research Institute
JKUAT	Jomo Kenyatta University of Agriculture And Technology

Ng/kg	Nanogram per kilogram
SSA	Sub-saharan Africa
WHO	World Health Organization
Zen	Zearalenone

OPERATIONAL TERMS

Milk	Unpasteurized whole raw milk
Small holder dairy farmer	Farmer with less than 20 milking animals
Presence of AFM1	Detectable levels of AFM1
Levels of AFM1	Levels between 2ppt-100ppt
Urban farm	Farm located inside the town
Peri urban farm	Farm located in the outskirts of town
Intervention farms	Farms receiving training and NovaSil binder
Control farms	Farms receiving no intervention

ABSTRACT

Milk consumption in Kenya is higher thanthat of other countries in East Africa. However, milk contamination with aflatoxin M1 (AFM1) is common, but the magnitude of exposure to AFM1 and associated health risks might not be well understood by specific groups i.e. farmers and need routine monitoring. Aflatoxins, which commonly contaminate animal feeds and human food, present a significant public health challenge in sub-Saharan Africa. Aflatoxin M1 (AFM1) can be found in milk from cows if fed on diets contaminated with aflatoxin B1 (AFB1). Aflatoxin contamination throughout the dairy value chain has a negative impact on food security and livelihoods. Therefore, this study was carried outto determine the effects of farmers' knowledge and use of binders on aflatoxin M1 contamination of cow milk produced in urban and peri-urban dairy farms. The study involved smallholder dairy farms in urban and peri-urban areas of Kasarani sub-county, Nairobi county, Kenya. A quasi-experimental design was used, including baseline, intervention and endline surveys. Farmer data was collected through questionnaires, while milk contamination data was collected through laboratory analysis using enzyme-linked immunosorbent assay (ELISA) for AFM1 in milk. A baseline survey was conducted with 100 farmers, from which 30 whose milk contained AFM1 levels greater than 20 ng/kg were randomly selected for inclusion in the study. Twenty of the thirty farmers were educated on aflatoxins and their effects on animals and humans, and they were given NovaSil® binders to mix with their animals' feeds for three months (intervention), while the remaining ten served as a control group. All farmers were visited twice a month for interviews and milk sample collection for three months. One month after the end of the trial period, the control group was trained as the intervention group for a day and provided with a binder that would last for three months. An endline survey was conducted ten months after the baseline to assess the intervention's effect on aflatoxin levels in milk and farmers practices and knowledge. During the baseline survey, 84 milk samples were collected from smallholder dairy farms and analyzed for AFM1. Ninety-nine percent of the samples (83/84) analyzed were contaminated with AFM1. The mean aflatoxin level was 84 ng/kg at the baseline, with 64% of the samples exceeding the EU legal limit of 50 ng/kg but within Kenyan standard of 500 ng/kg. Whereas 80% of the farmers were aware of aflatoxin, there was no correlation between farmers knowledge and AFM1 prevalence. The intervention group had a significant difference in AFM1 levels between the trial periods and the baseline, whereas the control group did not. The NovaSil® binder significantly reduced AFM1 concentrations in the raw milk produced by the farmers in the intervention group over the trial duration ($p < 0.01$). The intervention group had eight times more reduction in aflatoxin levels compared to the control group. The control farms were more likely to have milk with AFM1 levels exceeding the regulatory limit of 50 ng/kg than the intervention farms ($p < 0.001$). The farmers in the intervention group reported an improvement in milk yield, cow health and appetite. The training also improved the farmers understanding of how mould and aflatoxins affect humans and animals. In conclusion, the use of binders and the training significantly reduced the aflatoxin levels in milk during the

trial period, this did not continue when farmers were no longer provided with the binders after the trial period. Educating farmers on aflatoxins control and addition of binders in animal feeds is feasible in smallholder systems and can be promoted as an approach to on-farm mitigation of aflatoxins. There is a need to raise awareness of binders use and invest more in acceptable and locally sustainable marketing approaches for the binders.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Milk is the fresh, whole, lacteal fluid secreted by female mammary glands within 72 hours after parturition, with a mildly sweet taste and faint odor (Belitz, Grosch, and Schieberle 2009; Dash 2014). Milk contains diverse nutrients needed in the body, such as riboflavin, selenium, calcium, and magnesium, and thus plays an essential role in ensuring human nutrition and development, especially in childhood (FAO 2013). Cow milk is readily available and most commonly used for human consumption in Kenya. In Kenya, 80% of milk is produced by peri-urban and rural, smallholder dairy farmers (FAO 2011). Quality milk production is crucial to meet consumers' demands, whose need is nutritious and wholesome foods produced in a clean environment free from chemical contaminants and pathogens (Girma *et al* ., 2014; Hasan *et al* ., 2015). Heavy metals, aflatoxins and pest residues, are major milk contaminants derived from feed and fodder fed to animals (Dua *et al* ., 2012).

Aflatoxins are mycotoxins that are primarily produced by *Aspergillus flavus* species and are carcinogenic to human and animal (Baan et al. 2009; Klich 2007). Grains, various animal feedstuffs, nuts, and legumes are the common agricultural products infected by aflatoxins (Yunus et al. 2022). Aflatoxin exposure at high doses can result in severe health effects such as abdominal discomfort, vomiting, and death (Probst, Njapau, and Cotty 2007), whereas persistent sublethal exposure can result in immune system suppression, liver cancer, and growth retardation in children (Khlangwiset, Shephard, and Wu 2011). A study by Williams et al. (2004) found that over 5 billion people in low-income countries are at risk of chronic aflatoxicosis exposure, with contamination being the cause of over 125 deaths in 2004-2005 in Eastern province, Kenya (Azziz-Baumgartner et al. 2005).

The most common strains of aflatoxins that humans and other mammals are more prone to consuming are B1, B2, G1, and G2 (Atela *et al*., 2016). When consumed with food substances, the mammalian metabolic processes usually turn these strains into metabolites known as M1 and M2 which are carcinogenic (Kangethe *et al.*, 2017). Aflatoxin M1 (AFM1) is the main product of hydroxylation of aflatoxin B1 (AFB1) excreted in the milk of lactating animals after consuming AFB1 contaminated feed (Aycicek *et al* ., 2005). The risk of exposure to AFM1 in humans is through milk consumption (Fallah 2010). The percentage of AFB1 excreted as AFM1 by cows varies but has been reported in the range of 1-3% (Aliabadi et al. 2012).

An increasingly high proportion of dairy cattle in the world are kept in intensive farming systems, and with this, the aflatoxin problem is increasing among dairy farmers (Unnevehr and Grace 2013). Small-scale dairy farmers, especially intensive farmers, feed their dairy cows on commercial concentrates, often from uncertified agro-vet dealers. Dairy farmers in rural and peri-urban areas have inadequate knowledge of proper feed formulation and storage facilities (Lukuyu et al. 2011). A research study by Makau *et al*. (2016) showed that on-farm feed formulation and poor handling of feeds in peri-urban farms affect the quality of feeds.

The high level of AFM1 in Sub Saharan Africa (SSA) is a major source of concern for food safety. A risk assessment on AFM1 exposure in low- and middle-income dairy consumers in Kenya by Ahlberg *et al.*, (2018) found that 2.7% of children could theoretically be stunted due to AFM1 exposure from milk, though stunting has not been proven to occur after exposure to AFM1. Although there is no proof of causation, exposure to AFM1 from milk in Kenya has been found correlated to a decrease in growth (Kiarie et al. 2016). In Kenya, Sirma *et al*. (2018) reported that between 0.0014 and 0.0039 cancer incidence rates per 100,000 people a year could potentially be linked to the ingestion of AFM1 in milk. A more recent strategy is to utilize mycotoxin-binding compounds in animal feed because they decrease mycotoxin bioavailability by securing them in the animal's digestive system tract (Kabak and Dobson 2009), lowering mycotoxin production in milk. Study by Magnoli *et al* ., (2008) show that the toxicity from aflatoxin contaminated feeds decreased when clay additives such as zeolite, salt, bentonite, Novasil plus, and Astra Ben 20A were added to improve the physical qualities and prevent caking

1.2 Problem statement and justification

Milk safety is a major global concern since its highly susceptible to biological, chemical and physical contaminants. Aflatoxins are the main chemical contaminants in the dairy value chain. Aflatoxin contamination increases production costs, reduces milk quality, and causes health effects in humans and animals. In an intensive dairy farming system, high amounts of concentrates are fed to dairy cows to increase milk production. Along the dairy feed chain, aflatoxins could contaminate the concentrates; thus, the milk produced will be contaminated with AFM1.

The most toxic of the aflatoxins is AFB1 which has been reported as a natural carcinogen in animals and humans (Hussain et al. 2008). In animals, AFB1 causes toxic effects including immune status depression, growth impairment, poor dietary intake and weight gain (Atherstone *et al.*, 2016). AFM1 the main hydroxylated metabolite of AFB1 can be detected in milk and dairy products from dairy cattle fed on AFB1 contaminated feeds and is excreted within 12 hours after ingestion by lactating cows (Dashti et al. 2009; Iha et al. 2013). Dietary intake of AFB1 in feeds translates to about 3% AFM1 found in milk (Hoogenboom et al. 2010).

AFM1 is known to have carcinogenic, genotoxic and cytotoxic effects (Awad, Ghareeb, and Böhm 2012), with humans being exposed due to ingestion of contaminated milk and milk products (Langat et al. 2016). Various studies have reported high AFM1 levels in milk and poor knowledge about aflatoxins in Nairobi, implying that the population is at a high risk of exposure to aflatoxins (Kagera *et al.*, 2019; Kiama *et al.*, 2016; Kiarie *et al.*, 2016; Kuboka *et al.*, 2019; Lindahl *et al.*, 2018).

The most susceptible and exposed population are the infants who are often weaned on cow milk and they are not immune competent at this early age. Therefore, consumption of AFM1 contaminated milk may further suppress their immunity and make them more susceptible to other diseases (Kang'ethe *et al.*, 2009; Langat *et al.*, 2016). A study in Kisumu, Kenya, by Okoth & Ohingo (2004) reported a significant correlation between wasting and aflatoxin exposure in children under three years, while Gong *et al.* (2004) reported a correlation between stunting and aflatoxin exposure. Improving milk safety and quality is vital as it reduces production costs, negative health effects on humans and animals thus a need to explore combined mitigation measures along the value chain.

Therefore, this study aimed to assess the knowledge, attitude and practices of urban and peri-urban dairy farmers on aflatoxin in milk, determining effectiveness of training smallholder dairy farmers on safe milk production and NovaSil® binder use.

1.3 Objectives

1.3.1 General Objective

To determine the effects of training and use of Novasil binders on AFM1 levels in milk and farmers awareness, knowledge and practices on aflatoxin contamination in urban and peri-urban dairy farms in Kasarani sub-county.

1.3.2 Specific Objectives

1. To assess the farmers' awareness, knowledge, and practices regarding aflatoxins contamination in peri-urban and urban farms.
2. To assess the effect of training and use of aflatoxin binders on levels of AFM1 in milk.
3. To assess the effect of on-farm mitigation measures on farmers knowledge, awareness and practices and AFM1 levels in milk.

1.4 Research questions

1. What is the farmers'awareness, knowledge and practices on aflatoxins contamination?
2. What's the effect of training and adding aflatoxin binders in feeds on AFM1 levels in milk?
3. What is the effect of on-farm mitigations on farmers' knowledge, awareness and practices and AFM1 levels in milk?

CHAPTER TWO

LITERATURE REVIEW

2.1 Mycotoxins

Mycotoxins are toxic secondary metabolites of fungi that when ingested cause adverse negative effects on animals and humans (Hampikyan et al. 2010). The main fungi that produce mycotoxins are *Penicillium, Fusarium* and *Aspergillus spp*. Mycotoxin production is highly dependent on grain damage caused by pests and rodents, environmental conditions and abiotic factors such as moisture content and pH of the feeds (Bhat, Rai, and Karim 2010; Makau et al. 2016). Mycotoxin occurrence largely affects dairy farming through feeding animals with contaminated feeds, affecting animal productivity with animal feeds being often contaminated (Changwa *et al*., 2018; Lewis *et al*., 2005). The potential transfer of these toxins into animal by-products like milk, meat, eggs and organs which humans consume is a major concern causing either acute or chronic mycotoxicosis and may even cause death (Changwa et al. 2018; Njobeh et al. 2012).

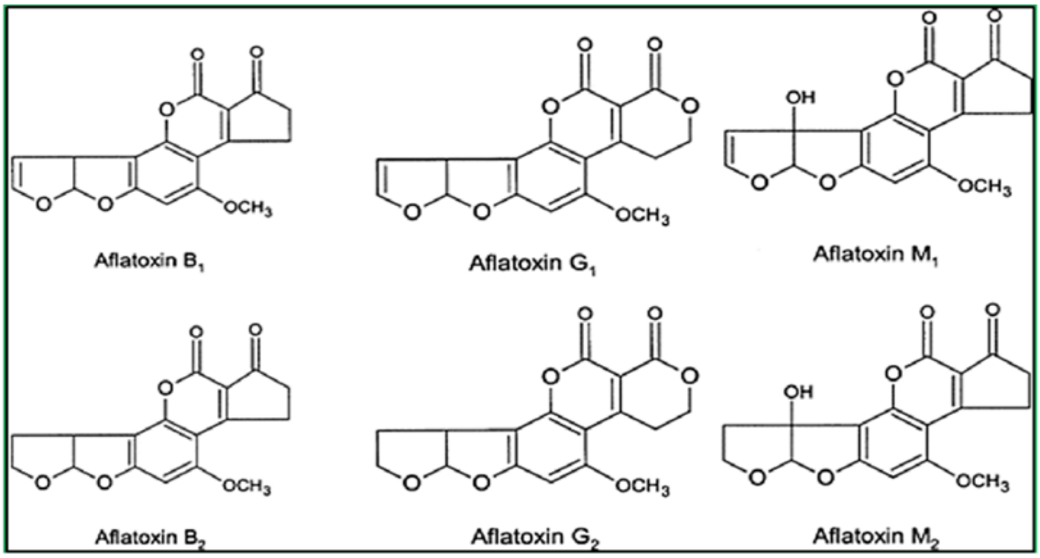
The well-known mycotoxins of significant agricultural importance and which have been found in feeds are fumonisins (FUM), zearalenone (ZEN), T-2 toxin (T-2), and deoxynivalenol (DON), produced by *Fusarium spp*, ochratoxin A (OTA), produced by Aspergillus and *Penicillium spp* and aflatoxin (AF), produced by *Aspergillus spp* (Changwa et al. 2018; Njobeh et al. 2012). Contamination of dairy feed with mycotoxins has been reported frequently in SSA posing a great danger to animal health and productivity (Kemboi et al. 2020). Because of their frequent occurrence in food and feed (Gruber-Dorninger *et al*., 2019) and high toxicity to animals and humans, AF and FUM have been extensively studied in SSA and has necessitated regulation in the majority of countries (Njobeh et al. 2012). According to Sirma *et al*. (2018), countries with mycotoxin problems, including most SSA countries, have relaxed enforcement of regulations, which may be because those countries set limits that are too high for them to carry out. Recent reports, however, have indicated the presence of other mycotoxins in dairy feed in South Africa, Kenya, Rwanda, Tanzania, Sudan, Ghana, and Nigeria (Changwa et al. 2018; Makau et al. 2016;

Nishimwe et al. 2019; Njobeh et al. 2012; Rodrigues, Handl, and Binder 2011). Since certain mycotoxigenic fungi can grow and produce mycotoxins in comparable environments, raw materials used to prepare compounded feed can be contaminated by multiple mycotoxins at the same time (Atherstone, 2016). Mycotoxins can have synergistic, additive, and antagonistic effects on one another, for instance, FUM has been observed to increase the absorption of AF and consequently the carryover to milk (Miazzo et al. 2005a).

2.2 Aflatoxins

Aspergillus and *Penicillium* are the main fungal classes that produce mycotoxins during storage. On the other hand, *Fusarium* produces toxins and contaminates crops in the field (Kemboi et al. 2020). The fungal genus *Aspergillus* contaminates grain during storage and produces a group of mycotoxins known as aflatoxins. Their optimal growth temperature of 25 ºC and water activity of 0.75 and above, but at 10-12 ºC, they can produce secondary metabolites (Lizárraga-Paulin and Martinez 2011). Aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) are among the well-known and naturally occurring types of aflatoxins (Strosnider et al. 2006).

The most prevalent aflatoxin is AFB1, which is liable for carcinogenicity, chronic toxicity, acute toxicity, immunotoxicity, and genotoxicity (Lizárraga-Paulin and Martinez 2011). Figure 2.1 shows the chemical structures of AFB1, AFB2, AFG1, AFG2, AFM1 and AFM2. A cyclopentenone ring fusion to lactone ring of the coumarin structure characterizes the B-toxins while an additional fused lactone ring is present in G-toxins, and at positions 8, 9 on terminal furam ring, the B and G toxins contain an unsaturated bond (Lizárraga-Paulin and Martinez 2011). The hepatic microsomal cytochrome P450 enzyme family converts ingested AFB1 and AFB2 into aflatoxin M1 and M2 respectively, which can be excreted in the milk of lactating animals (Strosnider et al. 2006). The high consumption of milk and dairy products by children makes the presence of AFM1 in milk extremely important. AFM1 levels in liquid milk, dried or processed milk products should not exceed 50 ng/kg, according to FAO guidelines (FAO 2011)



Source: (Musleh, Al-ouqaili, and Al-kubaisi 2017)

Figure 2.1: Chemical structures of aflatoxin B1, G1, B2, G2, M1 and M2

2.2.1 Prevalence of Aflatoxins in feed

Prevalence of aflatoxins is higher in tropical regions in latitudes between 40º N and 40º S of the Equator; also, the health risk is more in developing countries where most staple foods are affected by aflatoxins (Strosnider *et al* ., 2006). Among the staple foods affected by aflatoxins are cereals (wheat and maize) and their derivate, nuts, cassava, oilseeds, dried fruits, wines, legumes, milk and milk products (Wild and Gong 2009). Nuts and cereals, which are widely consumed and more prone to contamination, are significant sources of human exposure to aflatoxins (Lizárraga-Paulin and Martinez 2011). Aflatoxin exposure routes are through ingestion of contaminated food, breastfeeding, inhalation, and skin contact and they can have negative impacts on various organs (Gallo et al. 2015; Murugesan et al. 2015).

In 2004-2005 and 2010, Kenya had an aflatoxicosis outbreak in the rural eastern region due to the consumption of maize contaminated with aflatoxin (Kangethe et al. 2017; Probst et al. 2007; Strosnider et al. 2006). The 2004 outbreak indicated aflatoxin levels in patients with a range between 120 to 1200 pg/mg albumin, and they showed symptoms of liver dysfunction (Strosnider et al. 2006). The increased aflatoxin contamination has since 2004 been attributed to climate change (Obonyo &

Salano, 2018). Due to the high heat and humidity from the sparse rainfall, most of Kenya's agricultural products are now susceptible to aflatoxin contamination.

Lack of knowledge of proper feed storage conditions is a major challenge to farmers. Because of the warm and humid climate, which promotes the growth of *Aspergillus spp*, East African countries near the equator have a higher occurrence of AF than other mycotoxins (Kemboi et al. 2020). Gizachew *et al*. (2016) reported a 100% prevalence of AFB1 in compounded dairy feed in Ethiopia, brewer yeast, silage, maize, and pea hull, with all samples exceeding 0.5 µg/kg. In compounded dairy feed, Senerwa *et al.*, 2016 in Kenya reported a greater number of samples with AFB1 over WHO/FAO legal limit at 90.3% but noted variations across various agro-ecological zones.

The percentages of AF and AFB1 in moldy maize used as animal feed in Kenya and Tanzania were 56% and 29%, respectively (Dixon et al. 2021; Kang, Korhonen, et al. 2017); however, the means (3.84 µg/kg and 3.49 µg/kg, respectively) were under the regulatory limits set by the EAC and the EU. According to Rodrigues *et al*., (2011), compounded dairy feed and the raw materials used to make it had a 54% incidence of AF in Sudan. Similarly, Okoth and Kola. (2012) found that compounded dairy feed, cottonseed cake, and sunflower seed cake had 100% incidences of AF at levels ranging from 5.13 to 1123 ng/kg. In the study, 95 percent of the samples were over the permissible limit with cottonseed cake and compounded dairy feed having the highest percentages, at 51.2% and 41.9%, respectively; just 7% of the samples of sunflower seed cake were over the permissible limit. In Tanzania, 61.5% of sunflower-based dairy feed had AFB1 levels over the EAC and WHO/FAO legal limits, with a 65% total occurrence (Mohammed, Munissi, and Nyandoro 2016).

2.2.2 Factors enhancing aflatoxin contamination

It is approximated worldwide that a quarter of products from agriculture have aflatoxin contamination (Williams *et al* ., 2004; Wild and Gong, 2010). According to Wu et al., 2011 drought, extreme weather conditions and precipitation causes plant stress making them more vulnerable to fungal infection. Crops exposure to high moisture and temperatures leading to harvest and during storage of the crops facilitates growth of fungi and production of aflatoxin (Murugesan et al. 2015).

Aflatoxin exposure in developing countries is mainly because of conditions favoring aflatoxin production and failure to enforce existing regulatory limits for aflatoxin in feeds and agricultural products (Williams et al. 2004; Yard et al. 2013). The ideal temperature range for fungus growth is 36 to 39 °C, with a humidity level of at least 85% (Klich 2007). In the Eastern region of Kenya, the temperatures are high for most of the year and if cereals are not sufficiently dry, it could favor the growth of moulds during storage (Lewis et al. 2005). Various studies (Kangethe et al. 2017; Senerwa et al. 2016; Sirma et al. 2018b) have reported over 50% of feeds contaminated with AFB1 above the legal limit.

2.2.3 Prevalence of AFM1 in milk

Aflatoxin B1 (AFB1), has been reported to transfer to the milk of lactating cattle at significant levels that are of concern, this is crucial for global public health since children are the main consumers of milk. Upadhaya *et al*., (2009) reported AFB1 degradation to be 14% in cattle and 25% in goats rumen, and also the kind of feed affected the level of degradation. Similar to this, Jiang *et al*., (2012) reported that the kind of feed had an impact on the degree of AFB1 degradation and the rumen microbial community, with feeds containing cellulose, like roughages, degrading more quickly than those without. The difference between initially included AFB1 and residual AFB1 in the culture fluids without any produced metabolites tested for was used to calculate the AFB1 degradability rate (Ogunade et al. 2016; Rodrigues et al. 2011). The remaining AF is absorbed in the small intestines and most converted in the liver to AFM1, a significant metabolite which is excreted in urine and milk and is a class 1 human carcinogen (Rodrigues et al. 2011).

Less than 1% to 6.2% of AFB1 is carried over into milk (Britzi et al. 2013; Pei et al. 2009) depending on individual animal variability, animal species, feed type and feeding practices, actual milk production, the lactation stage, and presence of other mycotoxins (Britzi et al. 2013; van der Fels-Klerx and Camenzuli 2016; Miazzo et al. 2005b). According to a study by Hernandez-Camarillo *et al*., (2016); AFM2 a by-product of the hydroxylation of AFB2 was present in 20% (mean 0.2 µg/kg) cheese samples consumed in Mexico but it has been shown to poses less threat than AFM1. The same study reported AFM1 presence in 53% of the cheese (mean 3.0 µg/kg) thus, AFM1 is consequently of greater concern than AFM2 since AFB1 is more common in dairy feed than AFB2 is in SSA.

In Kenya, the presence of AFM1 in milk has been widely reported (Kiarie et al. 2016; Kuboka et al. 2019; J. Lindahl et al. 2018; Senerwa et al. 2016). Previous research in Kenya found AFM1 in a variety of dairy products at levels ranging from 34 to 370 ng/kg (Lindahl *et al*., 2018; Senerwa *et al*., 2016; Sirma *et al*., 2018.) and in low-income areas, 100% of the milk may be contaminated, putting poor children at risk (Ahlberg et al. 2018; Kiarie et al. 2016). Across the globe, over 60 countries have maximum permissible AFM1 level in milk (Iha *et al* ., 2011); however, the FAO/ WHO (FAO 2011) regulatory maximum AFM1 level for both powdered and liquid milk is 50 ng/kg. The AFM1 begins to appear in milk about 12-24 hours after AFB1 is ingested (Rahimi *et al* ., 2010). Sarımehmetoglu *et al*., (2004) report indicates that AFM1 has relatively high stability in processed and raw milk products.

The world requires more efficient use of resources to encourage better management practices in agricultural dairy farms. Incidences of AFM1 contamination in commercial milk are lower among many countries that regularly monitor AFM1 in milk; most of them found samples having AFMI levels higher than the acceptable level of 50 ng/kg (Dua et al. 2012). In most cases, contamination by AFM1 is higher in areas where cattle feed on high amounts of compound feed than in areas with broad pasture zones (Ramos and Ramad, 2003). AFB1 has been found in feeds at high levels, which is consistent with the high incidence of AFM1 in milk seen in East Africa.

AFM1 has been recorded in, Kenya Ethiopia, and Sudan at 100% incidence, with 66.4%, 91.8%, and 100% of the positive samples exceeding the regulatory limit of 50 ng/kg set by the EU (Ali, El Zubeir, and Fadel Elseed 2014; Gizachew et al. 2016; J. Lindahl et al. 2018). Other studies conducted in Kenya found that between 39.7% and 99% of milk samples contained AFM1, and between 10.4% and 64% of those samples exceeded the regulatory limit set by the EU of 50 ng/kg (Kagera *et al.*, 2019; Kirino *et al.*, 2016; J. Lindahl *et al.*, 2018) with the highest level reaching 6900 ng/kg, which is significantly higher than the EU and EAC limit. Tanzanian milk samples also had significant levels of AFM1, with 83.8% of all positive samples exceeding the legal limit set by the EU (Mohammed et al. 2016). AFM1 levels in imported milk powder in Sudan ranged from 10 to 850 ng/kg (Oluwafemi *et al.*, 2014) with 50% of samples exceeding EU regulatory limits and 33% exceeding CODEX and EAC regulatory limits of 500 ng/kg.

A high prevalence of AFM1 was found in raw milk and imported milk powder in Nigeria. Oyeyipo *et al.*, (2017) reported AF in repacked milk powder in five states in the South West region, Nigeria. Of the milk samples, 53.6% was contaminated with AFM1 but none exceeded the Nigerian regulatory limit of 500 ng/kg. However, the maximum level of 460 ng/kg was above the EU regulatory limit of 50 ng/kg. Interestingly, very high levels of AFB1, above the Nigerian and EU regulatory limit of 500 ng/kg, were reported in milk (29700–79400 ng/kg) and this can be explained by the frequent presence of *Aspergillus* species that were found contaminating the milk due to the open-air repackaging of the milk powder. In another study on raw milk from free-grazing cows in Abeokuta, Nigeria, Oluwafemi *et al*., (2014) reported a 75% occurrence of AFM1 with 64% exceeding the EU limit. High levels of AFM1 occur in milk in South Africa. Dutton *et al.*, (2012) reported a 100% incidence of AFM1 in milk from dairy farms, ranging from 20 ng/kg to 1500 ng/kg. Retail milk was also contaminated with AFM1, at levels of 10–3100 ng/kg. Similarly, Mulunda *et al*., (2016) in a study carried out in selected rural areas of Limpopo Province in South Africa reported 100% AFM1 occurrence with 90.6% and 62.1% of the positive samples above South Africa and EU regulatory limit of 50 ng/kg in Mapete and Nwanedi area, respectively (Mulunda *et al*., 2016).

2.2.4 Mitigation of aflatoxin occurrence in feeds

Various strategies have been devised to lessen the effects of mycotoxins because of the detrimental health and financial implications they have on the dairy sector as well as their relative stability to manufacturing procedures. Dairy farmers in SSA have been reported to have low awareness of mycotoxins, and little is being done to disseminate information on appropriate control strategies (Stepman 2018). In a study of urban areas in Kenya, Kirino *et al*. (2016) found that while 58% of milk traders were aware of AF, only a small percentage were aware of AF carry-over to milk, and farmers also reported feeding moldy maize to animals (Kangethe, *et al*., 2017) Similarly, Kangethe *et al*., (2009) found that 42% of people were aware of AF. In Benin, Ghana, and Togo, James *et al*., (2007) found that 20.8% of farmers, 26.7% of traders, 60% of poultry producers, and 25.2% of consumers were aware of AF. Changwa *et al*. (2018) showed that between 17% and 92% of people in South Africa were generally aware of mycotoxins. The consequences of AF and FUM were unknown to 92.4% of livestock producers and animal feed suppliers in Rwanda (Nishimwe et al. 2019). Additionally, Ethiopia and Tanzania have also reported having low levels of awareness (Stepman 2018). Thus the application of different mitigation methods can be hampered by this low degree of awareness.

These mitigation strategies can be broken down into two categories: pre-harvest, which aims to stop fungal contamination in the field, and post-harvest, aimed at preventing contamination and reducing or eliminating mycotoxin contamination during harvesting, processing, or storage (Hell, Mutegi, and Fondohan 2010). Since contamination prevention is the preferred approach, pre-harvest mitigation strategies are crucial but because this is not always enough in SSA, post-contamination options are also required (Kemboi et al. 2020). After harvesting, post-harvest strategies are implemented. After harvesting, moisture content is quickly reduced, which is crucial for halting the growth of fungi and the formation of mycotoxins. Cereals are thought to be safe at moisture levels between 10% and 13%. However, because of the high temperatures and humidity in the majority of SSA nations, effective drying and storing is frequently a problem (Hell et al. 2010). To reduce microbial contamination and the development of mycotoxin, feed must be stored in a dry environment with low humidity, appropriate aeration, and free from pests and rodents (Hell et al. 2010).

The tropical environment of SSA encourages the growth of mycotoxin-producing fungi and exacerbates food instability, which leads to actions like diverting moldy grains for use as animal feed. The majority of dairy feeds are typically purchased, harvested, and stored; nevertheless, the feed quality can be affected as a result of potentially adverse storage circumstances (Govinden and Odhav 2008). Decontamination is therefore the greatest method for preventing mycotoxins in the dairy supply chain (Mutua *et al.*, 2019; Kangethe, *et al.*, 2017). To remove the mycotoxin or to lower the bioavailability of the toxin, decontamination is administered to the previously contaminated diet (Strosnider et al. 2006). To decontaminate feed from mycotoxins, biological, physical and chemical methods have been widely used (Bhat et al. 2010; Xiong et al. 2013) Decontamination substances are known as detoxifiers, and they are classified as binders, which prevent absorption of mycotoxins from being absorbed in the gut system, and modifiers, which cause mycotoxins to be converted in the intestines into less hazardous metabolites.mycotoxins in the intestines into less toxic metabolites (Kemboi et al. 2020). Modifiers typically contain microbes and enzymes, whereas binders typically contain clay minerals or yeast products (De Mil et al. 2015).

2.5 Aflatoxin mitigation in milk

To reduce AFM1 contamination, various methodologies have been developed, with both direct and indirect approaches thoroughly reviewed (Jard et al. 2011). In the milk value chain, there are several mycotoxin-mitigation strategies, including trials involving good agricultural practices in pre-harvest and post-harvest management of feed ingredient crops, feed decontamination via dilution, chemical, physical and biological treatments but their success has been limited (Karlovsky et al. 2016; Kuboka et al. 2019). Sorting, de-hulling, ozone fumigation, thermal inactivation, and irradiation are common post-harvest mycotoxin decontamination strategies; however, those intended for rural smallholder farmers should be simple, practical, safe, effective, and affordable (Atherstone *et al.*, 2016; Mahuku *et al.*, 2019; Udomkun *et* *al*., 2017). Adding mycotoxin binders (clay-based enterosorbents) to the dairy animal diet may reduce AFB1 uptake in the animal body, resulting in less carry-over in milk (Diaz et al. 2004). When used and consumed by an animal, the binders remove mycotoxins in the feed by binding to them and preventing the animal's digestive system from absorbing them (Whitlow 2006). When clay enterosorbents were included in the diets of lactating dairy cattle and goats fed AFB1-contaminated feed, the concentration of AFM1 in milk was decreased significantly (Phillips *et al*., 2008).

According to studies, adding activated carbon (AC) and hydrated sodium calcium aluminosilicates (HSCAS) to AFB1-contaminated feed at a rate of 2% decreased the amount of AFB1 that transferred to milk as AFM1 by 50% and 36%, respectively (Galvano et al. 2001). In a study comparing the effects of AC, esterified galactomannan, calcium bentonite, and three HSCAS products, milk AFM1 concentrations were reduced by 5.4%, 59%, 31%, 65%, 50%, and 61%, respectively (Diaz et al. 2004). The AFM1 content in milk was decreased by 45%, 48%, and 4%, respectively, by adding two commercial HSCAS products, Novasil Plus® and Solis®, or an esterified galactomannan product (MTB-100) at 0.5% to dairy cows' diets (Kutz et al. 2009). Recently, researchers looked into the ability of saponite-rich bentonite to reduce AFM1 contamination in milk. The detoxification capacity of the bentonites used was effective, reducing contamination to below the European standard limits for AFM1 (50 ng/kg) with only minor changes to the nutritional properties of the milk. Bentonite residues found in milk (0.4%) posed no risk to human health (Carraro et al. 2014). Novasil has shown a higher specificity and efficacy for binding aflatoxins than other mycotoxins (Phillips *et al*., 2019).

Kuboka *et al*., 2022 conducted a study in Kenya that demonstrated the potential of adding Novasil aflatoxin binder to feeds to reduce aflatoxin contamination in milk with reduction rates of 34% and 45% with binder at 0.6% and 1.2% (w/w), respectively. In Kenya, no specific standards govern the use of mycotoxin binders, which are imported as feed additives but are not required (Mutua et al. 2019). The same study reported eight mycotoxin binders used/marketed, with the majority having a 25kg package and two having a 1 and 5kg minimum package. The study also discovered that while binders were not commonly used, most feed makers claimed to do so when they thought the raw ingredients posed a high danger. Providing Novasil to smallholder dairy farmers may have a widespread impact on AFM1 in milk sold, according to a field trial in Kenya (Anyango et al. 2021).

2.6 Conceptual framework

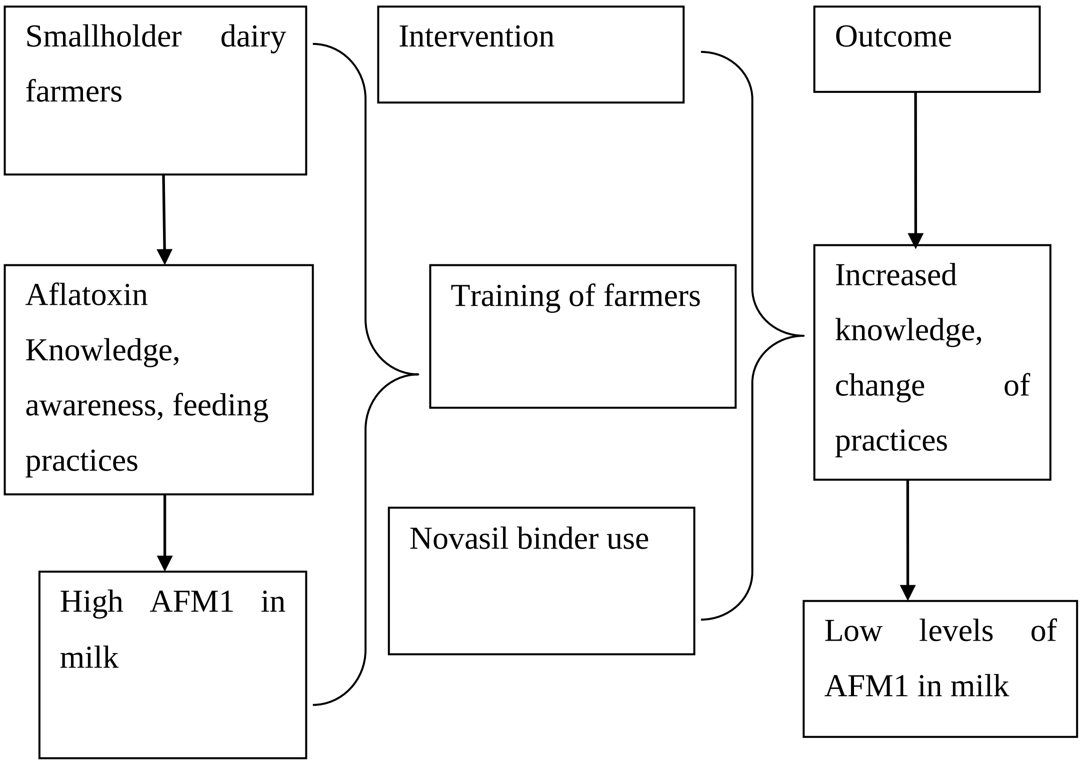


Figure 2.2: Conceptual framework

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was conducted in Kasarani sub-county, which was purposively selected since it has urban and peri-urban areas with intensive zero-grazing smallholder farms as reported by the Ministry of Agriculture, Livestock and Fisheries. Kasarani sub-county has five administrative wards, namely Kasarani, Mwiki, Clay city, Njiru, and Ruai, all included in the study (Figure 3.1: Map of Kenya and Kasarani sub-county).

(Adopted from IEBC, 2016)

Figure 3.1: Map of Kenya and Kasarani sub-county

3.2 Study Design

This study adopted a quasi-experimental design, including a baseline survey, trial period (time series) and an endline survey. The baseline survey was conducted in the month of April 2017 which is within the long rainy season, the trials were done between June and September 2017 while endline was in February 2018 which is mostly dry season. A comprehensive list of dairy farms in Kasarani sub-county was provided by the veterinary and livestock office and this formed the sampling frame. A simple random selection was done to identify one hundred eligible farms for the baseline survey.

After baseline survey analysis of milk for AFM1, thirty of the farms identified as producing milk with AFM1 levels above 20ppt were randomly selected to either receive training and NovaSil® binder (20 farmers; intervention) or to receive no intervention (10 farmers; control). The toxin binder was to be mixed with concentrates at two spoonfuls per two kilogram feeds. In the initial farm visit, farmers were provided with more details related to the study (frequency of visits, sampling of milk), There were fortnight visits to each intervention and control farms to collect milk samples to determine the presence of AFM1. Summary results were discussed during these fortnight visits and feedback provided to the farmers in the subsequent visit.

In the control farms, farmers were visited twice, during the baseline survey for sample collection and at the end of the study for additional sampling. Their selection was carefully done to minimize the risk of spill over from farms receiving the intervention. An evaluation was done four months after the stop of intervention to follow up on knowledge, awareness and practices regarding aflatoxin contamination as well as collecting milk samples for AFM1 analysis.

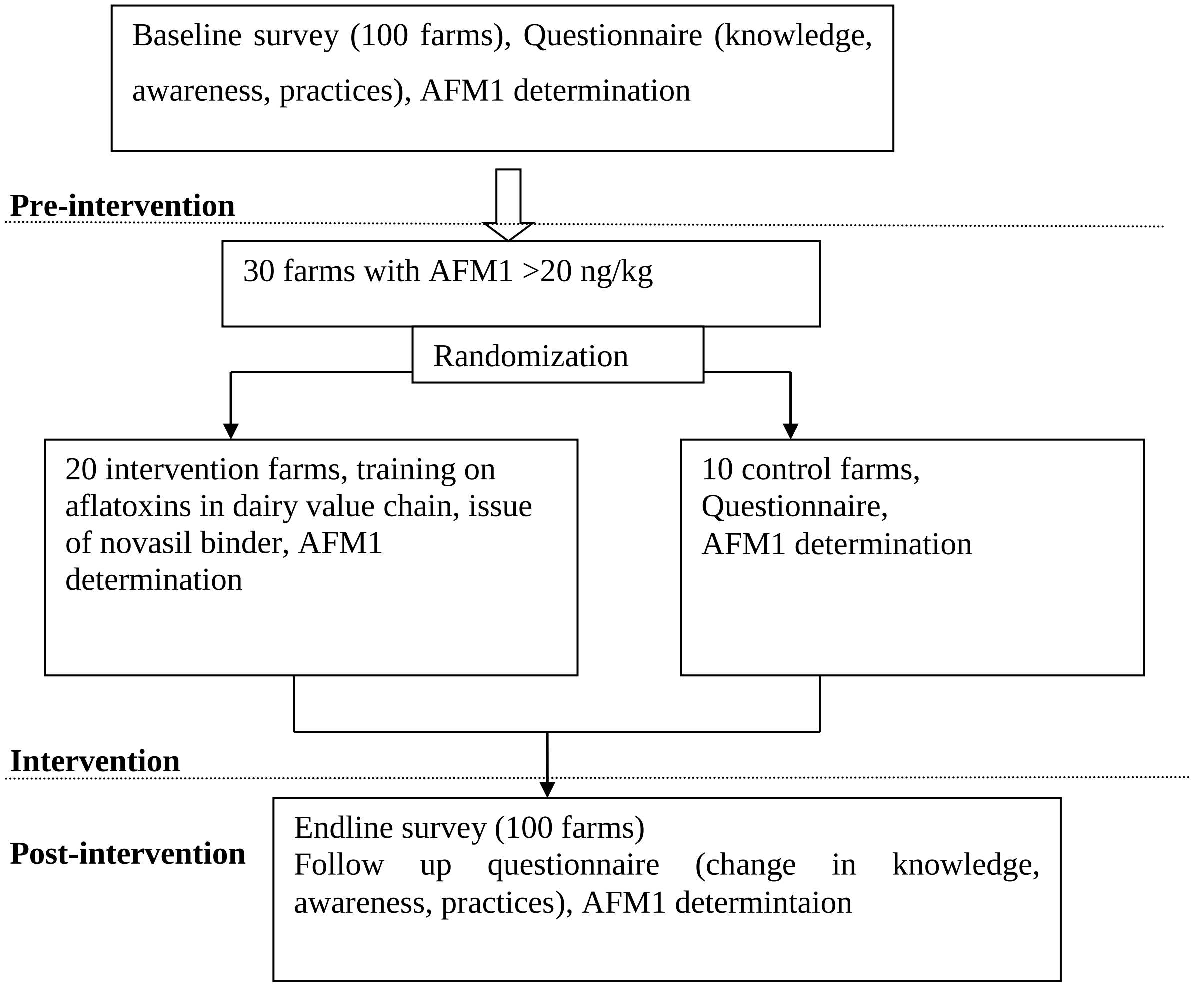


Figure 3.2: study design

3.3 Sample Size Determination of Farmers to Participate in the Baseline Survey

An average prevalence of 50% aflatoxin in milk reported in a study was used with defined precision of 10% precision and 95% confidence level (Gizachew et al. 2016). The approximate sample size was determined using the method described by (Naing et al., 2006; Audigé, 2005; Dohoo *et al*. 2003). Therefore the calculated sample size was 96 dairy farms. In addition, the number of farms were increased by 10% to accommodate sampling losses and refusal, finally 100 dairy farms were included in the study.

$n = z^2 P(1-P) / d^2$

Where

n- Sample size with infinite population correction

Z- Z score at 95% level of significance confidence (1.96)

P- Estimated prevalence of 50%

d^2 - Precision 10%

$1.96^2 x 0.5(1-0.5) / 0.1^2 = 96$

3.4 Sample Size Determination for the Intervention Study

Sample size was determined using the formula proposed by Metcalfe (2001); STATA sampsi 0.5 0.1, p(0.5) r(2) control farms with a hypothesized reduction of the percentage of farms above the EU limit from 50% to 10%. Using a 2-sample 2-sided binomial test to compare these at the 5% level of significance with 50% power requires 30 treatment farms and 15 control farms (using 2:1 ratio), adding some for drop-outs. Farmers whose milk had AFM1 levels above 20 ng/kg were used as a sampling frame. Due to resource constrains, 30 farms were included in the study intervention.

3.5 Inclusion and Exclusion Criteria

3.5.1 Inclusion Criteria for Intervention Farms

To participate in the study, farmers should have met the following criteria:

i. Must have commercial milk production so that they are not only having milk for their household only.
ii. Must be a small- scale farm with less than 20 milking animals.
iii. Must have at least one lactating cow, and likely to have it the whole study period.
iv. The cows must be fed on concentrates.
v. The cows should be intensively managed , no access to pasture grazing.
vi. Consent must be granted by the farm owner.
vii. Farmers willingness to be trained and use the binder provided.

viii. The milk should contain aflatoxins at baseline analysis.

3.5.2 Exclusion Criteria

i. Eligible farms owned by an institution.
ii. Eligible farms whose owners don't reside at the farm.

3.6 Sampling procedure for the Intervention study

Simple random sampling was used to select 20 intervention and 10 control farms from those whose milk contained AFM1 levels above 20 ng/kg. The control farms were selected carefully to minimize the risk of spillover from farms receiving the intervention. The administration of the binders was pre-tested in the International Livestock Research Institute (ILRI) animal facility before being applied in the field.

3.7 Data collection

3.7.1Baseline survey

Data from the dairy farmers were collected on paper questionnaires administered through face-to-face interviews with the farmers. The questions were designed in English and explained to the farmers either in English or Kiswahili depending on preferred language. Pre-testing of the questionnaire was done on five farms in Kasarani a week before the start of the baseline survey to check if the questions were clear to the farmer to give answers that would answer the research questions fully. The respondents were either household heads, spouses, children or farm workers who had knowledge or were involved with herd management. The questionnaire comprised of demographic questions, herd composition, feeding practices (types of feeds, source, amount of compounded feeds given, routine monitoring of feed storage conditions) daily milk production (how much is produced, consumed within household, sold). Some of the questions were designed to judge farmers' knowledge and awareness of aflatoxins transfer to milk and their impacts on animal and human health. their sources and effects on animal and human health. The respondents' awareness was determined by whether they had heard about aflatoxins, while knowledge was taken as the correct information regarding aflatoxins, Different questions were also asked regarding presence and effects of mouldy feeds on animal health and diseases observed by the farmers.(Appendix II).

3.7.2 Intervention study

Fortnight visits were made to the farms to collect milk samples for AFM1 analysis and follow-up questions were asked to check on binder use status and any effect of using binders on cow behaviour (Appendix III). One month after the end of the intervention period, the control group was given the same intervention package as the intervention group, i.e., novasil binder, to last three months and a one-day training on aflatoxins occurrence and their effects on animal and human health.This was done to compensate for farmers time taken during the study period.

3.7.3 Endline survey

An endline survey was conducted four months after the intervention period to evaluate if the intervention effect remained among the trained group and if there was any change of knowledge and practices among the farmers regarding aflatoxins control (Appendix IV). Milk samples were also collected to analyze for AFM1 levels.

3.8 Milk collection and analysis

3.8.1 Collection of milk samples

Representative duplicate milk samples from each farm were aseptically collected into 50 ml sterile plastic falcon tube and placed in a cool box containing ice packs to keep the milk cool during transportation to the laboratory at International Livestock Research Institute (ILRI), where it was stored in a freezer at −20 degrees celsius awaiting analysis. The milk sample intended to be collected was fresh milk which was milked in the morning or at the time of the visit.

3.8.2 Aflatoxin M1 analysis

Qualitative and quantitative data on AFM1 was collected through laboratory analysis of the milk using the Aflatoxin M1 Low Matrix ELISA kit (Helica Biosystem Inc., San Diego. USA) following the manufacturer's instructions. According to the manufacturer, the ELISA kit used had a lower detection limit of 2 ng/kg AFM1 concentration which was assumed to be accurate. Milk samples exceeding the highest standard of 100 ng/kg were diluted using the aflatoxin-free skim milk provided in the kit and retested in duplicates. This method has been previously described (Kagera et al. 2019; Kiarie et al. 2016; Senerwa et al. 2016). This kit's accuracy testing was previously done by Tadesse *et al.*, (2020) through spiking of samples which showed the recovery rates ranging between 70-156% with negative samples not exceeding 4 ng/kg.

The milk samples were thawed at room temperature before use. A 2 ml aliquot of each milk sample was centrifuged at 2000 rpm for 5 minutes to allow separation of the upper fatty layer, and the lower plasma layer of the milk was used in the assay. Before use, all reagents provided in the kit were kept at room temperature for 30 minutes; aliquots of 200 µl of each standard and samples were dispensed into AFM1 antibodies pre-coated plates in duplicates. The microplate was covered to avoid evaporation and protect it from excess UV light and incubated for 2 hours at room temperature. The liquid contents of the wells were discarded into a sink, and the wells were washed three times using the reconstituted wash buffer. Thereafter, the wells were tapped face down on absorbent paper to remove residual wash buffer. One hundred microlitres of the conjugate was added to each well, and the plate was covered and incubated for 15 minutes at room temperature. Another washing with reconstituted wash buffer was done three times, and 100 µl of enzyme-substrate was added to each well. The microplate was covered to avoid direct light and incubated for 15 minutes. The reaction was stopped by adding 100 µl stop solution, which caused the blue colour of the well's contents to turn yellow. Each microwell's optical density (OD) was read using a microplate reader at 450 nm using an air blank. The AFM1 concentration in each well was calculated using a logarithmic standard curve (requiring an R^2 value of above 95%), and the average of the duplicates was used as results.

3.9 Measurement of variables

Demographic and farm characteristics were measured and presented as percentages. The proportions of farmers whose milk samples were contaminated with AFM1 above the recommended limits were determined by the frequencies of milk samples collected and presented as percentages of farmers with contaminated milk. Feeding practices of farmers were measured in percentage of farmers feeding their cattle on various feeds. This was then compared before and after intervention to observe if there was any change. Levels of AFM1 were determined by ELISA where counts were obtained in nanogram per kilogram for each milk sample collected.

3.10 Data analysis and presentation

Data analysis was performed using STATA (version 14.0; StataCorp, TX, USA). Various variables data were presented as means for continuous variables or proportions for categorical variables. Chi-square ($\chi 2$), *t* tests and analysis of variance (ANOVA) were used to compare means, proportion of categorical variables and to examine differences in the responses from farmers. A p-value level of at least 0.05 was used in the assessment of statistical significance.

3.11 Ethical considerations

All procedures and protocols used in the study were reviewed and approved by the JKUAT Board of Postgraduate Studies (BPS) and ILRI's Ethical Review Committee in Kenya, approval number 2017-10. To adhere to the ethical principles, written and verbal consent was sought from the local administration, county livestock stakeholders, and the participating farmers. The farmers were informed of the purpose of the research study, potential benefits and their ability to participate or withdraw from the study at any time they wished. Before the beginning of the interviews, the farmers were issued with an informed consent form to read through and understand the aim of the research and sign (Appendix). A copy of the consent form together with the information about the intended research was left with the farmers. There was no payment made to the participants, thus participation was on a voluntary free-will basis. All personal information collected was confidential and solely used for research purposes by the research team. The names of the farmers were coded so that no one could trace the information to the farmers. The benefits of the study included educating farmers on aflatoxin occurrence, mitigation, its negative effects on humans and animals and provision of binder to mix in the animal feeds. The principal investigator stored, cleaned and analyzed data and only shared it with the supervisors. Ethical principles were emphasized and maintained at all stages of the study.

CHAPTER FOUR

RESULTS

4.1 Farmers' awareness, knowledge and practices on aflatoxins

4.1.1 Respondents' characteristics

The respondents' ages ranged from 20 to 75 years with an average age of 50. The educational level of the respondents varied from no education (3%) to the highest, with 29% having attended higher education. More female respondents (18%) completed secondary school education than male respondents (11%). The male respondents who completed only upper primary were more (16%) than their female counterparts (10%) (Figure 4.1).

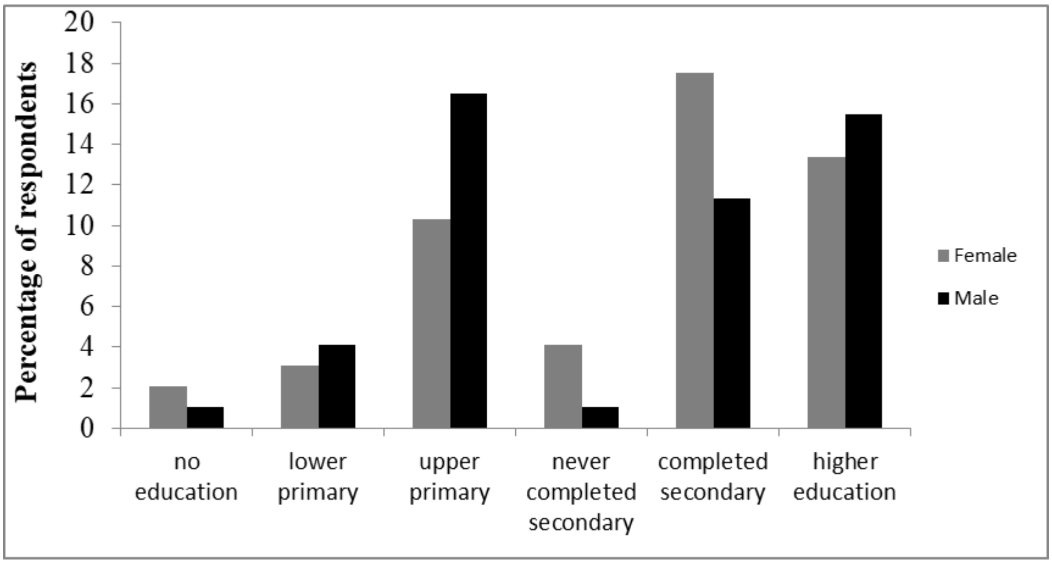


Figure 4.1: Education level of female and male respondents in Kasarani, Kenya

4.1.2 Farms characteristics

The total number of farms surveyed was 100, corresponding to a response rate of 100%. The number of cattle owned in the interviewed farms ranged between 1 and 60 animals, with an average of 6 (sd 7) animals per farm. On average, 47.50% of the owned animals were lactating and milked at the time of the interview. The number of milked cows per farm ranged between 1 and 18, with an average of 3 cows. Most

(72%; n=100) dairy farmers reared exotic breeds, and a few kept crossbreeds (25%) and locals (3%). The exotic breeds included Holstein-Friesians, Guernsey, Jerseys, Ayrshire, and Fleckvieh. Other livestock found were goats, sheep, poultry, pigs, and donkeys.

Depending on milk production, farmers milked their cows twice or thrice daily. The average daily milk production per household was 27 litres, with an average selling price of 64 Kenyan shillings per litre. 4.1.3 Farmers' knowledge and awareness about aflatoxin.

The respondents' awareness was determined by whether they had heard about aflatoxins, while knowledge was taken as the correct information regarding aflatoxins, e.g. their occurrence and effects on human and animal health. Overall, 80% of the respondent said they had heard of aflatoxin, of which 52% were women and 48% were men. Most respondents (55%) gave the correct information regarding aflatoxin, 45% of whom were men and 55% were women. Overall, 58% of the respondents said that aflatoxin in some food and feed types poses a danger to humans, of which 41.38% were male and 58.62% were women. According to the respondents, the food and feed types likely to be contaminated with aflatoxin included maize (68%), concentrates (36%), fodder and forage (35%), while 15% mentioned cereals in general, grains and flour (Figure 4.2).

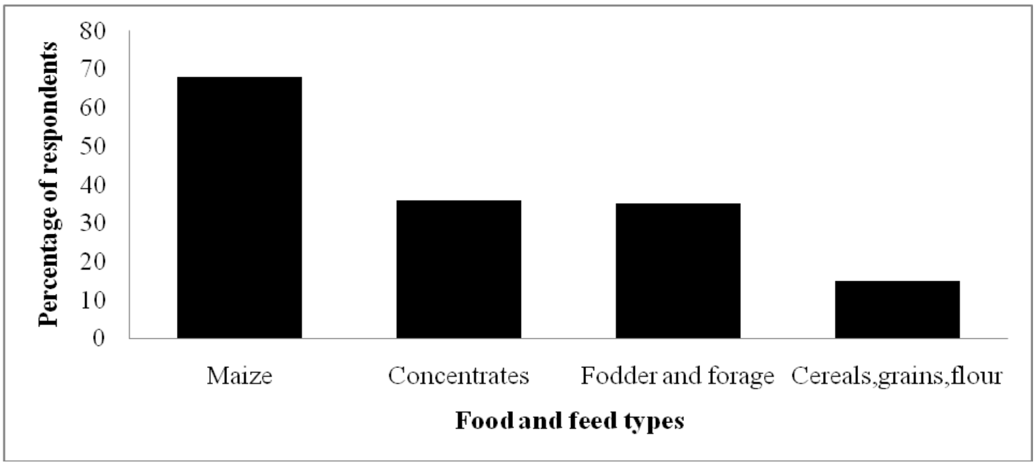


Figure 4.2: Percentage of dairy farmers in Kasarani identifying food and feed products prone to aflatoxin contamination.

4.1.4 Feeding practices

Feeding practices among farmers were studied and data is presented in Table 4.1. Most of the respondents (89%) reported that they supplemented their animals' diet with commercial concentrates such as dairy meal, wheat bran and maize germ. The management systems of the animals varied across farms, with the majority practicing zero grazing (93%) and only 2% practiced pasture grazing. Farmers who practiced pasture grazing also supplemented feed for their animals with commercial or compounded feeds. The feed types provided to the cattle and their sources varied across the farms, and these included concentrates (99%), cut-carry pasture (97.9%), hay (98.8%), and silage (56.3%). The commonly purchased feed type was concentrates (92.6%), while cut-carry pasture was obtained mainly from own farms (52.8%). The different feed types used in the households were stored either on concrete floors or on raised surfaces of which more than 50% of the hay (53%), cut-carry pasture (60%), and concentrates (60%) were reported to be stored on raised surfaces.

Table 4.1: Smallholder dairy farmers feeding practices in Kasarani, Kenya, and how the feed is sourced and stored.

Feed type	%Households using	Origin of the product			
		% On-farm formulation	% Purchased	% stored on the floor	% stored on raised surface
Hay	98.78	11.54	88.46	22.06	77.94
Cut-carry pasture	97.87	52.81	47.19	20	80
Concentrates	98.98	7.37	92.63	27.71	72.29
Silage	56.25	100	0	66.67	33.33

4.1.5 Aflatoxin M1 concentrations in relation to awareness

During the baseline survey, 84 milk samples were collected from all the five wards. The overall mean concentration of AFM1 in the cow milk from Kasarani sub-county was 83.66 ng/kg (64.68) with a maximum level of 255.96 ng/kg which was within the Kenyan standard of 500 ng/kg. Overall, 64% of the milk samples exceeded the FAO/WHO limit of 50 ng/kg with a mean of 120.65 ng/kg.

Though a majority of the respondents (64%) were aware of the effects of aflatoxin, milk samples from their farms were more contaminated (average level of 90.35 ng/kg) compared to those from farms where respondents were not aware of aflatoxin effects (average level of 71.62 ng/kg). However, awareness was not significantly associated. ($p=0.12$) with AFM1 levels (Table 4.2)

Table 4.2: Aflatoxin M1 contamination in milk and the level of respondent awareness in Kasarani, Kenya

	Status	Number of samples	Mean AFM1 (ng/kg)	Standard deviation	Min	Max	Median
Overall	Not aware	30	71.62	61.45	2.12	176.76	59.70
	Aware	54	90.35	66.02	<LOD	255.96	88.36
	Total	**84**	**83.66**	**64.68**	**<LOD**	**255.96**	**80.17**
<50 ng/kg	Not aware	14	16.76	17.03	2.12	48.91	9.94
	Aware	16	17.36	17.18	<LOD	49.22	9.09
	Total	**30**	**17.08**	**16.81**	**<LOD**	**49.22**	**9.55**
>=50 ng/kg	Not aware	16	119.63	42.22	58.76	176.76	121.24
	Aware	38	121.08	53.38	50.46	255.96	104.82
	Total	**54**	**120.65**	**49.95**	**50.46**	**255.96**	**107.52**

4.2 Effect of training and adding aflatoxin binders in feeds on aflatoxin M1 levels in milk

Overall, the milk production decreased during the intervention period while AFM1 levels decreased significantly ($p < 0.001$) between baseline and last visit intervention period (Table 4.3).

Table 4.3: Mean milk production (L± standard deviation) /farm and aflatoxin levels (ng/kg ± standard deviation) in milk.

	Control			**Intervention**		
Visit Number	**N**	**Average milk yield**	**AFM1 (ng/kg)**	**N**	**Average milk yield**	**AFM1 (ng/kg)**
Baseline	10	35.50 ± 30.83	90.87 ± 39.34	20	56.61 ± 56. 71	132.86 ± 59.97
1	8	32.63 ± 26.49	98.32 ±52.14	20	38.35 ± 42.19	82.14 ± 54.72 ***
2	10	26.30 ± 25.66	75.13 ± 46.65	20	39.38 ± 36.40	97.95 ± 73.09 **
3	10	24.60 ± 26.20	81.87 ± 51.86	20	38.54 ± 38.16	81.48 ± 66.69 ***
4	9	26.61 ± 24.67	68.22 ± 81.21	20	39.35 ± 37.78	101.52 ± 83.04 **
5	8	27.63 ± 25.75	97.52 ± 94.67	20	32.5 ± 30.00	88.09 ± 92.64 ***
6	8	28.38 ± 26.18	81.66 ± 70.08	20	32.08 ± 25.02	59.93 ± 56.82 ***

Note: Asterisks **, *** Significant difference between the baseline and each visit at $p < 0.01$, $p < 0.001$ respectively, using the test on log (AFM1)

During the trial period, there was a significant difference ($p<0.01$) in the AFM1 levels between the intervention period and the baseline for the intervention group. In contrast, there was no significant difference (p=0.75) in the control group (Table 4.4).

Table 4.4: Comparison of mean aflatoxin levels (ng/kg ± standard deviation) during baseline and trial period

Description	Mean AFM1 levels at baseline	Mean AFM1 levels during the trial	Mean difference(AFM1)	t	p-value
Intervention group	132.21± 59.32	59.93± 56.82	72.28	3.94	0.0003
Control group	90.87± 39.34	81.66± 70.08	9.21	0.33	0.7463

4.3 Effect of on-farm mitigations on farmers' knowledge, awareness and practices and AFM1 levels in milk?

4.3.1 Farmers' feedback on the use of Novasil binder

The use of binders twice a day was reported in all the intervention farms, corresponding to the number of times they fed the cows with concentrate feeds each day. All the farmers in the intervention group (100%) reported that they used the binder, that it was easy to use the binder, that it was easy to know how much binder to mix with feeds using the spoon provided during the study, and the cows fed well on the feeds mixed with binder. Following the instructions during training, most farmers did not share their binder portions with others. Cows in the intervention farms were reported to eat better (75% versus 29%), they were perceived to be healthier (74% versus 35%) and had better milk production (44% versus 27%) as compared to those in the control group (Table 4.5).

Table 4.5: Farmers feedback on their cows during the intervention period.

		Intervention	Control
Feeding of cows ***	Better	90 (75.7 %)	14 (29.2%)
	Same	23 (19.3%)	31 (64.6%)
	Worse	6 (5.0%)	3 (6.25%)
Health of cows ***	Better	89 (74.8%)	17 (35.4%)
	Same	25 (21.0%)	29 (60.4%)
	Worse	5 (4.2%)	2 (4.2%)
Milk yield ***	Better	53 (44.5%)	13 (27.1%)
	Same	19 (16%)	18 (37.5%)
	Worse	47 (39.5%)	17 (35.4%)

Note: Asterisk *** $P < 0.001$ in Chi^2 test. Data are presented as absolute numbers and percentages of total respondents to the questions.

4.3.2 Respondents Knowledge and Awareness on aflatoxins

Results of pre- and post-intervention studies to understand effects on farmers' knowledge, awareness and practices on moulds and aflatoxins are presented in Table 4.6. Wherever data was obtained with respect to comparison between control and intervention groups at baseline and endline survey, measures were taken to understand the role of intervention on the selected effectiveness indicators. Farmers' awareness of aflatoxin was high and proximately close during baseline and endline at 85.71 and 88.17%. However, the knowledge of aflatoxins at baseline was low (37.5%), with an increase (85.37%) during the endline survey. For both baseline and endline surveys respectively, the effects of aflatoxin in humans reported were as follows: cause disease 44 and 48%, death 21 and 20%, gastrointestinal problems 25 and 26%, stunting in children 1%, and cancer 6%. The foods and feeds reported to be prone to aflatoxin contamination during the baseline and endline surveys respectively were: maize 85 and 65%, cereals 19 and 23%, milk 1%, concentrates 45 and 44%, and fodder and forages 44 and 27%. In comparison to the baseline results, there was an increase in knowledge about the impact of moulds on cattle: cause disease (50 versus 35%), reduce milk production (15 versus 5.4%), and cause stomach upset (25 versus 19%) after the intervention .

4.3.3 Practices for aflatoxin mitigation

Data on feed monitoring during storage and possible actions on mouldy feeds was taken only in the endline survey. During the endline survey, it was found that in overall, most respondents (94.62%) acknowledged they monitor feed conditions during storage, despite being in close proximity (95.24%) to the control group. Moulds growth (95.45%), moisture content (15.91%), pests (39.77%), store ventilation (5.68%), and foreign substances (1.14%) were among the feed storage aspects reported to be monitored. The most common actions reported for mouldy feeds were disposal (87.10%), with the majority of respondents (91.67%) being from the control group, feeding animals (7.53%), and only the control group reported mulching (2.15%). During the baseline and endline surveys, 37 and 43% of respondents reported seeing moulds on their farms, respectively (Table 4.7).

Table 4.6: Percentages of respondents knowledge and awareness about aflatoxins pre and post intervention

	Baseline survey			Endline survey		
Attribute	**Control %**	**Interve-ntion %**	**Overall %**	**Control %**	**Intervention %**	**Overall %**
Heard of aflatoxins	75.71	90	80	85.71	93.33	88.17
Right information on aflatoxins	45.28	22.22	37.5	83.33	89.29	85.37
Impact of aflatoxins on humans						
Cause disease	41.51	48.15	43.75	44.44	53.57	47.56
Death	18.87	25.93	21.25	18.52	21.43	19.51
Gastrointestinal problems	26.42	22.22	25	24.07	28.57	25.61
Stunting in children	1.87	0	1.25	0	3.57	1.22
Cancer	7.55	3.7	6.25	7.41	3.57	6.10
Foods and feeds highly susceptible to aflatoxin contamination						
Maize	84.91	85.19	85	62.96	67.86	64.63
Concentrates	45.28	44.44	45	38.89	53.57	43.90
Fodder And Forage	37.73	55.56	43.75	25.93	28.57	26.83
Cereals, Flour, Grains	24.53	7.41	18.75	24.07	21.43	23.17
Seen mould	37.14	36.67	37	46.03	36.67	43.01
Impact of moulds to cattle						
Disease	38.46	10	35.1	51	45.45	50
Milk Reduction	7.69	0	5.4	13.79	18.18	15
Stomach Upset/Diarrhoea	15.38	10	18.9	24.14	27.27	25
Death	3.85	6.67	8.1	3.45	18.18	7.50

Note: Data presented as percentages of total respondents to the questions.

Table 4.7: Percentages of respondents response on practices about moulds and aflatoxins

Attribute	**Control %**	**Intervention %**	**Overall %**
Monitor feed storage conditions	95.24	93.33	94.62
Conditions monitored during storage			
Moulds growth	96.67	92.86	95.45
Moisture/dryness	15	17.86	15.91
Pests	43.33	30	39.77
Ventilation	5	6.67	5.68
Foreign substance	0	3.33	1.14
Action if feeds are mouldy			
Dispose	91.67	86.67	87.10
Feed animals	6.67	10	7.53
Mulching	3.33	0	2.15

4.3.3 AFM1 concentration

During the endline survey, 77 milk samples were collected. The overall mean AFM1 level in the milk was 201.56 ng/kg, with 185.30 (±163.39) ng/kg for the control group and 230.01 (±276.88) ng/kg for the intervention group. There was no significant difference (p = 0.13) in AFM1 levels in milk from intervention farms at endline and baseline. There was a significant difference (p = 0.03) in AFM1 levels in milk between the endline and baseline for the control farms, with levels being higher in the endline survey. Even though the control group was not provided with the binding agent until after three months, it was found that using binders and training during the intervention period significantly (p = 0.029) reduced the aflatoxin levels in milk (Table 4.8). Overall there was an increase in AFM1 levels as compared to the baseline, an indication that the feeds used was contaminated and the farmers may not be using toxin binders after end of intervention period.

Table 4.8: Comparison of mean aflatoxin levels (ng/kg ± standard deviation) during baseline and endline

Description	Mean AFM1 at baseline	Mean AFM1 at endline	Mean difference (AFM1)	t	p-value
Intervention group	132.21± 59.32	193.84±160.08	61.63	-1.58	0.1285
Control group	90.87 ±39.34	162.24 ±71.63	71.37	-2.53	0.0286

Note: Data are presented as mean AFM1 concentration (ng/kg) in milk, pre and post intervention for control and intervention farms

CHAPTER FIVE

DISCUSSION

5.1 Baseline survey

This study reports on farmers' knowledge, awareness and practices on aflatoxins and prevalence of AFM1 in milk from urban and peri-urban smallholder dairy farms. The required household-respondent target sample of 100 farmers for this study was well attained, this could be attributed to early briefing and involvement of area livestock officers, which motivated the respondents to participate in the study.

The high level of aflatoxin awareness (84%) was comparable to the level reported by Amimo *et al*. (2011) of 93% and higher than those reported by Ayo *et al.* (2018) and Kamala *et al*. (2016); this may be due to increased awareness as a result of aflatoxicosis epidemic in Kenya that killed people (Probst *et al* ., 2007). It might also be related to increasing farmer organizations training on aflatoxin mitigation methods (Anyango et al. 2021). More than 50% of the respondents were aware of aflatoxins, the sources, types of feed, and food that can be easily contaminated with aflatoxin, and the human health implications.

In contrast to a prior investigation by Kang'ethe *et al*. (2015), which revealed varying awareness in different urban centers; (Machakos 31.7%, Nyeri 55.6%, and Eldoret 12.7%), none of the respondents allegedly understood that milk might also be infected with aflatoxin. Different levels of awareness are shown in the report, which may indicate that some regions are better informed than others. In this study, most farmers practiced zero-grazing, thus relying more on purchasing pasture, fodder, and concentrates with only a few making their on-farm formulations; this is comparable to an earlier report by Makau *et al*. (2016). Zero-grazing is the preferred production method for farmers in urban and peri-urban areas due to rising pressure on land for human settlement and a lack of resources for farmers to run large-scale dairy farming operations.

This study showed that milk produced from urban and peri-urban dairy farms in Kasarani sub-county is contaminated with AFM1. Most (64%) of the milk samples collected had AFM1 levels above the FAO/WHO maximum limit of 50 ng/kg but within the Kenyan limit of 500 ng/kg. The high levels of AFM1 in milk may be due to feeding practices. Farmers who formulated their feeds may have used low-quality ingredients, which could have been contaminated with aflatoxins due to poor storage at the source and on the farm. Research shows that dairy farmers have a habit of feeding animals spoilt maize and selling it as animal feed to other farmers (Kiama, 2016).

Though it was not in the scope of this study to determine the levels of AFB1 in the animal feeds, previous studies have documented their occurrence in the feeds (Kang'ethe & Lang'A, 2009; Makau *et al.*, 2016). The prevalence of AFM1 contamination (100%) in this study were above those reported in Kenya by Makau *et al.*, (2016) (68%) in Nakuru and in another study by Senerwa *et al.*, (2016) (39.7%). This variation in contamination levels may be brought about by various methods of managing cattle, feed and feed additives suppliers, and feed storage conditions along the value chain (Makau et al. 2016). Aflatoxin levels were higher in farms with more awareness which could be related to commercial feeds, which are highly contaminated with aflatoxins in Kenya (J. F. Lindahl et al. 2018). Consequently, the increased knowledge of aflatoxin contamination points along the dairy value chain has not given farmers the tools they need to implement on-farm mitigation (Anyango et al. 2021).

5.2 Intervention and Endline survey

This study reports on combined intervention that included training and provision of a commercial toxin binder to smallholder farmers in Kenya in order to assess its effect on the occurrence of AFM1 in milk from urban and peri-urban dairy farms. Despite the feedback from most farmers, there was no significant difference in milk yield between the intervention and control groups. Aflatoxin has detrimental consequences on dairy animals, one of which is decreased milk production (Anyango et al. 2021). These effects could have been lessened in the intervention group by giving the

NovaSil® binder, but the study was unable to demonstrate this. However, it was shown that feeding binder to cows reduced their exposure to aflatoxin without having a negative impact on milk production (Maki *et al*., 2016). During the intervention period, milk yield decreased over time but this was not statistically significant. Additionally, it's probable that both the control and intervention farms had seasonal influences that impacted the production of milk as reported by a previous study (Senerwa et al. 2016).

There was no control over the feeds used in this field trial, and even though the farmers were instructed to feed 1 teaspoon per 2 kg feed at first, then 2 teaspoons, the researchers had no control over how much the cows were actually fed. This was intentional, as the goal was to observe the effects under normal farming conditions. The mean AFM1 levels in milk produced by the intervention group decreased over time, with farmers having an average of 59.9 ng/kg during the final visit compared to 101.5 ng/kg during the third visit. Under the low dose regime of binder administered to the cows (1 teaspoon for 2 kg of feed), AFM1 levels at visits 1, 2, and 3 appeared to rise. A drop in AFM1 was also seen when the dosage was increased to 2 teaspoons per 2 kg tin of food. This was done since the feeds' AFB1 content was probably larger than anticipated. The intervention farms in this study demonstrated a considerable decrease in AFM1 levels in milk. Similar findings were made in the US, where dairy cows fed an AF-contaminated feed and NovaSil® binder had significantly lower AFM1 concentrations in their milk without compromising the content or quality of the milk (Maki *et al*., 2016).

Farmers in this study reported no abnormal signs after feeding the cows NovaSil®) binder. This was similar to results of Maki *et al*., (2016), who reported that cows showed no abnormal behavior or clinical signs of aflatoxicosis. However, other studies on the use of NovaSil® binder have been conducted under controlled conditions, whereas this is the first study on the use of NovaSil® binder by smallholder dairy farms in East Africa. There was a significant difference in AFM1 levels between the baseline and each trial point for the intervention group, which was provided with novasil binders and frequent training on aflatoxins.

The AFM1 levels difference between the baseline and endline surveys shows variation contamination of the commercial feeds used in the farms. Novasil binder is a natural clay that binds aflatoxins and functions as a "chemical sponge," absorbing mycotoxins in the gastrointestinal tract and inhibiting uptake in the blood and subsequent distribution to target organs (Anyango et al. 2021). The training improved farmers' understanding of binder use and impacted how feeds were handled, which helped lower AFM1 levels. The training also enhanced farmers understanding of how moulds and aflatoxins affect humans and animals. This is similar to report previous showing that training on pre and post-harvest management of cereals and feed crops leads to improved knowledge and change of practices on aflatoxin control (Anitha et al. 2019; Pretari, Hoffmann, and Tian 2019)

In the dairy value chain, aflatoxin is a public health problem, and mitigation will need a One Health approach; a collaborative, multi-sectoral, and trans-disciplinary approach that works at the local, regional, national, and global levels to attain the best possible health outcomes while acknowledging the connections between humans, animals, plants, and their shared environment (OHCEA 2019). Although it was outside the scope of this study, it is possible that variations in the actual levels of AFB1 in the various feed batches administered to the cows contributed to variations in the AFM1 concentration rates secreted in the milk throughout the trial. In Kenya, the maximum residue limit for AFM1 in milk used is 500 ng/kg, which the East African Community adopts (EAC) while the FAO/WHO and Codex Alimentarius Commission (CAC) limit is 50 ng/kg (Gong *et al* ., 2015, Grace, D.;2015). There is a drive to constantly apply mycotoxins regulations globally to facilitate international trade and reduce human exposure (Wu, F., 2004). From this study, the FAO/WHO limits are too tough to consider in Kenya for smallholder dairy farmers to attain, even with mycotoxin binders. The study showed the potential of using mycotoxin binders on the farms and follow-up training.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This is the first pilot study in Kenya to report on training and the use of novasil binders to mitigate aflatoxins in small scale dairy farms. This study found that a majority of farmers who mainly practiced zero grazing, were aware of aflatoxins but were less knowledgeable about aflatoxins. During the study, farmers reported death, disease, gastro intestinal problems, stunting in children and cancer as effects of aflatoxin. They also mentioned maize, cereals, concentrates, milk, fodder and forages to be susceptible to aflatoxin contamination.

All milk samples analysed during the study had detectable AFM1 levels which were within the East African standards making it safe for human consumption in the region. However more than half of the milk samples analysed exceeded the EU standard which could limit trade with EU countries. The use of novasil binders and regular trainings during the intervention period, showed a significant reduction of AFM1 levels in milk from the intervention farms when compared to baseline levels.

The farmers knowledge levels had significantly improved at the end of the study in respect to effect of moulds on cattle and feeding practices. Majority of the respondents reported to be monitoring feed condition during storage mostly for mould growth and increased moisture content, with most of them disposing off any mouldy feeds.

6.2 Recommendations

There is need for improving farmers knowledge on aflatoxins mitigation measures, its occurrence in feed and milk and its consequences on human and animal health.

Feed ingredients and finished products should be thoroughly monitored for mould growth to prevent aflatoxin contamination in the dairy value chain.

Since toxin binders show promising results in lowering aflatoxin in milk, there is need to establish potential outlets where farmers can obtain the mycotoxin binders in small quantities to encourage their use and prevent adulteration.

There is need to conduct more research on the cost-effectiveness of toxin binders in the smallholder dairy context, to promote their appropriate use and understand their effect on the nutritional composition of milk.

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APPENDICES

Appendix I: Consent form

Building capacity in urban and peri-urban dairy farmers to produce and sell aflatoxin safe milk

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 and Kisumu Counties. This study has two parts 1) baseline survey to help understand smallholder milk production systems in the area and 2) a follow up field trial with a few of the farmers to analyze the effect of selected aflatoxin control interventions on milk quality and safety. We are visiting you because your farm has been selected to participate in the baseline survey. If you accept to participate in the baseline study, we will ask you a few questions related to feeding, milk production and utilization. We will also request you to provide us with a sample of milk for further laboratory testing, mainly for bacterial testing and aflatoxin contamination. The research team promises to respect privacy and confidentiality of your information. This information we talk about will be shared with our research team members, but we will remove all names so that no one be able to trace back the information to you.

Informed Consent Form

~not to be attached the questionnaire~

Do you have any questions about the research we wish to conduct? Once again, we thank you for accepting us on your farm 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

		Farmer Initials	signature
YES	Verbal		
	Written		
NO			

Appendix II: Baseline survey questionnaire

NAME OF THE ENUMERATOR _____________________________

DATE: ___/___/2017

CHECK IF: Adequate farmer introduction has been done ____√ | and Consent is granted _____√

1.1 Location of the farm

County:	**Ward:**	**Village:**	**GPS:** Latitude **GPS:** Longitude

1.2 Household details

a) Respondent details	Gender:	Age:	
	Highest level of education: [ ]	Relationship to household head:: [ ]	Sources of income: [ ] [ ] [ ]
	1=never 2= primary (lower) 3=primary (upper) 4=secondary school (not completed) 5= secondary school (completed) 6= college / university	0= respondent is household head 1=wife 2=husband 3=son 4=daughter 5=farm worker 6=other, specify	1=employed full time 2=employed casual 2= farming 3=other
b) If respondent is not household head	Gender of household head:	Age:	

c) **Role in dairy farm**	What is your role in feeding cattle? [ ] [ ] [ ]	Role in selling milk [ ] [ ] [ ]	How responsible? [ ] [ ] [ ]
	1=Decided what feed to buy 2=Buy/acquire feed 3=Feed animals	1=Decide how much milk to sell 2=Decide where to sell milk 3=Sell milk 4=Control all money from the sale 5=Control some money from the sale	1=Own cattle 2=Co-own cattle 3= involved with cattle 4=not involved cattle
d) Previous training on dairy production: Yes/ No	What aspect of the training was done : *1=health 2= production 3= milk hygiene & safety 4= other*	Specify which institution (or group) provided the training:	Which year was the training done :

1.2 Who in the family is tasked with the following activities? (multiple numbers possible)

Feeding of the animals	
Milking of cows	
Cleaning of the milking items	
Selling of milk produced	
Transporting milk to market	

CODE: 1= husband 2= wife 3= male worker 4=female worker 5= male relative 6= female relative 7= other, specify

1.3 Herd details

1.3.1 Details of cattle owned

	Adult males	Adult females			Calves & weaners
		milked	Dry cows	Heifers	
Number on farm					
Specify breed(s) kept *1= local 2=exotic, specify which one 3= crossbreeds*					
Management system *1= pasture grazing 2=tethering 3= zero-grazing / cut and carry*					

1.3.2 How many of the following livestock species do you keep?

Goats: [ ] Sheep: [ ] Poultry: [ ] Donkeys: [ ] Pigs [ ] other, specify: ___________ [ ]

1.4 Milk production

1.4.1 Amounts of milk produced

a. How often are the cows milked in a day		
b. Indicate the amount of milk produced in a day (in Litres) for the top 3 cows	Cow #1	
	Cow #2	
	Cow #3	
c. How much, in a day, is produced by the other milking cows		

1.4.2 The estimated total amount of milk produced on a typical day by all the cows on your farm, like yesterday, is __________ Litres

1.4.3 Description of sold milk

What price is the milk sold at per litre	
How long is the milk stored before being sold (hrs)	
How is the milk transported to market	

0= Customer or trader comes to the farm to pick milk 1= walk to deliver the milk 2= use own bicycle 3= use public vehicle 4= use own vehicle	

1.4.4 Who buys the milk that you produce

Who buys	Quantity (L) sold per day
1. Neighbor for home use	
2. Milk trader	
3. Hotels or shops	
4. Bulking Centre	
5. Other : ___________	

1.5 Feeding

1.5.1 Feeding and feed storage practices

Feed type used by the farmer						
	Open grazin g	Hay bales	Cut-carry-pasture	Concentrates/ compound feed	Silage	Molasses
Does this feed option apply (check)						
What is the source of the feeds	NA					
How is the feed stored	NA					
Do you routinely monitor the condition of your feed during storage for any spoilage *[yes] [no]*	NA					
If yes, what conditions do you routinely monitor for during storage	NA					
What actions would you take if you noticed your stored feed had moulds	NA					

CODES

Source of feeds: 1= on-farm formulations 2= purchased, specify price per unit 3= other sources, specify

How is the feed stored: 1= on the floor 2= on raised surfaces 3= other, specify

Storage conditions routinely monitored for: 1= moisture 2= warmth 3= ventilation 4= moulds growth 5= dryness 6= pests / animal

Actions If stored feed had moulds: 1= dispose of the feed 2= still give animals the feed 3= mix with good feed

1.5.2 Observe if there is a feed storage facility within the farm; if not, ask and describe how / where the feed is stored

1.5.3 Supplementation with concentrate / commercial feeds *(applies to what the farmer is using at the time of the study visit)*

Feed type	Description of the feed (brand)	Quantities (kg) given per day/ cow	What do you use to measure the portion you feed	How is the feed provided to the animals
1.				
2.				
3.				
4.				

CODE

Description of feed: 1=bought commercial (specify brand type) 2= on-farm formulation (specify ingredients)

How is the feed provided to animals: 1= alone 2= as a mix with other feeds

1.5.4 What else do you routinely add to your feeds?

1.6 Awareness of moulds and aflatoxins

1.6.1 Have you ever seen moulds on cattle feed, in your farm______________ [yes] [no]

1.6.2 If yes, do you think it has any impacts on cattle, and if so, what impact(s)

1.6.3 Have you heard of aflatoxins _________ [yes] [no]

1.6.4 If yes, what are they?

1.6.5 If yes to 1.6.3 above, which products (food types, feed types etc.) would you expect to be easily contaminated with aflatoxins?

1.6.6 Do you think the presence of aflatoxins in these foods poses any danger to humans? Which danger(s)?

1.7 If you get invited to participate in the next stage of the study, we will facilitate getting you an additive to help in milk safety some of which may cost you some money to acquire.

Aflatoxin binders	**A form of clay that, when mixed with feeds, sticks to any aflatoxins present so that much of the aflatoxins pass out in the cattle faeces and can't enter the milk**
	1.7.3 Would you be willing to acquire and use aflatoxin binding agents
	1.7.4 How much (per KG) would you be willing to contribute to get a substance that will reduce aflatoxins for your cow

SAMPLING OF MILK

Collect 2 x 40 ml in sterile falcon tubes from milk that is meant for household consumption or 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, 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….

Appendix III: Trial Questionnaire

Building capacity in urban and peri-urban dairy farmers to produce and sell aflatoxin safe milk

DATE (current visit): ____________ CODE: _____________

DATE (last visit): _______________

1.0 Milk production

1.1 How many cows gave milk yesterday?	How many litres did they produce in total yesterday? ________ How many litres did you sell yesterday? ___	How much did you sell the milk per litre for yesterday's milk? _______	In your view, has there been a change in milk quantity since the last time we visited? (yes / no) If yes, what change _______

1.2 What management changes have you made on your farm since the last visit

1= changed feed type (yes/no) 2= changed the person who feeds the cows (yes/no) 3= changed the person who does the milking (yes/no)

Other change: ______________________________

2.0 Using the binders **-** *for controls, please SKIP to question 4.0*

2.1 Tell us what you have been doing with the binder (**1**=using **2**=not using)

If **using**, how many times a day _________________________________

If **not using**, please tell us why ____________________________________

2.2 How do you view the feeding of your cows with the binder

	Difficult	No opinion	Easy	Did not do this
Knowing how much binder to add to the feed				
Using the spoon provided to measure the binder				
The actual mixing of the binder with feed				
Cows eating feed mixed with the binder				

Observation: Conduct a visual inspection to confirm the farmers report (**1**=report is correct **2**= report is not correct 3= not possible to confirm)

2.3 Have you encountered any other challenges while using the binder on your farm? If yes, describe ________________________

2.4 Observe and approximate how much of the binder is left (e.g. 1, ½, ¼ of the large or small box) ____

2.5 Have you shared out your binder? (Yes / no), if yes, to who __________________________________

3.0 In your view, how do you rate the following?

	Worse	same	better
Feeding your cows now			
The health of your cows			
Milk yield of your cows			

SAMPLING OF MILK

Collect 2 x 40 ml in sterile falcon tubes from milk that is meant for household consumption or 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, chilling	
Indicate the approximate date and time when the sample is collected	

Appendix IV: Endline Questionnaire

SURVEY CODE:

NAME OF THE ENUMERATOR

___________________________ DATE: ___/___/2018

CHECK IF: Adequate farmer introduction has been done ____√ | and Consent is granted _____√

1.2 Location of the farm

County:	Ward:	Village:	GPS: Latitude
			GPS: Longitude

1.2 Household details

a) Respondent details	Gender:	Age:	
	Highest level of education: [ ]	Relationship to household head:: [ ]	Sources of income: [ ] [ ] [ ]
	1=never 2= primary (lower) 3=primary (upper) 4=secondary school (not completed) 5= secondary school (completed) 6= college / university	0= respondent is household head 1= wife 2=husband 3=son 4=daughter 5=farm worker 6=other, specify	0=employed casual 1=employed full time 2= farming 3=other
b) If the respondent is not a household head	Gender of household head:	Age:	

c) **Role in dairy farm**	What is your role in feeding cattle? [ ] [ ] [ ]	Role in selling milk [ ] [ ] [ ]	How responsible? [ ] [ ] [ ]
	1=Decided what feed to buy 2=Buy/acquire feed 3=Feed animals	1=Decide how much milk to sell 2=Decide where to sell milk 3=Sell milk 4=Control all money from the sale 5=Control some money from the sale	1=Own cattle 2=Co-own cattle 3= involved with cattle 4=not involved cattle

1.3 Herd details

1.3.1 Details of cattle owned

	Adult males	Adult females			Calves & weaners
		milked	Dry cows	Heifers	
Number on farm					
Specify breed(s) kept *1= local 2=exotic, specify which one 3= crossbreeds*					
Management system *1= pasture grazing 2=tethering 3= zero-grazing / cut and carry*					

1.3.2 How many of the following livestock species do you keep?

Goats: [ ] Sheep: [ ] Poultry: [ ] Donkeys: [ ] Pigs [ ] Other, specify: ____________ [ ]

1.4 Milk production

1.4.1 Amounts of milk produced

a. How often are the cows milked in a day		
b. Indicate the amount of milk produced in a day (in LITERS) for the top 3 cows	Cow #1	
	Cow #2	
	Cow #3	

1.4.2 The estimated total amount of milk produced in a typical day by all the cows on your farm, like yesterday, is __________ Liters

1.4.3 Description of sold milk

What price is the milk sold at per litre	
How long is the milk stored before being sold (hrs)	

1.5 Feeding

1.5.1 Do you routinely monitor the condition of your feed during storage for any spoilage

[yes] [no]___________________________

1.5.2 If yes, what conditions do you routinely monitor for during storage *[1= moisture 2= warmth 3= ventilation 4= moulds growth 5= dryness 6= pests / animal 7=other, please explain]* _____________________________

1.5.3 What actions would you take if you noticed your stored feed had moulds *[1= dispose of the feed 2= still give animals the feed 3= mix with good feed 4=other, please explain*

____

Do you routinely add anything to your feeds today? Yes/No. If yes, specify below

1.6 Awareness about moulds and aflatoxins

1.6.1 Have you ever seen moulds on cattle feed, in your farm__________ [yes] [no]

1.6.2 If yes, do you think it has any impacts on cattle and if so what impact(s)
____

1.6.3 Have you heard of aflatoxins _________ [yes] [no]

1.6.4 If yes, what are they?

1.6.5 If yes to 1.6.3 above, which products (food types, feed types etc.) would you expect to be easily contaminated with aflatoxins?

1.6.6 Do you think the presence of aflatoxins in these foods poses any danger to humans? Which danger(s)?

1.6.7 What can a farmer do to reduce mould on their farm?

1.6.8 What can a farmer do to reduce aflatoxins in the milk their cows produce?

1.6.7 Is there any particular thing you do today or have been doing on your farm to reduce mould growth in the feed?

1.6.8 Is there any particular thing you do today or have been doing on your farm to reduce aflatoxin contamination in the feed?

1.7 Use of Novasil - *to be asked to all farmers who were issued with novasil binder*

1.7.1 If we issued you novasil binder and requested you mix it with feed for your animals, did you encounter any problems using it? If yes, which ones? How could the binders be made to work better?

	Problems encountered in the use of novasil binder	For each, probe for farmer suggestions on how the problem could be solved
1		
2		
3		

How much would you be willing to pay for something added to the feed to reduce aflatoxins?

a. If this reduces aflatoxins and my cows improve production, I would be willing to pay _____ KSH per day
b. If this reduces aflatoxins and the milk gets safer, but I can't sell more milk, I would be willing to pay _____ KSH per day
c. If this reduces aflatoxins and I can sell milk at a higher price, I would be willing to pay _____ KSH per day

SAMPLING OF MILK

Collect 2 x 40 ml in sterile falcon tubes from milk that is meant for household consumption or 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, 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….

Appendix V: Ethical Clearance

ILRI
INTERNATIONAL
LIVESTOCK RESEARCH
INSTITUTE

31 March 2017

Our Ref: ILRI-IREC2017-10

International Livestock
Research Institute P.O. Box
30709 00100 Nairobi, Kenya.

Dear Dr. Johanna Lindahl,

Re: FoodAfrica2: WP 5 Measuring and mitigating the risk of mycotoxins in maize and dairy products for poor consumers in Kenya

Thank you for submitting your request for ethical approval to the ILRI Institutional Research Ethics Committee (ILRI IREC). ILRI IREC is accredited by the National Commission for Science, Technology and Innovation (NACOSTI) in Kenya.
This is to inform you that ILRI IREC has reviewed and approved your study titled **'FoodAfrica2: WP 5 Measuring and mitigating the risk of mycotoxins in maize and dairy products for poor consumers in Kenya'**. The approval period is March 31, 2017 to March 30, 2018 and is subject to compliance to the following requirements:

- Only approved documents will be used;
- All changes must be submitted for review and approval before implementation;
- Adverse events must be reported to ILRI IREC immediately;
- Submission of a request for renewal of approval at least 30 days prior to expiry of approval period; and
- Submission of an executive summary report within 90 days upon completion of the study.

Please do not hesitate to contact ILRI IREC on ILRIResearchcompliance@cgiar.org for any clarification or querry.

Yours Sincerely,

Dr. Silvia Alonso
Chair, ILRI Institutional Research Ethics Committee

Documents received & reviewed:

- Research Compliance Form and IREC Form 2
- Protocol & Plan
- Informed Consent Form
- Questionaire

Appendix VI: Farmer Training Manual

Classical Aflatoxin messages by the government extension system

Aflatoxin

Aflatoxins are poisonous chemicals produced by molds that grow on foods and feeds. The mold can grow on crops before or after harvesting. In Kenya, this is most of a problem in maize.

Aflatoxin can cause liver cancer and is bad for child growth and development .If a child gets too much aflatoxins it may be too short and also will be likely to get other illness because its body may not be strong enough to fight diseases.

The mold appears as grey-green or yellow on kernels but, moldy grains do not always have toxins, and even clean looking grains can have toxin. Washing, cleaning, heating or processing does not eliminate aflatoxin.

Note: Aflatoxin is soil borne. Moldy grains are not always aflatoxin contaminated. Even clear looking grains can be contaminated. Washing, cleaning, heating or processing does not eliminate aflatoxin In agriculture, there are four important mycotoxins impacting on food and feed safety.

Mycotoxigenic fungi

Mycotoxin	commodity	Fungal source
Deoxynivalenol/nivalenol	Wheat; maize barley	Fusarium, graminearum, f. culmorum
Zearalenone	Maize wheat	As above
Ochratoxin	Barley; wheat, coffee	Aspergillus, penicillum
Fumonisin	maize	Fusarium, moniliforme
Aflatoxin B1B2	maize, peanuts	Aspergillus flavus
Aflatoxin B1B2 G1G2	Maize, peanuts	Aspergillus parasiticus

How to recognize aflatoxin

Observe grey-green or yellow-green mold growing on corn kernels

Products prone and high risk:

Corn or maize, rice, nuts and pulses, wheat, sorghum, millet, beans, soya beans, ground nuts, peanuts, spices, milk, eggs, meat products due to contaminated feeds

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Economic significance

- Reduces nutrition value of produce.
- Contaminated grains destroyed; hence reduced income.

- Toxic even at low levels.

Poisoning symptoms

- Swollen stomach
- Fatigue
- Eyes turn color to yellow
- Swollen legs

Advanced stages

- Liver Cancer
- Reduced immunity
- Ultimate Death due to aflatoxicosis in human, livestock and domestic animals.

Pre-disposing factors for aflatoxins

1) Climate

- Storage of grains at temperature 10-40 degree Celsius
- Harvesting and storage of maize with moisture contend above 13% at high humidity; 65-70%
- Moisture stress during growing period leading to shiveled grain size
- The uneven grain surface create ideal conditions for fungal growth
- Smooth surface discourage growth of aflatoxin fungi
- Poor agronomic practices
- Wrong choice of varieties to be grown in different AEZ making produce prone to invasion prior to harvest.

2) Pest infestation

Rodents, birds and insects attack while crop is either in the field or during storage.

3) Time of harvesting

Grain or pulses harvested in rainy season and also with high moisture content are more.

4) Harvesting methods

Mechanized harvesting as opposed to manual harvesting results in higher percentage of broken grains increasing the chances of aflatoxin contamination.

5) Storage structures

- Poor storage facilities that are not water proof
- Use of improper storage bags
- Use of contaminated bags
- Poorly ventilated stores

6) Type of seeds

Different varieties of cereals and pulses require different drying regimes

7) Shelling

- Hand shelling is less susceptible to fungal attack since percentage of broken grains is low.
- Mechanical shelling especially with poorly calibrated shellers leads to higher percentage of broke grains.

NB: shelling by beating of maize cob should be discouraged as it leads to a higher percentage of broken grains.

Recommendation

1) Information awareness

General public and especially farmers should be informed of the dangers associated with aflatoxin poisoning. Early detection of poisoning from resulting illness symptoms need immediate medical attention.

2) Training

Extension officers, farmers, produce and feed handlers need continuous training on techniques that reduce aflatoxin formation in food and feed.

3) Proper storage management

- The stores should be dry, clean, raised, well aerated, have leak proof roofs, fitted with rat guards, free of climbing weeds in a well-drained environment
- Storage bags should be placed on pallets to enhance aeration. Use of jute or sisal bags reduces probability of fungi attack compared to those grains stored in propylene bags.
- Change grains occasionally, to discourage formation of pockets of moisture.

4) Transportation

- Transport grains/pulses in closed or well covered vehicles to cut contact with rains or external moisture sources.
- Confirmatory test should always be done for suspected grain consignments.

5) Surveillance

Routine surveillance of food and feed stores should be mandatory. All grain handlers should be registered for ease of monitoring

6) Grading

All broken and discolored grains should be sorted out. Cobs should not touch soil during harvesting. All decaying kernels and shriveling grains with paperly skin are undesirable and more susceptible. All foreign materials should be removed before storage.

7) Regulations

Allowable levels of aflatoxin in various food and feeds should be clearly stated.

8) Resistant cultivars

Breeding of more tolerant varieties of cereals and pulses to fungi attack should be explored.