EFFECTIVENESS OF A MILK SAFETY INTERVENTION BY SMALLHOLDER DAIRY FARMERS ON BACTERIAL AND AFLATOXIN M1 CONTAMINATION IN MILKIN KISUMU COUNTY, KENYA

BY

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DECLARATION

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

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DEDICATION

I dedicate this work to my father Mr. Fredrick Owino Odhiambo, my daughter Arhvehmariah Alena and my entire family members.

ABSTRACT

Milk, important in the human diet especially that of children, is prone to contamination by bacteria and aflatoxins. The leading public health hazard from poor milk safety are microbial (bacterial) followed by chemical milk contaminants; and aflatoxin M1 (AFM1) which is implicated in stunting, immune suppression and carcinogenicity. Young children, infants, pregnant women, elders and immunocompromised people are the primary groups at risk for milk safety problems. Similar to much of Kenya, milk production in Kisumu County is dependent on smallholder dairy farmers who sell unregulated milk with high risk of contamination with aflatoxin and bacteria. No single intervention offers a comprehensive solution to reducing both AFM1 and total bacterial counts in milk. The simultaneous use of Mazzican, a hygienic milk container, and NovaSil mycotoxin binder offers promising results and investigation of their potential to improve milk safety is warranted. The main objective of the study was to assess the effectiveness of a milk safety intervention comprising of training in milk hygiene and feeding practices; and use of Mazzican, plus NovaSil binder, on the bacterial and aflatoxin M1 contamination in milk produced by smallholder dairy farmers in Kisumu County. Specific objectives were to determine: the proportion of farmers producing milk contaminated with AFM1 and bacteria at levels above the recommended limit; the milk hygiene and feeding practices of farmers before and after intervention; the effect of Mazzican and NovaSil binder on milk safety; and the willingness of farmers to use the intervention. A quasi-experimental study design was used. Milk samples and data on milk handling and dairy feeding practices were collected at baseline from 100 urban and peri-urban smallholder dairy farmers randomly selected from a list obtained from the County veterinary office. Thirty of these farmers producing milk with AFM1 levels above 20ppt were selected for the intervention. Farmers in the treatment group (n = 20) were trained on safe milk handling and feeding practices and use of Mazzican container and NovaSil binder while control farmers (n = 10) were not subjected to the intervention. Milk samples were collected fortnightly from all study farmers (n = 30) for three months. Data on milk hygiene and feeding practices was collected using a questionnaire. Enzyme-linked immunosorbent assay was used to asses AFM1 and milk cultures for total bacterial counts (TBC).Independent t-test was used to determine effects of Mazzican and NovaSil binder. Of the total 77 milk samples collected 28.6% had AFM1 levels above the recommended limits, 36.4% of samples had TBC above one million and 13% had both high levels of AFM1 and TBC. There was improvement in farmers' milk handling practices: cleaning of the milking shed, cleaning of udder before milking, checking of mastitis and storage equipment used ($p<0.05$).NovaSil binder use reduced AFM1 levels by 188.76 ppt ($p<0.001$) while use Mazzican reduced TBC by 5.2×10^7 Cfu/ml ($p<0.05$). Most farmers (86.2%), were willing to use the intervention in future. Findings of the study indicate that use of NovaSil binders, Mazzican containers and good milk handling practices improves safety of milk produced by smallholder dairy farmers in Kisumu; and can be scaled up.

Key words: Aflatoxin M1, total bacteria, milk contamination, smallholder dairy farms, NovaSil binder, Mazzican, dairy feeding practices, milk handling practices.

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LIST OF ABBREVIATION AND ACCRONYMS

AFB1	:	Aflatoxin B1
AFM1	:	Aflatoxin M1
BPW	:	Buffered peptone water
CFU/ML	:	Colony forming unit per milliliter
DVS	:	Director Veterinary Services
ELISA	:	Enzyme-linked immunosorbent assay
EU	:	European Union
FAO	:	Food and Agriculture Organization
GAP	:	Good agricultural practices
GMP	:	Good manufacturing practices
HCC	:	Hepatocellular carcinoma
HPLC	:	High-performance liquid chromatography
IARC	:	International Agency for Research on Cancer
ILRI	:	International Livestock Research Institute
KDB	:	Kenya Dairy Board
KMT	:	Kisumu milk trader
PPT	:	Parts per trillion
PW	:	Peptone water
SPSS	:	Statistical Package for the Social Sciences
TPC	:	Total plate count

OPERATIONAL TERMS

Milk: Unpasteurized whole raw milk

Small holder dairy farmer: Farmer with less than 20 milking animals

Presence of bacteria: Viable colonies on agar plates of milk cultures

Levels of total bacteria count: Total number of colonies of two consecutive dilutions

Presence of AFM1: Detectable levels of AFM1

Accepted levelsofAFM1: Levels between 0ppt-49ppt

Urban farm: Farm located inside the town

Peri-urban farm: Farm located in the outskirts of town

Intervention farms: Farms receiving training, Mazzican and NovaSil binder

Control farms: Farms receiving no intervention

Safe milk: Milk with AFM1 levels<20ppt and in TBC of <1 million Cfu/ml

Milk safety intervention: Intervention with Mazzican, NovaSil binder and training

Effectiveness: Outcome of the intervention and willingness to use it in future

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CHAPTER ONE
INTRODUCTION

1.1 Background Information

Food safety is an increasing global public health concern requiring attention of many disciplines. Milk forms a major part of human diet and is considered as nature's single most complete regularly consumed food (Pal & Awel, 2014). Milk is important especially for children and expectant mothers due to its nutrition richness. However, it has been shown to be contaminated with bacteria (L. Kurwijila *et al.,* 2016) as well as aflatoxins (E. Kang'ethe, 2016). Like much of Africa, milk production in Kenya is heavily dependent on smallholder dairy farmers, accounting for 80% of total production (Muia *et al.,* 2011). Smallholder dairy farmers are most vulnerable to poor microbial quality in milk because the sanitary measures taken during handling of milk at different stages are generally substandard (Bereda *et al.,*2012). This is due to lack of farmers' education on safe handling techniques and proper personal hygiene practices. Training farmers in safe handling techniques and proper personal hygiene practices, including hand washing, would contribute to reduced microbial contamination (Nyokabi *et al.,* 2021) and therefore should be included in initiatives to improve milk safety. Farmers should also be sensitized on good animal feeding practices, in order to prevent aflatoxin M1 (AFM1) contamination. Quality control at farm level will reduce contamination hence increase safe quality milk for consumption.

Mycotoxins are metabolites of fungi which evoke pathological changes in humans and animals. There are different forms of mycotoxins such as: aflatoxins, ochratoxins, citrinin, fusarium, ergot, patulin. *Aspergillus spp*., the fungi producing aflatoxins occur naturally in soil (Reddy & Salleh, 2010). Aflatoxin is the most toxic mycotoxin, with aflatoxin B1 (AFB1) being the most carcinogenic and is transferrable to milk as the metabolite aflatoxin M1 (AFM1). AFM1 and AFB1 have been classified as group 1 carcinogens (Wild*et al.,* 2015).

Kenya leads globally in acute aflatoxicosis recording 317 cases reported and 125 deaths, with a case fatality rate of 39% in 2004 compared to 13 deaths that had occurred in China (Gieseker, Control, & Prevention, 2004). Long term exposure to aflatoxins may contribute to neurological impairment, immunosuppression, child mortality (FAO, 2015) and carcinogenicity of vital body organs (Maleki *et al.*,2015). In addition, stunting and wasting in children has been associated with long term exposure to aflatoxin in Kisumu (M. Obade *et al.*,2015b; Okoth & Ohingo, 2004). On the other hand, acute aflatoxin exposure has been associated with mortality (Lawley, 2013). Therefore, appropriate measures should be instituted to reduce exposure to aflatoxins, especially in young children who are highly vulnerable (M. Obade *et al.*, 2015a).

The prevalence rate of AFM1 in milk worldwide was 79% between 1988-2020 (Salari, Kazeminia, Vaisi-Raygani, Jalali, & Mohammadi, 2020) and Kenya AFM1 prevalence was estimated to be 72% in one study, translating to 3.744 billion litres out of 5.2 billion produced (E. Kang'ethe, 2016). Of this, 20% had AFM1 levels above 50 parts per trillion (ppt), translating to 748 million liters that should be discarded annually (McGuire, 2015), which would lead to both food insecurity and economic loss. In Kisumu AFM1prevalence in raw milk has been estimated to be 40%, which is four times higher than in Eldoret, Nakuru and Nairobi having 10%, 8%, and 8% respectively (Rademaker *et al.*, 2016). Reduction of exposure to AFM1 in milk may be achieved through training farmers on good livestock feeding practices such as proper feed storage, discarding of mouldy feeds, and good ventilation of storage rooms as well as daily cleaning of feeding areas of the dairy cows.

Unlike pasteurized milk which is considered safer for human consumption, raw milk often contains microorganisms, which may cause 96% of food-borne diseases such as shigellosis, tuberculosis, brucellosis and diphtheria (Dhanashekar *et al.*, 2012). Bacterial contamination of

raw milk can originate from different sources such as mastitic animals, air, feed, soil and feces (Torkar & Teger, 2008). Milking personnel and insufficiently cleaned milk containers remain the major sources of bacterial contamination of raw milk (Coorevits *et al.,* 2008; Reta *et al.*, 2016). According to the Kenya Bureau of Standards (KEBS), the allowable total bacterial counts (TBC) should be less than1 million Cfu/ml in raw milk (Rademaker *et al.,* 2016). The percentage of samples exceeding the allowable total bacterial count limits observed in Kisumu was 38.5% compared to other towns such as Eldoret 50%, Nakuru 50% and Nairobi with 100% (Rademaker *et al.,* 2016). Despite Kisumu county recording lower levels than other towns cited, it is desirable that raw milk should be sterile therefore reduction of these levels is highly recommended considering lack of a centralized milk processing plant in the County.

Despite several interventions, prevention and control of aflatoxins and bacterial contamination in milk and feed remains difficult. Aluminium cans were designed to address bacterial contamination but are often too costly, necessitating the smallholder's dairy farmers to use non-food grade jerri cans which accelerate spoilage leading to poor bacteriological quality in milk. Mazzican made of food grade material, affordable and easy to clean offers a suitable alternative for smallholder farmers. On the other hand, good agricultural practices, proper storage, decontamination of feed through dilution and chemical treatment of feeds have been put in place to prevent aflatoxins but are insufficient in reducing aflatoxin contamination. Mycotoxin binders are natural adsorbents with ability to decrease bioavailability and exposure to aflatoxins (Mallmann *et al.,* 2012). Addition of mycotoxin binders can act as a safety measure for livestock feed manufacturers and farmers and an assurance to customers (Jacela *et al.,* 2010). No single intervention offers a comprehensive solution in milk safety, hence combining several measures is recommended (Mutua *et al.,*2019). Willingness by smallholder dairy farmers to use Mazzican in

reducing bacteriological contamination in milk was 98% in Tanzania (L. Kurwijila *et al.*, 2016), indicating that it is a promising intervention with potential for scaling up. NovaSil binder has not been scaled out to the community level therefore there is no data on willingness of smallholder farmers to use it. There is need to try this intervention in a Kenyan setting to determine its potential effectiveness in reducing milk contamination without interfering with the daily farmer activities.

1.2 Statement of the Problem

Unlike centrally processed milk, that produced by smallholder dairy farmers is uncontrolled and is not subjected to testing by public health officers at household level, thus is a potential source of exposure to bacteria and AFM1 posing health risks to consumers. Kisumu County records high levels of bacterial and AFM1 contamination in milk and could benefit from measures to reduce such contamination. Several interventions targeting bacteria and aflatoxin contamination in milk have been ineffective due to cost and difficulty in cleaning milk containers in the case ofthe former; and reduction of nutritional value of animal feed by chemical and dilution treatments in the case of the latter. No single intervention offers a comprehensive solution. NovaSil binders are shown to reduce AFM1 levels but studies have only been done under controlled environments, thus there is need to assess its effectiveness in community settings. Studies on use of Mazzican have shown improved bacteriological quality in milk. Combined use of Mazzican and NovaSil binder may improve milk safety because they can easily be incorporated by smallholder dairy farmers in their daily activities of feeding and milking of dairy cows. To augment these interventions, training farmers on feeding practices and hygienic milk handling practices may enhance sustained reduction of aflatoxin and bacterial contamination, respectively; because these farmers are responsible for small-scale production of milk that is

directly available to consumers and are best placed to achieve reduction of milk contamination. There is need, therefore, to assess whether using this combined intervention comprising training, Mazzican container and NovaSil binder, successfully reduces the production of unsafe milk; and whether smallholder farmers are willing to continue using the intervention, which is key to its sustainability and potential for up-scaling.

1.3 Study Objectives

1.3.1 General Objective

To determine effectiveness of a combined milk safety intervention by smallholder dairy farmers on bacterial and aflatoxin M1 contamination in milk in Kisumu County, Kenya.

1.3.2 Specific Objectives

i. To determine the proportion of farmers producing milk with levels of total bacterial counts and aflatoxin M1 above the recommended limits.
ii. To evaluate the milk hygiene and feeding practices of farmers between the control and intervention group
iii. To determine the effects of Mazzican and NovaSil binder on total bacterial counts and AFM1 levels in milk respectively.
iv. To determine the willingness of farmers to use the intervention for safer milk.

1.4 Research Questions

i. What is the proportion of farmers producing milk with levels of total bacterial counts and aflatoxin M1 above the recommended limits?
ii. What are the milk hygiene and feeding practices of farmers between the control and trial groups?
iii. What are the effects of Mazzican and NovaSil binder on total bacterial counts and AFM1 levels in milk respectively?

iv. What proportion of farmers is willing to use the intervention in future, for safer milk?

1.5 Study Justification

Poor milk quality is a problem in Kisumu County. The study will help generate data on the prevalence of farmers producing unsafe milk in regards to bacterial and aflatoxins contamination in Kisumu County; reflecting the extent of milk contamination that will inform future studies aimed at improving milk safety. It will also provide evidence on effectiveness of Mazzican, NovaSil binder; safe milk hygiene and feeding practices which will help in policy development and implementation for safer milk production at farm gate level. It will increase awareness on milk safety with special concern to milk hygiene, feeding practices, bacteria and aflatoxins because farmers in this will be trained. Findings of the study will indicate viability of the use of these interventions to improve safety of milk produced by smallholder dairy farmers in Kisumu, hence increasing safe milk production and food security. Whereas the effect of the intervention on milk safety will provide information on whether this intervention can improve milk safety, findings on willingness of farmers to use intervention will establish acceptability and sustainability of the intervention for scale-up potential hence justify its potential effectiveness.

CHAPTER TWO
LITERATURE REVIEW

2.1 Prevalence of Aflatoxin M1 and Bacteria in Milk

Aflatoxin B1 (AFB1) is considered a class 1 carcinogen and can cause acute and chronic illness in people and animals (Min *et al.,* 2011). In the United States the recommended level for aflatoxins in foods meant for human consumption is 20 μg/kg (20 ppb); while the most recent outbreak of aflatoxin poisoning in Kenya was linked with the intake of foods containing levels as high as 8,000 ppb (Control & Prevention, 2004), which is 400 times higher than the recommended level. In a study in Addis Ababa, Ethiopia a total of 110 milk samples were collected and analyzed by ELISA for aflatoxins. Results showed that all the milk samples were contaminated with aflatoxin M1 with over 90% exceeding the EU limit of 50 parts per trillion (ppt). This correlated with the animal feed which had 7-419 ppb (Gizachew *et al.,* 2016); implicating animal feeds in aflatoxin contamination of milk.

A study conducted in Nakuru County, Kenya, found that in the peri-urban dairy system, 33/68(48.5 %) of the milk samples were contaminated, with the AFM1 concentration ranging between 17 and 83 ppt. All milk samples from the peri-urban dairy system had AFM1 contamination levels below the EU limits of 50 ppt, ranging between 0 and 41ppt (Makau *et al.,*2016) thus concluding that Nakuru County peri-urban population may be exposed to AFM1. High levels of AFM1 found in milk in the urban and peri-urban areas may be attributed to the increased number of farmers practicing intensive farming due to inadequate land capacity compared to their rural counterparts who may practicing open grazing. Reported prevalence of aflatoxin M1 prevalence in milk was lowest in Kwale (13.6%) and highest in Tharaka-Nithi (65.1%). The proportion of milk samples with AFM1 above the EU standard varied from 3.4%

(Kwale) to 26.2% (Tharaka-Nithi); the highest was 6999ppt (Senerwa *et al.*, 2016). A study in Nairobi found a prevalence of 49.9% with a mean concentration of 128.7ppt (Kirino *et al.*,2016). In Bomet, AFM1 contamination prevalence rate of 43.8% was found (Langat *et al.*, 2016). The mean AFM1 concentrations in raw milk in Kisumu County is 92.57, Eldoret 10.74, Nakuru 96.80 and Nairobi 185.38 ppt (Bebe *et al.*, 2018).This was attributed to poor feed storage, failure to test milk and feeding of contaminated feeds to cows (Kirino *et al.*, 2016; Rademaker *et al.*, 2016).

Another study in Kisumu County on aflatoxin exposure in pregnant women revealed that women in Kisumu East, West and Nyando sub counties were exposed to aflatoxin levels above EU limit with women from Nyando sub-county being highly exposed followed by Kisumu East while Kisumu West was the least exposed. If diet consumed by the pregnant women closely relates to what their families eat then not only the pregnant women are exposed but the entire community at large (M. Obade *et al.*, 2015b). However, low levels of aflatoxin were found in raw milk samples in Kisumu County (Obade *et al.*, 2015). Despite reports on low levels of AFM1 contamination, chronic exposure to low levels of aflatoxins has been associated with health risks such as organ damage such liver, impaired immune function, stunting in children, cancer, low production in animals and acute aflatoxicosis causes death (Upadhaya *et al.*,2010).

Previous studies have shown that there are health risks involved in the consumption of raw milk and the population of Kisumu is exposed to bacteria and AFM1 contamination through milk. Due to the complexity of the dairy chain, milk contamination can occur in different levels of production, therefore there is need for development of adequate control plans for monitoring the microbial quality and safety of milk from production to processing.

Raw milk has been known as a vehicle for pathogens for more than 100 years (Jayarao *et al.*, 2004). Milk meant for human consumption must be free from any pathogenic organisms to be safe. The main pathogens occurring in milk in these times are Shiga toxin-producing *Escherichia coli*, *Salmonella species*, *Listeria monocytogens*, *Campylobacter species* and coagulase positive *Staphylococcus species*(Fusco *et al.,* 2020). The illnesses caused include: brucellosis, tuberculosis, typhoid, paratyphoid and diphtheria (Sarkar, 2015). The high fatality rate (about 20%) and hospitalization rate (>95%) make listeriosis one of the deadliest foodborne diseases (ECDC, 2018). The presence of these pathogens in milk is of public health concern especially with regards to wide spread consumption of milk across populations (Omore *et al.*, 2002). High-quality milk contains a low bacteria count, and is free of human pathogens and antibiotic residues. A study in Accra and Kumasi found the prevalence rates of the various bacteria identified in milk were 1.7–25.9% and 2.6–39.5% respectively (Addo *et al.,* 2011). In Nairobi the mean levels of total viable count in the urban areas was7.52 and 8.18 log10cfu/ml in the slum areas (Wanjala *et al.*,2017). A study across major towns in Kenya on milk quality established that 38.5% of milk samples exceeded allowable bacterial load (Bebe *et al.,* 2018). High prevalence of bacterial contamination has been attributed to unhygienic handling of milk and lack of cooling facilities in Kisumu County (Rademaker *et al.,* 2016).

2.2 Public Health Importance of Milk Contamination

Food borne illness is a serious public health threat. The Centers for Disease Control and Prevention (CDC) estimates that 76 million foodborne illnesses, including 325,000 hospitalizations and 5,000 deaths, occur in the United States each year relates to milk. The leading public health hazard from poor milk safety are microbial (bacterial) followed by chemical milk contaminants (Nyachuba, 2010). Young children, infants, pregnant women, elders

and immune compromised peoples are the primary groups at risk for milk safety problems(Girma *et al.,* 2014). Even though pasteurization is considered a perfect prevention method of biological milk safety hazards, hazards could remain because of insufficiently cleaned equipment, and unhygienic milk handling practices. Toxic chemicals are often from biological sources such as molds and some bacteria. In Kenya, common zoonotic agents commonly associated with consumption of raw milk from cattle that were assessed included: *Brucellaabortus* (the cause of a flu-like illness known as brucellosis) E. coli O157:H7 (may cause bloody diarrhea and acute kidney failure) and *Mycobacterium bovis* (a cause of tuberculosis).

Aflatoxin is a contributor to the burden of foodborne illnesses, deaths and DALYs in Africa with a prevalence of 0.4%, 0.4% and 15% per 100,000 inhabitants respectively (Havelaar *et al.*, 2015). Hepatocellular carcinoma (HCC) is the third leading cause of cancer deaths worldwide, with prevalence 16–32 times higher in developing countries, and aflatoxin exposure contributing to 17% of HCC in sub-Saharan Africa (Liu & Wu, 2010). Acute aflatoxin poisoning leading to acute disease and deaths has continued to occur in several parts of Kenya. However, there is emerging evidence implicating chronic aflatoxin exposure as an important factor in infant growth stunting and immune suppression (Sirma *et al.,* 2019).

The consumption of smaller dosages overtime produces no obvious symptoms as would happen with acute dosage. Thus, it has not attracted much attention in Kenya in terms of public health priorities. Several authors have reported the presence of mycotoxins, especially aflatoxins in the milk of farm animals, when they consume contaminated feeds (Bhat *et al.*, 2010). This activity is common since substandard farm produce is not discarded but instead fed to farm animals. A study by in Sierra Leone (Elzupir & Elhussein, 2010), indicated that the major source of

mycotoxin ingestion by infants was milk. Due to the fear for microbial and chemical contamination in raw milk, consumers may shy away from buying raw milk. Usually milk produced by smallholder dairy farmers is with the intention for direct human consumption. Therefore, control systems and educational systems of teaching how to handle the fresh milk, the hygiene of the cows and the milking, the udder health of the cows, the prevention of zoonotic infection of the cows and the milk, and good feeding practices are necessary. There is need to address the safety of the milk produced by smallholder dairy because this milk is not subjected to regulation.

2.3 Effect of Mazzican on Total Bacterial Counts in Milk

Unhygienic handling is a common problem leading to poor milk quality especially in small holder dairy production and marketing systems without cold chains. Milk containers made from aluminum or stainless steel are usually recommended to address this problem because they are easy to clean and sanitize. However, these metal containers are expensive and unaffordable for many smallholders who often resort to using cheaper but less hygienic containers made from non-food-grade plastics that are also not easy to clean and sanitize (Kurwijila *et al.*,2016). To encourage production of low bacteria count milk, farmers are advised to use good milk production practices. This includes good personal hygiene, udder health and use of sanitary milk vessels. A more affordable food grade plastic container (the "Mazzican") was developed. Kurwijila (2016) tested the Mazzican and found that it is an appropriate vessel for improving the hygiene of milk handling during milking and transportation to the market.

A field testing was done in Mvomero District in Tanzania where fourteen farmers supplying milk to milk traders were provided with Mazzican for use in milking and to deliver milk to traders. Results showed that there was a dramatic reduction of 76.3% in the total bacterial count in raw

milk samples from the pastoralist farmers as a result of switching from jerry cans to the use of Mazzican (Kurwijila *et al.,* 2016). The results confirmed that the Mazzican is a much better container than plastic jerry cans for use in milking, transportation of raw milk and maintaining good bacteriological milk quality under smallholder and traditional cattle milk producer's conditions in Tanzania.

A study in Kiambu County Kenya, revealed that all small-scale farmers used plastic jerri cans to hold their milk, while all large-scale agents used aluminum cans, but microbial results did not differ significantly between small- and large-scale agents despite their diverse equipment (Orregård, 2013). Therefore, it is essential to try an alternative to the current intervention especially for smallholder dairy farmers who are using jerry cans which are hard to clean and are made of non-food grade plastics. Kisumu County, where dairy industry is also dominated by smallholder dairy farmers (Oloo, 2010), is a suitable location for testing the can in Kenya. Because recommended aluminum or stainless containers are expensive and unaffordable to small-scale farmers, there has been a need for developing high quality, yet affordable milk, containers to ensure the milk quality is maintained. Since only one small study has been done to establish the efficacy of Mazzican, further evidence shall be provided by this study.

2.4 Effect of NovaSil Binder on Aflatoxin M1 levels in Milk

In the world, an increasingly higher percentage of dairy cattle are kept in intensive farming systems and are fed on commercially acquired feeds which often are highly contaminated with aflatoxins (Unnevehr & Grace, 2013). Quality and safety of food for both human and animal consumption is a major public health concern with the increase in significant hazards associated with chemicals; aflatoxins being one of them. Mycotoxin binders come in handy when farmers have feeds that are contaminated with or without their knowledge and do not wish to destroy

these feeds. Most toxin binders are mineral clays that prevent the aflatoxins from being absorbed by the intestine. Ingestion of bentonite clay is a promising approach among dietary intervention strategies to reduce aflatoxin exposure from food and feed. A good toxin binder can actually restore the nutritional values of aflatoxin contaminated feedstuffs. Bentonite clays, which are rich in montmorillonite, have been effectively used in dairy cows to diminish the negative effects of aflatoxin exposure (Diaz *et al.,* 2004; Kutz *et al.,* 2009). Findings from a recent clinical intervention study showed that a montmorillonite rich Ca-bentonite (NovaSil) was effective in reducing aflatoxin biomarkers in serum and urine with negligible nutrient interactions in humans naturally exposed to aflatoxins via contaminated foods (Afriyie-Gyawu *et al.,* 2008; Wang *et al.,* 2008).

A study in Mexico was conducted to characterize and compare twelve different additives distributed in Mexico as mycotoxin binders utilizing agents. NovaSil was showed to be the most effective due to its high binding capacity, high absorption efficacy, less activation time and a higher inclusion rate (Marroquin-Cardona *et al.,* 2009). The efficacy of NovaSil clay to reduce aflatoxin B1 biomarkers of exposure was evaluated in 656 blood samples and 624 urine samples collected from study participants during a 3-month phase IIa clinical intervention trial in Ghana. NovaSil was delivered before meals via capsules. Serum AFB1–albumin adduct was measured by radioimmunoassay and urinary AFM1 metabolites were quantified by immunoaffinity-high-performance liquid chromatography (HPLC)-fluorescence methods.

Levels of AFB1–albumin adduct in serum samples collected at baseline and at 1 month were similar (p ¼ 0.2354 and p ¼ 0.3645, respectively) among the placebo, low dose (1.5 g NovaSil day), and high dose (3.0 g NovaSil day) groups. However, the levels of AFB1–albumin adduct at 3 months were significantly decreased in both the low dose group ($p < 0.0001$) and the high

dose group ($p < 0.0001$) compared with levels in the placebo group. Levels of AFM1 in urine samples collected at baseline and at 1 month were not statistically different among the three study groups. However, a significant decrease (up to 58%) in the median level of AFM1 in urine samples collected at 3 months was found in the high dose group when compared with the median level in the placebo group ($p < 0.0391$). In addition, significant effects were found for dose, time, anddose–time interaction with serum AFB1–albumin adduct and dose–time interaction with urinary AFM1 metabolites. The results suggest that capsules containing NovaSil clay can be used to reduce effectively the bioavailability of dietary aflatoxin based on a reduction of aflatoxin-specific biomarkers (Wang *et al.*, 2008). There is no research on smallholder dairy farmer use of NovaSil binder in Africa as well as Kenya. Since ingestion of bentonite clay is a promising approach among dietary intervention strategies to reduce aflatoxin exposure from food and feed, it is needful to conduct further research to establish its effectiveness.

2.5 Conceptual Framework

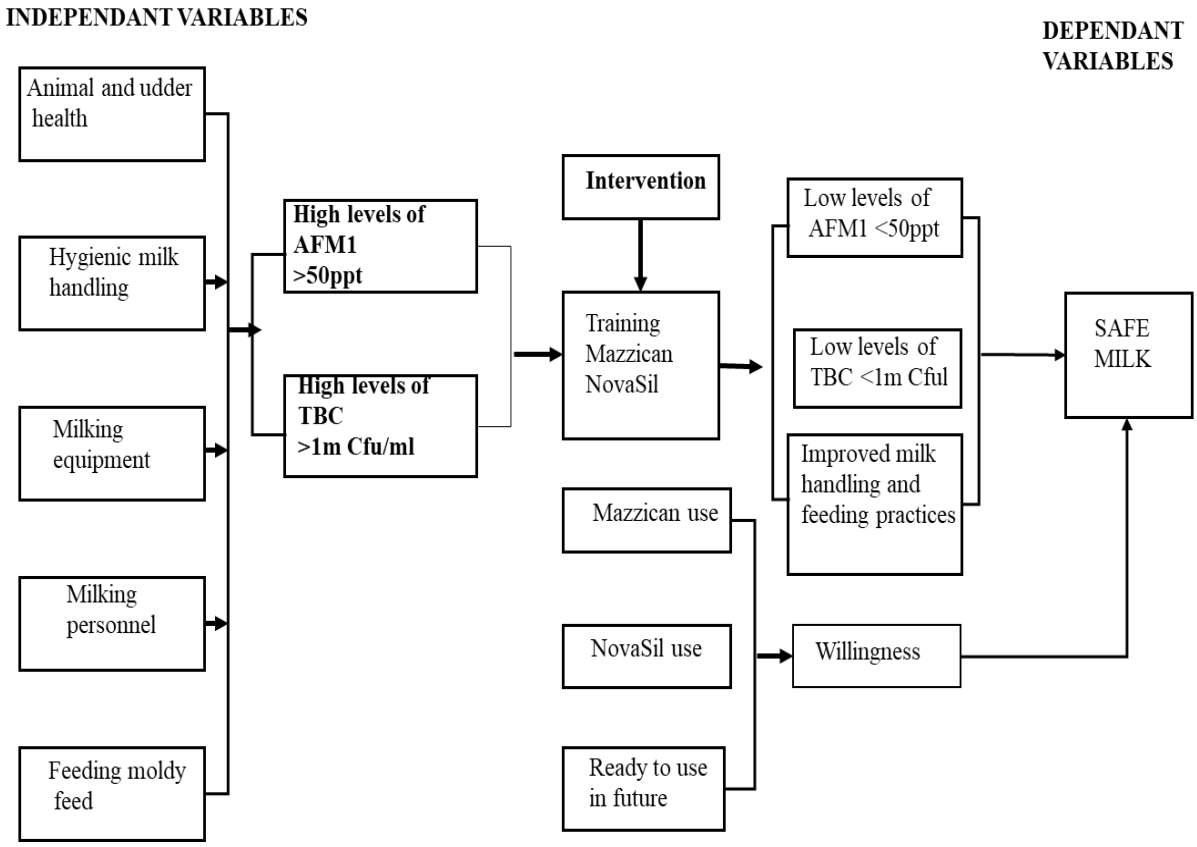


Figure 2.1: Conceptual framework

CHAPTER THREE
METHODOLOGY

3.1 Study Area

Kisumu County has a latitude of -0.091702 ° and a longitude of 34.767956 °. The county consists of 7 sub-counties i.e. Kisumu West, Kisumu Central, Kisumu East, Seme, Muhoroni, Nyando and Nyakach West. It borders Siaya County to the West, Vihiga County to the North, Nandi County to the North East and Kericho County to the East. Its neighbour to the South is Nyamira County and County is to the South West. The County has a shoreline on Lake Victoria occupying northern, western and a part of the southern shores of the Winam Gulf (Appendix IV). The total population of Kisumu County is 968,909 (according to the 2009 National Census). The major economic activities are subsistence farming, livestock keeping, fishing, rice farming, sugar cane farming, and small-scale trading, education, tourism and health.

The county produces 30 million litres of milk annually. The 95% dairy farmers in Kisumu comprising of those in the urban and peri urban areas practice intensive farming due to lack of adequate land for grazing

3.2 Study Design

This study employed a quasi-experimental design. A baseline survey to identify eligible farmers was conducted from a randomly selected group of farmers obtained from a list provided by the veterinary office. One hundred farmers were randomly selected for the baseline survey to determine milk contamination levels, dairy feeding and milk handling practices. Thirty of the farmers identified as producing milk with AFM1 levels above 20ppt were randomized to either receive training, NovaSil binder and Mazzican (20 farmers; intervention) or to receive no intervention (10 farmers; control). There were biweekly visits for three consecutive months to each farm to collect milk samples for determination of both total bacterial counts and levels of

AFM1. Proportions of farmers producing unsafe milk before and after the study were used in combination with willingness to continue using the intervention in future; as a measure of effectiveness.

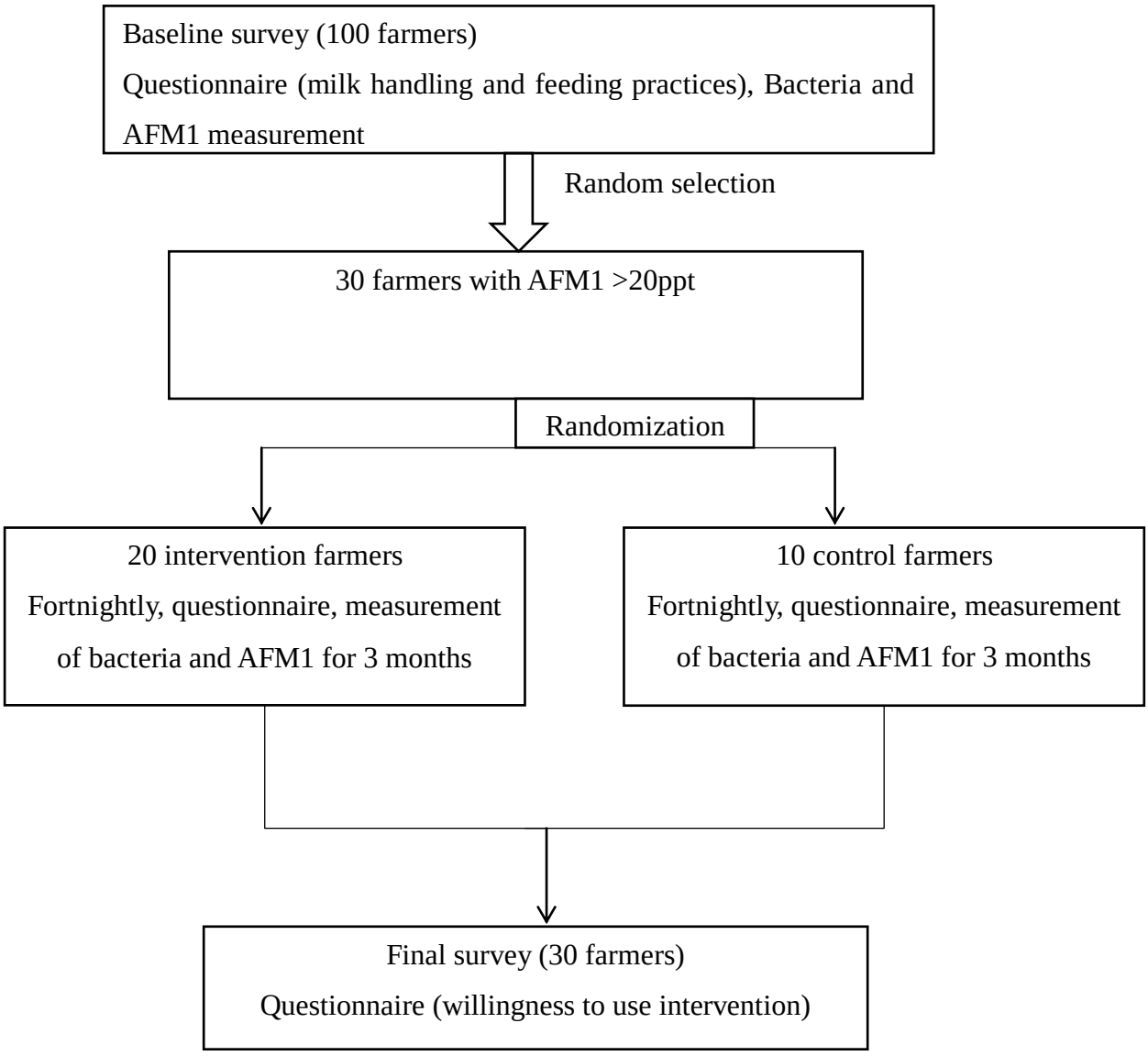


Figure 3.1 Study design

3.3 Target Population

Urban and peri-urban smallholder dairy farmers in the selected study sites were targeted. The documented number of urban and peri-urban small holder dairy farmers in these sub counties is 143 farmers according to the Kisumu Veterinary and Production Department.

3.4 Sample Size Determination

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

Urban and peri-urban areas of Kisumu County in the four sub-counties i.e, Kisumu East, Kisumu West, Nyando and Kisumu Central, in the study have a population size of 143 dairy farms with an average of two dairy animals per farm. The sample size of farms to participate in the baseline survey was calculated using the formula suggested by Daniel (Metcalfe, 2001) indicated below, assuming that 70% would have detectable aflatoxins in the milk and with 10% precision.

$$n' = \frac{NZ^2P(1-P)}{d^2(N-1)+Z^2P(1-P)}$$

Where

n=Sample size with finite population correction,

N=Population size

Z= Z statistic for a level of confidence

P= Expected proportion

d= Precision

n= 143*95^2*0.7(1-0.7)

$$\frac{}{0.05^2(143-1) + 95^2(1-0.7)}$$

n =100 farmers

Lists of dairy farmers in the selected sub-counties were obtained from the county veterinary and livestock production department. Computer based simple random sampling was utilized to identify the 100 farmers from the list hence giving every farmer an equal chance of being part of the baseline survey from which farmers in the intervention study were selected.

3.4.2 Sample Size Determination for the Intervention Study

The number of farmers for inclusion in the intervention study will be calculated using the formula proposed by Metcalfe (2001):0.7 0.2, p (0.5) r(2).Assuming a reduction of farmers producing unsafe milk from 70% to 20%; and using a low power of 50%, and a ratio of 2, a sample of 20 farmers was included in the intervention group, and 10 in the control group. Hence a total of 30 farmers were required for the quasi-experimental study. Farmers producing milk with AFM1 levels above 20ppt were used as a sampling frame to obtain the 30 farmers that were included in the intervention study.

3.5 Inclusion and Exclusion Criteria

3.5.1 Inclusion Criteria for Intervention Farmers

To participate in the study, farmers were expected to meet the following criteria:

i. Must have commercial milk production so that they are not only having milk for their household
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 period.
iv. The cows must get some feed likely to contain aflatoxins, not only fed grass, hay or silage

v. The cows should be intensively managed, no access to pasture grazing.

vi. Consent must be granted by the owner of the farm.

vii. Farmers should be willing to adhere to guidelines in utilizing the inputs provided including the binders and the milk safety cans.

viii. The milk should contain aflatoxins on the baseline analysis

3.5.2 Exclusion Criteria for Farmers

i. Eligible farms whose owners are not resident on the farm

ii. Eligible farms owned by an institution

3.6 Sampling Procedure for the Intervention

From farmers producing milk with AFM1 levels above 20ppt, 10 farmers from the sub-county having more than 10 eligible farmers were randomly selected for the control group to avoid spillover of the intervention; and 20 farmers from all other selected sub-counties were randomly selected for the intervention group.

3.7 Data Collection Tools

A structured questionnaire was used where farmers were asked questions on milk hygiene and feeding practices; Mazzican, NovaSil binder usage and their willingness to use them in future. AFM1 levels was determined by optical density readings from an ELISA reading machine and entered in an excel template for calculation in parts per trillion. Total bacterial counts data was collected by viable counts on the culture plate and data recorded in an excel template for each farmer.

3.8 Piloting of Data Collection Instruments

Questionnaires were standardized and pretested on 20 farmers in Kasarani sub county, Nairobi County. Each question was scrutinized during the pretesting and corrective measures taken to eliminate ambiguity and ascertain conformity with objectives. Investigator bias was minimized

by training each field staff and going through difficulties in the questionnaire beforehand. This ensured that there was uniformity in techniques and approach. Social desirability bias was minimized by explicitly explaining the objectives of the study to the participants and the importance of giving correct information.

3.9 Training of Farmers

Farmers were centrally trained on hygienic milk production including: animal and udder health, cows to be always kept clean and healthy incase a cow is suspected to be sick a qualified veterinary practitioner should be contacted immediately. Checking of mastitis should be done in every milking session and mastitic milk should not be sold or drunk. Mastitis can be controlled by observing general hygiene and proper milking procedures.

Udder hair should be kept short by trimming. Milk from animals undergoing antibiotic treatment should not be consumed or sold until the withdrawal period has elapsed. Hygienic milk handling; maintaining the milking environment clean free from dust and mud, no milking of cows if you are suffering from communicable diseases, no mixing of colostrum with normal milk, wash hands with soap and clean water before milking, wash udder with a clean cloth and warm water, dry the udder with a clean dry cloth, make the first draw into a strip cup to check for mastitis, use clean containers for milking, after milking dip the tits into an “antiseptic dip”, during milking the milker should not have long nails, sneeze, smoke, spit or cough directly on the milk, the cow should be released from the milking area as soon as the milking was finished, milk should be sieved after milking and covered to avoid contamination and finally move the milk to a clean and cool area.

Cleaning of milking containers: thoroughly scrub the container with warm water and soap, dip-rinse in boiling water and air-dry the container in inverted position on a clean rack in the open.

Cows should be well-fed and watered; a good balance of forage and concentrated is important. Concentrates should not be moldy or caked. Cows should not be fed with silage during milking as that will give rise to off-flavors in the milk. Proper feeding practices such as discarding moldy feeds, good aeration of stored feed and storing feeds on raised surfaces. Local awareness of the link between aflatoxins in feed and human health was sensitized. Farmers were also trained on use of Mazzican and NovaSil binder. They were also be provided with more details related to the study (for example, frequency of visits, sampling of milk, access to the binders, how much of these were to be given, the mixing with feed and replacement of cans when lost or damaged.

3.10 Milk Collection and Analysis

3.10.1 Collection of Milk Samples

Milk samples from farmers at their individual farms were collected aseptically in 50ml sterile falcon tubes by trained research assistants, put in cooler boxes then stored in a freezer at -3°C to -6°C in Kisumu East veterinary office then later transported frozen to ILRI laboratory for analyses. Milk samples were collected in duplicates 20-30mls each, one to measure aflatoxin M1 and the other bacteria from each farmer. The intended milk sample to be collected was fresh milk, milked either in the morning or at the time of visit. Farmers were called in advance to be informed of our visit so as to keep a little milk for analyses.

3.10.2 Bacteriological Examination

Bacteriological examination was conducted to determine total bacterial count using total plate count agar (PCA). PCA was made according to manufacturer's instructions; 23.0g of PCA premix was weighed on a weighing balance and put in sterile 1l bottle, 1000ml of distilled water was added and sterilized in autoclave at 121°C for 15minutes. 15ml to 20ml of the medium was poured into sterile petri dishes and allowed to solidify then inverted for storage at 8-15°C. Before use the agar plates were dried in a cabinet for 30 minutes. Peptone salt water (PW) was used for

serial dilutions; 1.0g of peptone and 8.5g of sodium chloride was weighed on a weighing balance and put in 1l bottle and 1000ml of distilled water added. The components were dissolved and heated, if necessary then were sterilized in the autoclave at 121°C for 15minutes. Buffered peptone water (BPW) was used for in initial suspension of the sample. 25.5g of the premix was weighed and put in a 1l bottle, 1000ml of distilled water added and sterilized in the autoclave at 121°C for 15minutes.

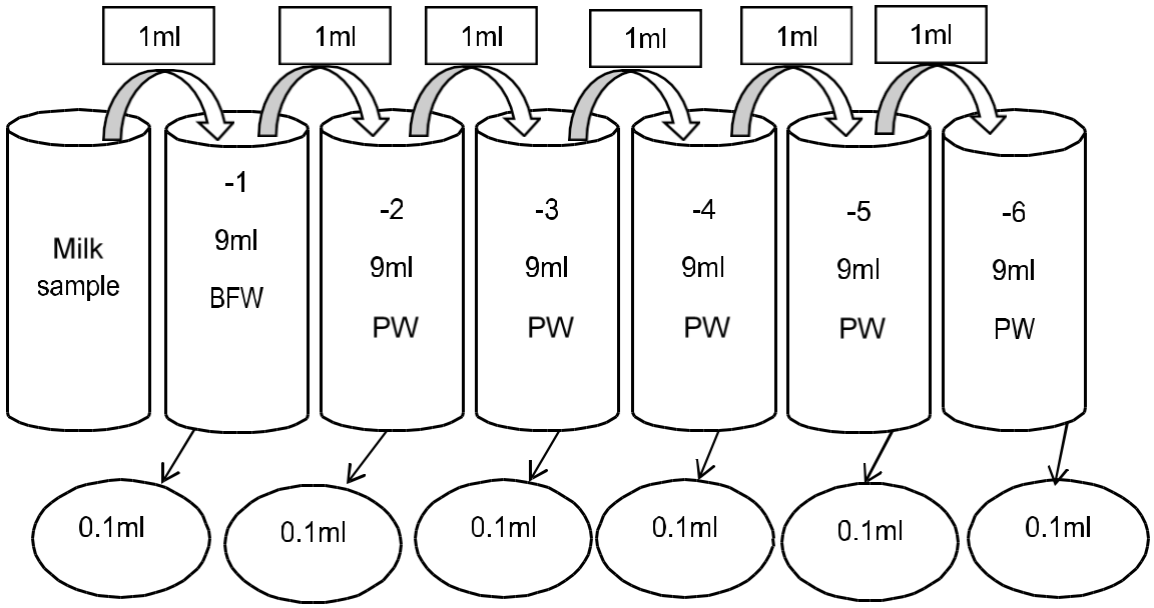


Figure 3.2: Dilution steps

Every dilution was plated in duplicates using the spread plate technique and incubated for 48hours at 30°C. Bacteria colonies of two consecutive dilution steps were counted and presented as colony forming units per milliliter (Cfu/mL). The essential objective was to ascertain the milk hygiene at the sources.

3.10.3 Aflatoxin M1 Analysis

Quantification of AFM1 was done using a commercial Helica® AFM1 ELISA quantitative kit (Helica Biosystems, Inc., Santa Ana, CA 92704, USA, Catalog No. 961AFLM01M-96) according to the manufacturer's instructions. The samples were thawed prior to being analyzed. Reagents were brought to room temperature before use. Samples and 200μl aliquots of the standards were dispensed into appropriate wells in duplicate. The plate was covered with asealing tape to avoid evaporation while providing protection from excess light, then incubated at ambient temperature (19°-25°C) for 2 hours. The contents of the wells were then discarded and washed thrice by filling with PBS-Tween 20® and using a multi-channel pipette.

Wells were tapped on a layer of absorbent paper facing down to remove any residual wash buffer. A 100μl of conjugate was added to each well, resealed and incubated for 15minutes at ambient temperatures. Washing was repeated as earlier described, after which 100µl of enzyme substrate was added to each well and incubated for 15 minutes. The result was a colour change, from clear solution to blue. Reaction was stopped by adding 100µl of "stops" solution and the optical density of each micro well was read with a micro plate reader of 450nm using a differential filter of 630nm.

Samples with AFM1 values above the highest standard concentration were further diluted and the assay conducted again until the AFM1 value quantification fall between the lowest (2ppt) and the highest (100ppt) aflatoxin values in the standards. The limit of detection of AFM1 was 2ppt for this testing method.

3.11 Measurement of Variables

Demographic and farm characteristics were measured and presented as percentages. Proportions of farmers with contamination ofAFM1 and total bacterial count above the recommended limits were determined by the frequencies of milk samples produced by farmers above the

recommended limit. These were presented as percentages of farmers with contaminated milk. Milk hygiene and feeding practices of farmers were measured in percentage of farmers practicing different milk hygiene and feeding practices. 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 parts per trillions for each milk sample collected. Samples with detectable AFM1 (>2ppt) were considered to be contaminated with aflatoxin. Level of AFM1 were determined in ppt and presented as a continuous variable. The levels were categorized according to the manufacturer's manual as; below limit of detection (<LOD), 2-49ppt and above 50 ppt (>50ppt). Total bacterial counts were obtained through milk cultures and were presented as counts in Cfu/ml for each sample. The counts were categorized as Grade 1 (1-199,999 Cfu), Grade 2 (200,000-1,000,000Cfu),) and Not graded (1,000,001 and above) according to Kenya bureau of statistics (KEBS). Willingness of using the intervention in future was determined by the frequency of farmers who agreed to use the intervention after one month upon completion of the intervention.

3.12 Data Analysis and Presentation

Data was entered in Excel and exported to and analyzed in SPSS. Comparisons and cross-tabulations were done and results presented in form of tables and graphs Descriptive statistics was used to analyze data and results presented by measures of central tendency. Percentage of samples with bacteria present, each Grade of Cfu, and AFM1 contamination was presented. Mean, median, maximum and minimum values of Cfu and aflatoxin levels were determined. Outcome of Mazzican and NovaSil was measured by the difference between the control and intervention using t-tests and regression analysis. Where information obtained was qualitative, the analysis established patterns, trends and relations of the information was obtained.

Categorical and nominal data was analyzed using Chi-square test or logistic regression. An alpha value of 0.05 was considered statistically significant.

3.13 Ethical Considerations

The proposal was submitted to the Maseno University School of Graduate Studies and to the Maseno University Ethics and Review Committee and ILRI for approval. Efforts were made to adhere to ethical principles. Informed consent was sought from all participants, local administration, and county dairy stakeholders. The farmers were informed of the intent of the research, its potential benefits and their ability to participate/withdraw from the research at any time they may wish. Study participants were required to sign an informed consent before the beginning of interviews after having read and understood the purpose of the intended research. Each farmer remained with a copy of the consent and brief information of the intended research. Participation in the study was on voluntary basis or free will as no participant was paid in any form. All personal information was kept confidential and solely used for the purpose of the research. The research team respected privacy and confidentiality of information obtained and was only shared with the research team members. All names were removed so that no one would be able to trace back the information to farmers. There were no identified risks on the study. Benefits of the study included provision of training, Mazzican milk container and NovaSil binder for those selected to be part of the trial study. All participants were trained on milk handling and good feeding practices at the end of the study hence all benefited. Data were stored, processed and analyzed by the principal investigator and only shared to supervisors. Ethical principles were emphasized and maintained at all stages of the study.

CHAPTER FOUR

RESULTS

4.1 Demographic Characteristics

During the baseline survey, a total of 97 farmers were interviewed corresponding to a response rate of 97% (n=100). Those interviewed were aged between 20 and 83 years, with a median of 47.5 and most were men (66%). Most of those that had attained upper primary education were men. Most (47.9%) of the respondents were the head of the households, 24% were wives to the household heads, 21.9% were the farm workers, 6.3% were sons and relatives of the household.

Table 4.1: Demographic and farm characteristics of smallholder dairy farmers

Characteristics	**Frequency n(%)**
Gender of farmer	
Male	64(66.0)
Female	33(34.0)
Education level	
Primary	26(26.7)
Secondary level	42(43.3)
Tertiary level	29(30.0)
Occupation	
Farmer	71(73.2)
Formal employment	12(12.4)
Casual employment	14(14.4)
Farm characteristics	Mean ±sd
Livestock species	
Cattle	5.4±6.9
Sheep	2.6±04
Goats	1.3±9.4
Pigs	1.1±5.9
Poultry	29.4±69.3
Milk production (l)	13.0±15.8
Price per liter(Ksh)	63±29.9
Number of milking cows per farm	2.83±1.95

Data on demographic characteristics of respondents presented as absolute numbers (n) and percentages in bracket.

Different livestock species were kept by study farmers. Most (90.7%) households practiced zero

grazing, 47.4% also practiced tethering and 22.8% pasture grazing. The minimum number of milking cows per farm was 1 while the maximum was 10. Most farmers (94%) milked their cows two times per day i.e. morning and evening, 4.8% milked once daily, while only 1.2% milked three times daily. The range of milk production per day was between 1- 80 liters. The maximum milk yield per cow was 27 liters while the least produced 250 milliliters per day. Farmers used different transport modes to deliver milk to their customers ranging from customers coming to purchase milk from the farm to being transported to the market via motorcycles or bicycles. In most cases (73.4%) it was the customers who visited the farms to purchase the milk.

4.1.1 Milk Safety Intervention

In the initial survey, milk samples were obtained from77 of the 97 farms visited. From the 77 farms, 30 (20 intervention and 10 control) farms having AFM1 levels above 50ppt were randomly selected and visited biweekly for 3 months.

4.2 Proportion of Farmers Producing Milk with Levels of Aflatoxin M1 and Total Bacterial Counts above the Recommended Limits

This objective sought to determine the proportion of farmers producing unsafe milk.

4.2.1 Aflatoxin M1 Contamination in Milk

Milk contamination with Aflatoxin M1 was categorized according to the Helica categories (Imtiaz & Yunus, 2019). The mean AFM1 contamination was 32.06 ppt ±sd 43.48 ppt. The minimum AFM1 level found was 0.36 ppt and the maximum 164.13 ppt. Farmers producing milk contaminated with AFM1 above the recommended limit of 50 ppt were 28.6%.

Table 4.2: Proportions of raw milk samples in the Helica categories of AFM1 contamination level

Levels of AFM1 in raw milk	Total n (%)
	n = 77
Below limit of detection(<LOD)	30 (39.0)
2-49ppt	25 (32.4)
Above 50ppt	22 (28.6)

Data presented as absolute numbers (n) and percentages in bracket. Raw milk samples were tested for AFM1 by ELISA. Milk samples with 50 ppt were considered to be contaminated. The proportion of farmers producing milk with exceeding permissible limit was 28.6%.

4.2.2 Bacteria Contamination in Milk

The EAC Grades for raw milk were used to categorize levels of contamination found in the milk. Raw milk is judged as being of low quality if it contains more than 1 million Cfu/ml (<$\log_{10}$ 6Cfu/ml). A total of 36.4% samples were of low quality. The mean total bacterial count was 1.5×10^{7} Cfu/ml sd ± 5.4×10^{7} Cfu/ml. The minimum count was 333.8 Cfu/ml while maximum was 2.3×10^{8} Cfu/ml. The proportion of farmers producing bacteria contaminated milk was 36.4%.

Table 4.3: Proportion of farmers producing milk in the KEBS/EAC categories of Grades for raw milk samples

EAC Grades for raw milk	Totaln (%)
	n=77
Grade 1 (1-199,999Cfu/mL)	41 (53.2)
Grade 2 (200,000-1,000,000Cfu/mL)	8 (10.4)
Not graded (1,000,001 and above)	28 (36.4)

Data presented as absolute numbers (n) and percentages in bracket. Milk samples with 1.0×10^{6} Cfu/ml (>$\log_{10}$ 6 Cfu/ml) were considered to be contaminated.

4.2.3 Aflatoxin M1 and Bacterial Contamination in Milk

Proportions of farms producing milk with aflatoxin and/or bacterial contamination are provided in table 4.4. Contamination of both AFM1 and bacteria at levels above the recommended was found in 13% of milk samples.

Table 4.4: Proportion of farmers producing milk contaminated withAFM1 and/or bacteria.

Contamination	Total n (%)
	n = 77
AFM1 only	22 (28.6)
Bacteria only	28 (36.4)
Both aflatoxin and bacteria	10 (13.0)
No contamination	17 (22.0)

Data presented as absolute numbers (n) and percentages in bracket. Raw milk is safe if it has low levels of AFM1 (<50ppt) and TBC (<$\log_{1\ 0}$ 6Cfu/ml). A total of 44 (57.2%) of samples collected had AFM1 and TBC levels beyond the recommended limits with 10 (13.0%) having both high levels of AFM1 and TPC above the recommended limits.

Similarity of farms selected for participation in the trial was assessed to ensure that farms included in the trial had similar levels of AFM1 and bacteria contamination. There was no difference in contamination levels for either Aflatoxin or bacterial contamination. Results are presented in table 4.5.

Table 4.5: Proportion of farmers in the trial producing milk contaminated with AFM1 and TBC at baseline

Levels of AFM1 and TBC in raw milk	Total n (%)	Control group n (%)	Trial group n (%)	*p* value
	n = 30	n = 10	n = 20	
Below limit of detection(<LOD)	0 (0.0)	0(0.0)	0 (0.0)	
2-49ppt	13 (43.3)	5 (50.0)	8 (40.0)	0.602
Above 50ppt	17 (56.7)	5 (50.0)	12 (60.0)	
Grade 1 (1-199,999Cfu/ml)	18 (53.2)	6 (60)	12 (60)	
Grade 2 (200,000-1,000,000Cfu/ml)	3 (10.4)	1 (10)	2 (10)	1.000
Not graded (1,000,001 and above)	9 (36.4)	3 (30)	6 (30)	

Data presented as absolute numbers (n) and percentages in bracket. Milk samples with 50 ppt were considered to be contaminated. Milk samples with 1.0×10^{6} Cfu/ml were

considered to be contaminated. Comparison of levels of AFM1 and TBC between groups was performed using chi-square test. Statistical significance was set a $p \leq 0.05$.

4.3 Milk Hygiene and Livestock Feeding Practices of Farmers

Objective 2 sought to assess hygiene and livestock feeding practices of farmers to determine if the intervention resulted in improvement.

4.3.1 Milk Hygiene Practices

Data on farmer hygiene practices is presented in table 4.6. There was no difference in hygiene practices between farmers in the intervention and control group at baseline.

Table 4.6: Milk hygiene and feeding practices farmers at baseline

Farmer practice	Activity	Control group n(%)	Trial group n(%)	*p* value
		n = 10	n =20	
Cleaning of milking	Daily cleaning of milking shed	7 (70.0)	19 (95.0)	0.058
	Thorough cleaning	6 (60.0)	18(90.0)	0.053
Washing of hands before milking	Yes (Water and soap)	6 (60.0)	14 (70.0)	0.584
	No	4 (40.0)	6 (30.0)	
Cleaning udder before milking	Yes (warm water)	9(90.0)	(100.0)	0.150
	No	1 (10.0)	0(0.0)	
Drying teats	Yes (With towel)	8 (80.0)	15 (75.0)	0.760
Cleaning of milking equipment	Yes(water and soap)	10(100.0)	19(95.0)	0.472
	No	0(0.0)	1(5.0)	
Checking mastitis	Yes	7 (70.0)	14 (70.0)	0.417
	No	3 (30.0)	6 (30.0)	
Milk processing	Yes	10(100.0)	20(100.0)	1.000
Storage equipment	Non-food grade container	10 (100.0)	20 (100.0)	1.000
Feed monitoring	Yes	5(50.0)	13(65.0)	0.429
	No	5(50.0)	7(35.0)	
Storage facility	Yes	7(70.0)	16(80.0)	0.352
	No	3(30.0)	4(20.0)	
Action on moldy feed	Discard	7(70.0)	16(80.0)	0.542
	Still give animals	3(30.0)	4(20.0)	

Data presented as absolute numbers (n) and percentages in bracket. Milk handling and feeding practices of farmers at baseline. Chi- square test was performed to determine if there were any differences at baseline between the two groups so as to adjust for baseline differences at trial level.

Milk hygiene practices at the end of the intervention are presented in table 4.7. Significantly more farmers in the intervention group practiced farmers daily cleaning of the milking shed, cleaning of the udder before milking and routine checking of mastitis. There were no differences between groups in the other practices assessed.

Table 4.7: Farmers' milk hygiene practice sat the end of the intervention.

Cleaning activity	Activity	Control group n(%)	Trial group n(%)	*p* value
		n = 10	n =20	
Cleaning of milking shed	Daily cleaning of milking shed	6 (60.0)	20 (100.0)	**0.002**
	Thorough cleaning	6 (60.0)	17 (85.0)	0.127
Washing of hands before milking	Water and soap Water alone	10 (100) 0 (0.0)	18 (90.0) 2 (10.0)	0.301
Cleaning udder before milking	Warm water, Not done	8 (80.0) 2 (20.0)	20 (100.0) 0 (0.0)	**0.038**
Drying teats	With towel	7 (70.0)	18 (90.0)	0.166
Cleaning of milking equipment	Water and soap Do not do actual cleaning	9 (90.0) 1 (10.0)	20(100.0) 0 (0.0)	0.244
Checking mastitis	Yes No	7 (70.0) 3(30.0)	20 (100.0) 0	**0.010**

Data presented as absolute numbers (n) and percentages in bracket. Farmers in the trial group were trained on standard milk hygiene and handling practices while those in the control group continued with their usual practices. The milk hygiene and handling practices between groups were compared using chi square test with $p<0.05$ considered statistically significant. Any group that had $p>0.05$ there was need to adjust for any baseline differences.

All intervention farmers 100% washed the milk equipment with water and soap which was 10% higher than those in the control group. Farmers in the intervention group were, more likely to

dry the animals' teats before milking (OR=3.9; 95% CI:) and to check for mastitis than those in the control group where (100% and 70% farmers, respectively) regularly checked.

4.3.2 Milk Processing and Storage

Data on milk processing and storage practices are presented in table 4.8

Table 4.8: Farmers' milk processing and milk storage practices after intervention.

Cleaning activity	Activity	Control group n(%)	Trial group n(%)	*p* value
		n = 10	n =20	
Milk processing				
	Boiling	4(40.0)	7 (35.0)	
	Refrigeration	5 (50.0)	10 (50.0)	0.918
	Not processing	1 (10.0	3 (15.0)	
Storage equipment	Jug	3 (30.0)	0 (0.0)	
	Jerrican	7 (70.0)	2 (20.0)	**0.000**
	Mazzican	0 (0.0)	18 (80.0)	

Data presented as absolute numbers (n) and percentages in bracket. Comparison between groups was performed using chi-square test. There was no statistical difference on milk processing strategies used by farmers p=0.918. In contrary, There was a significant statistical difference in storage equipment used by farmers in two groups p<0.001. Baseline differences were adjusted for the two groups on storage equipment used by farmers.

4.3.3 Feeding and Feed Storage Practices

Farmers were asked on the types of feeds they gave their dairy cows and the source of concentrate feeds. This was sought because binders are used alongside concentrate feeds. All farmers (100%) measured concentrate feeds using a 2kilogram tin.

Table 4.9: Proportions of types of feeds used by farmers and source of concentrate feeds

Type of feed	Frequency n(%)
Hay	27(90.0)
Cut and carry	30(100.0)
Concentrates	30(100.0)
Dairy meal	24(80.0)
Cotton seed cake	3(10.0)
Maize germ	3(10.0)
Silage	5(16.7)
Molasses	18(60.0)

Source of concentrate feed	
Local vendors	30(100.0)

Data presented as absolute numbers (n) and percentages in bracket of different types of feeds used by farmers.

Table 4.10: Farmers' dairy feeding and feed storage practices after intervention.

Feeding practice	Activity	Control group n(%)	Trial group n(%)	*p* value
		n = 10	n =20	
Feed monitoring	Routinely monitored	7(70.0)	16 (80.0)	0.542
Storage facility	Storage facility present	7 (70.0)	16 (80.0)	0.542
Action on moldy feed	Discard moldy feed	6 (60.0)	15 (75.0)	
	Still feed to cows	3 (30.0)	2 (10.0)	
	Use as manure	1 (10.0)	3 (15.0)	0.379

Data presented as absolute numbers (n) and percentages in bracket. Results show farmers in the two groups routinely monitored feeds while in storage however there was no significant difference p=0.429. There was no statistical difference in farmers having a storage facility p=0.542. Despite training of farmers that moldy feed should be discarded, 10% in the trial group still fed their animals.

There were no differences in dairy feeding and feed storage practices between farmers in the intervention and control groups at the end of the intervention.

4.4: Effect of Mazzican and NovaSil Binder on TBC and AFM1 Level

The objective to determine the effect of Mazzican and NovaSil binder on TBC and AFM1 levels in milk was to assess whether the intervention resulted in improved milk safety. The mean levels of both TBC and AFM1 at baseline for both control and trial farms were similar.

Table 4.11: Difference in aflatoxin and bacterial contamination between intervention and control groups at baseline and at the end of the intervention

Group	Control Group	Trial Group	*p* value
	n = 10	n = 20	
Mean TPC at baseline	1.9×10^{7} $\pm6.1\times10^{7}$	1.3×10^{7} $\pm5.1\times10^{7}$	0.751
Mean AFM1 at baseline	68.64±59.07	58.98±36.75	0.585
Mean TBC(Cfu/ml)	3.5×10^{7} $\pm7.8\times10^{7}$	$5.6\times10^{3}\pm3.3\times10^{3}$	**0.046**
Mean AFM1(ppt)	191.18±113.75	10.02±12.08	**0.001**

Independent t-test showed that there was a statistically significant difference in means between the control and trial group for TPC and AFM1 levels in raw milk; p=0.046 and p<0.001 respectively

At the end of the intervention, there was statistically significant difference in the mean levels of TBC between the control and intervention farms. Assumed variances were equal across the two groups, p=0.54.Milk produced by farmers who used Mazzican had lower levels of TBC Cfu/ml compared to those who did not t(28)= -11.273, p<0.01. Bivariate linear regression showed that use of Mazzican reduced observed TBC in milk by 5.2×10^7 Cfu/ml$p$<0.05 and the range of reduction was between 1.6×10^7 -8.8×10^7 Cfu/ml.

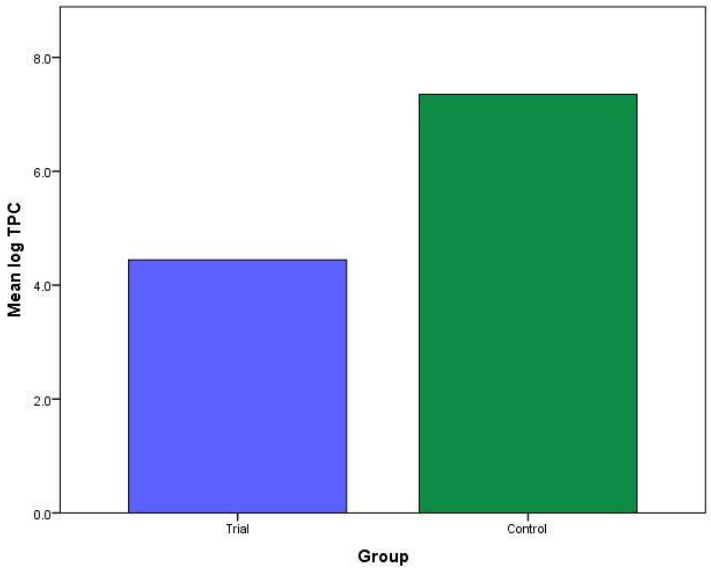


Figure 4.1: Mean log TPC (Cfu/ml) in milk produced by farmers in the control and trial group.

Levene's test for equality of variances showed that there was an assumption of equal variances p= 0.076. There was a difference in the mean AFM1 levels in milk produced by the intervention farms between the two trial groups t (28)= -8.265, p<0.001(table 11).

Bivariate linear regression showed that use of NovaSil binder reduced AFM1 levels in milk by 188.76ppt and the range of reduction expected is 136.23-241.29ppt p<0.001.

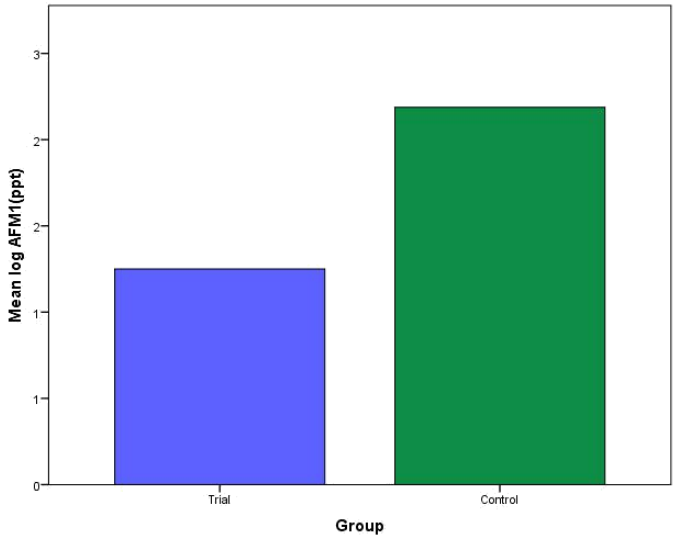


Figure 4.2: Mean log AFM1 (ppt) in milk produced by farmers in the control and trial group

At the end of the intervention there was a difference between the two groups in mean AFM1 and TBC levels in milk produced table 4.12.

Table 4.12: Proportion of farmers in the trial producing milk contaminated with AFM1 and TBC at after intervention

Levels of AFM1 and TBC in raw milk	**Total n(%)**	**Control group n(%)**	**Trial group n(%)**	***p* value**
	n = 30	n = 10	n = 20	
Below limit of detection(<LOD)	0 (0.0)	0(0.0)	7(35.0)	
2-49ppt	14 (48.3)	1 (11.1)	13 (65.0)	**0.007**
Above 50ppt	15 (51.7)	8 (88.9)	0 (0.0)	
Grade 1 (1-199,999Cfu/mL)	17 (56.7)	0 (0.0)	17(85.0)	
Grade 2 (200,000-1,000,000Cfu/mL)	3 (10.0)	0 (0.0)	3 (15.0)	**0.000**
Not graded (1,000,001 and above)	10 (33.3)	10 (100.0)	0 (0.0)	

Data presented as absolute numbers (n) and percentages in brackets. Chi-square test showed that there were differences in AFM and TBC between the two groups

4.5 Willingness of Farmers to use the Intervention for Safer Milk Production

Willingness of farmers to use the intervention in future was assessed to determine the potential for scaling up the intervention. Results are presented in table 4.13. Eighty-seven percent of farmers were willing to use the intervention. Farmers were asked where they would prefer to procure the intervention tools.

Table 4.13: Proportion of farmers willing to use the intervention in future

	Frequency
	n(%)
Willingness to use intervention	26 (86.7)
Farmers still using the binder after final visit	27(90.0)
Farmers still using Mazzican after the final visit	30(100.0)
Suggested source of procurement of Novasil	
From veterinary and livestock production office	27(90.0)
Factory	3(10.0)
Challenges of binder usage	
Lack of supply source	30(100.0)
Inadequate concentrates to mix with the binder	5(16.7)
Reduction of milk viscosity	3(10.0)
Milk being light upon boiling	2(6.7)
Uses of Mazzican by farmers	
Checking mastitis	28(93.3)
Milking	28(93.3)
Milk storage	25(83.3)
Transportation of milk to the market	20(66.7)
	Mean
Amount farmers were willing to pay for Mazzican (Kshs)	500
Amount farmers were willing to pay for NovaSil binder (Kshs)	300/2kg

Data presented as absolute numbers (n) and percentages in brackets. The proportions of farmers willing to use the intervention in future, who were still using the intervention after the final visit, challenges farmers faced while using the intervention and the price they were willing to pay for the Mazzican and binder.

At the end of the study, 10% of farmers were not using NovaSil binder and when asked why, they said it was due to lack of supply when it ran out of stock. Farmers were asked if they faced

any challenges while using the intervention tools, they reported a few challenges regarding the binder use such as lack of supply from local agrovets, lack of concentrate feeds to mix with the binder, reduction in milk viscosity and milk turning black upon boiling as in **table 12**. All parts of the Mazzican were present and clean during the final visit. Mazzican was used by farmers to check mastitis, milking, storage and transportation of milk to the market.

CHAPTER FIVE
DISCUSSION

5.1 Introduction

This study sought to determine effectiveness of a combined milk safety intervention by smallholder dairy farmers on bacterial and aflatoxin M1 contamination in milk in Kisumu County. The respondents comprised smallholder dairy farmers in the urban and periurban areas of Kisumu County. Their ages ranged from twenty to eighty- three years old with majority being 27 years old. More males than females practiced dairy farming in this population. In addition, more males than females were heads of households and had attained at least upper primary education. As also observed by Muraya *et al.*, (2018), farming was the respondents' major source of income for these Kisumu farmers. Dairy cattle were kept under intensive farming due to inadequate land capacity in the urban and peri-urban areas. The dairy cattle reared included exotic breeds, crosses between exotic and local breeds and local breeds. Beside dairying farmers also kept other livestock species (table 1) which supported their source of income and food. Milking was done 1, 2 and 3 times a day with an average production of 13litres per farm per day and a range of 1 to 80 litres per day. Milk produced was majorly sold to neighbors and in local markets; and was also consumed by farmers' family members. This study corroborates reports by Thornton (2010), that dairy production plays an important role in improving farmers' livelihoods in Kenya.

The first objective sought to determine the proportion of farmers producing milk with levels of total bacterial counts and aflatoxin M1 above the recommended limits in order to determine the proportion of farmers producing unsafe milk as defined in this study. A total of 70.2% smallholder dairy farmers in the study sites produce, sell and consume milk contaminated with

aflatoxin and or bacteria. Milk produced was more likely to be contaminated with bacteria than aflatoxin M1.

The findings indicate thatof the 70.2% contaminated raw milk samples, 61% had detectable levels of aflatoxin M1contamination ranging from 0.36-164 ppt; and 28.6% of farmers produced milk that had AFM1 levels above the recommended limits. It would be desired that no samples could have any detectable levels of AFM1. The mean level (32.06ppt) of AFM1 in milk in Kisumu was in the range observed in previous studies in other countries by Al Zuheir & Omar(2012) and GonÇAlves *et al.*,(2017), in Palestine and Brazil respectively, that recorded comparable or lower prevalence rates (Duarte *et al.,* 2013) of AFM1 in milk with samples above the EU limit of AFM1 levels at 36.2%, 20% respectively compared to the current study. This indicates AFM1 although occurring in a wide range, with levels in Kisumu being in the middle range, and lower than other areas, milk produced by small-holder farmers is a source of exposure to aflatoxin. Due to the health related risks of aflatoxin M1 exposure even in small amounts then reducing the high proportion of farmers producing AFM1 contaminated milk is recommended.

Assessment of bacteriological quality of milk produced by smallholder dairy farmers reflects the hygienic quality of raw milk they produce. This study reported that 36.4% of samples collected had TBC levels above the recommended limit of 1 million Cfu/ml for total bacteria counts in raw milk. This was comparable with a study in Kenya where 38.5% samples exceeded the allowable load limits in Kisumu (Bockline 2018). A study in Nairobi had 94.9% of raw milk samples having total viable counts above the recommended limit (Wanjala *et al.,* 2017). This study is in contrast with that in Zambia where all samples collected were below the recommended limit (Knight-Jones *et al.*,2016), this means that with standard milk storage containers, improved

personal and cow hygiene, good milk handling and unlimited access to appropriate infrastructure for milk chilling and storage farmers may be able to produce quality milk.

The unique finding was that 13% of farmers produced milk that had both high levels of AFM1 and TBC. Most studies that have been done focus on bacteria and AFM1 contamination independently, this is the first study to obtain the percentage of samples that are contaminated with high levels of both bacteria and AFM1in raw milk produced by smallholder dairy farmers.

The second objective sought evaluate the milk hygiene and feeding practices of farmers between the control and intervention group after training. Milk handling practices such as cleaning of milking shed, washing of hands before milking, cleaning udder before milking, drying teats, cleaning milking equipment, checking mastitis are important for bacteriological quality of milk. This study reported that after training there were improvements in cleaning of the milking shed, cleaning of udder before milking, checking mastitis and the use of Mazzican for milk storage. These improved practices were also reported in studies done in Uganda, Rwanda and Ethiopia (Byarugaba *et al.*,2008; Mpatswenumugabo *et al.,* 2017;Kebede & Megerrsa 2018; Bereda *et al.,* 2012). Farmers regularly cleaning and providing dry conditions are vital to minimize the growth of pathogenic microorganisms because these practices expose the teat end to wet and muddy barns increase the risk of occurrence of mastitis and milk contamination (Bereda *et al*., 2012). Training did not result in difference in other milking and milk handling practices such as thorough cleaning of the milking shed, washing of hands before milking, drying teats with towel, cleaning of milking equipment and milk processing procedures. However, more farmers in the intervention group performed these practices than those in the control group. Both boiling and refrigeration are good milk storage practices. This study reported no difference in milk processing procedures between the two groups. Milk was majorly either refrigerated or boiled

before being sold or consumed. This result is similar to that reported in Wolayta Sodo where there was no difference in the milk processing practices even after training the farmers in chilled or boiled their milk immediately after milking (Benta & Habtamu, 2011). In the current study, farmers were trained only once rather than continuously and this may have contributed to change only in some behaviours.

Findings in this study showed that farmers give different types of feeds to their dairy cows. Feeds such as forage, hay, concentrates, molasses and silage were used by farmers. Similar results on types of feeds were also reported in Ethiopia and Kenya (Duguma & Janssens, 2016; Muia *et al.,* 2011; Muraya *et al.,* 2018). Njarui (2011) reported between 88 and 92% of farmers provided their cows with concentrates which is in agreement with the current study. In Kenya, Muia (2011) in Nyandarua reported a similar percentage (38%) in use of hay grass but lower levels of use of fodders and concentrate supplements corresponding to 41 and 44% respectively. Most intervention farmers reported that they would discard feed when they noticed mold growth while a few reported to mix it with good feeds and still give the cows or used them as manure. This was similar for both the control and intervention farmers. It is evident that to some of the dairy farmers in both groups, the presence of molds on feeds did not motivate destruction of feeds, and that they would still give it to their animals, albeit in small quantities by mixing it with good feed. Similar results were also reported whereby farmers regularly fed cows with low quantities moldy feeds to potentiate milk production in Kenya (Kiama et al., 2016) which leads to AFM1 contamination in milk.

The number of farmers with feed storage facilities was not different in the two groups. More farmers in the intervention group were seen to routinely check their feed while in storage, compared compare to those in control group. Use of storage facilities by farmers is encouraged

to reduce exposure of feeds to unpredictable environmental conditions temperature, pests and animals. The present findings were similar to that by Mwende *et al.*,(2016) where 64% of peri-urban farmers had storage facilities. Routine feed monitoring while in storage is important because it reduces mold growth, infestation with pests and animals and dryness.

In summary, this study showed that training improved farmer behavior in milking and milk handling practices. However, there were no improvement in feeding practices, thorough cleaning of the milking shed and drying teats. This may be attributed to the one day training that was given to farmers which could be insufficient and if farmers would have been trained for more days there would be more time to emphasize on the recommended standards. However, lack of improvement in these practices should be explored.

The third objective sought to determine the effect of Mazzican and NovaSil binder on TBC and the levels of AFM1 in milk. This study reported a significant decrease of 188.76ppt in the level of AFM1 in milk between the intervention and control group. This is the first community based study that shows that use of NovaSil binder reduces AFM1 in milk produced by dairy farmers. This shows that it is a viable intervention by small-scale dairy farmers to reduce aflatoxin contamination in milk. The bacteriological quality of milk produced by farmers who used Mazzican improved considerably compared to those who did not. This shows that with all factors remaining the same, introduction of the Mazzican had a positive impact on improving milk quality of the intervention farmers. This corroborates findings in Tanzania where there was a 76.3% reduction in the TBC in raw milk from the farmers who used Mazzicans. The use of Mazzican can therefore greatly reduce bacterial contamination in milk.

The current study therefore shows that the introduction of combined Mazzican and NovaSil binder effectively reduces the levels of bacterial and AFM1 contamination in milk produced by smallholder dairy farmers to safe levels. Since all the available studies are controlled trials and this study is the first study to roll out NovaSil binder at the community level hence more studies are recommended to confirm the effectiveness of its use in the community.

The fourth objective sought to establish if farmers were willing to use the intervention in future for sustainability. Majority of farmers, 86.2%% were willing to use the intervention in future so as to produce and sell safe milk. Farmers who used Mazzican found them to be durable, efficacious, easy to clean and acceptable. Mazzicans are being used in several countries in the region including Ethiopia where a modified product is in use. Similar findings reported in Mvomero District by Lusato (2016) found the containers to be acceptable and efficacious. Sustainability is assured as Mazzican and Novasil binder are locally-produced. Farmers in this study also confirmed to use NovaSil binder in future since they are unable to test regularly for aflatoxin contamination at farm level.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary of findings

1. Most of the smallholder dairy farmers in the urban and peri-urban areas of Kisumu County produce milk that is contaminated with AFM1 and or bacteria.
2. Training farmers on hygienic milk handling and feeding of dairy cows resulted in improved cleaning of the milking shed, cleaning of udder before milking, checking mastitis and the use of Mazzican for milk storage. However, thorough cleaning of the milking shed, washing of hands before milking, drying teats with towel, cleaning of milking equipment and milk processing procedures did not improve. It also did not improve farmers' dairy cows feeding practices.
3. Use of Mazzican and NovaSil binder reduced bacteria and AFM1 levels in milk to safe levels.
4. Majority of farmers were willing to use the intervention in future so as to produce and sell safe milk

6.2 Conclusions

1. A high proportion of smallholder dairy farmers in the urban and peri-urban areas of Kisumu County produce milk that is contaminated with bacteria, AFM1 or both. This means that smallholder dairy farmers are a major source of exposure of persons consuming milk produced in their farms to bacteria and AFM1.
2. Training farmers on hygienic milk handling and dairy cow feeding practices improved cleaning of the milking shed, cleaning of udder before milking, checking mastitis and the use of Mazzican for milk storage

3. The use of Mazzican and NovaSil effectively reducedbacteria and AFM1 in milk thus farmers are able to produce and sell safer milk.
4. The farmers are willing to use the intervention in future therefore establishing that the intervention is acceptable and has potential to be sustainable and therefore can be scaled up.

6.3 Limitations of the Study

The intervention sample size was based on milk contamination with AFM1 which favoured detection of reduction in TBC and AFM1 but may have compromised the ability of the study to detect small differences in farmer behavior with respect to hygiene in milking practices may have been limited. This was a trade-off in this study. Confirmation of the effectiveness of training especially for behaviours whose differences could not be detected by this study should be done in a larger study.

6.4 Recommendations

6.4.1 Policy recommendation

The large proportion of smallholder dairy farmers in the urban and peri-urban areas of Kisumu County point to the need for interventions to reduce milk contamination with bacteria and AFM1 in this group of farmers.

There is also need for continuous education to farmers on good milk handling and feeding practices for safer milk production in order to successfully achieve adoption of hygienic milk handling and good dairy cows feeding practices by these farmers.

Use of Mazzican and NovaSil binder should be scaled up among smallholder dairy farmers to reduce bacterial and AFM1 contamination of milk they produce.

6.4.2 Recommendation for Further Studies

Further studies are recommended to explore why some milk handling and feeding practices did not improve despite training.

More studies should be done to confirm the effectiveness of NovaSil binder at the community level.

REFERENCES

Addo, K., Mensah, G., Aning, K., Nartey, N., Nipah, G., Bonsu, C., . . . Smits, H. (2011). Microbiological quality and antibiotic residues in informally marketed raw cow milk within the coastal savannah zone of Ghana. *Tropical Medicine & International Health, 16*(2), 227-232.

Afriyie-Gyawu, E., Wang, Z., Ankrah, N. A., Xu, L., Johnson, N. M., Tang, L., . . . Phillips, T. D. (2008). NovaSil clay does not affect the concentrations of vitamins A and E and nutrient minerals in serum samples from Ghanaians at high risk for aflatoxicosis. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess, 25*(7), 872-884.

Al Zuheir, I. M., & Omar, J. A. (2012). Presence of aflatoxin M1 in raw milk for human consumption in Palestinian. *Walailak Journal of Science and Technology (WJST), 9*(3), 201-205.

Applebaum, R. S., Brackett, R. E., Wiseman, D. W., & Marth, E. H. (1982). Responses of dairy cows to dietary aflatoxin: feed intake and yield, toxin content, and quality of milk of cows treated with pure and impure aflatoxin. *Journal of Dairy Science, 65*(8), 1503-1508.

Bebe, B. O., van der Lee, J., & Kilelu, C. W. (2018). Milk Retailing Practices and Quality Compliance in Urban Kenya.

Benta, D. B., & Habtamu, T. M. (2011). Study on prevalence of mastitis and its associated risk factors in lactating dairy cows in batu and its environs, Ethiopia. *Global veterinaria, 7*(6), 632-637.

Bereda, A., Eshetu, M., & Yilma, Z. (2014). Microbial properties of Ethiopian dairy products: A review. *African Journal of Microbiology Research, 8*(23), 2264-2271.

Bereda, A., Yilma, Z., & Nurfeta, A. (2012). Hygienic and microbial quality of raw whole cow's milk produced in Ezha district of the Gurage zone, Southern Ethiopia. *Wudpecker Journal of Agricultural Research, 1*(11), 459-465.

Bhat, R., Rai, R. V., & Karim, A. A. (2010). Mycotoxins in food and feed: present status and future concerns. *Comprehensive Reviews in Food Science and Food Safety, 9*(1), 57-81.

Bockline Omedo Bebe, J. v. d. L. a. C. W. K. (2018). Quality compliance of traded milk in urban retail outlets in Kenya. *3R Kenya Project Practice Brief 009 Wageningen Livestock Research, Wageningen University & Research, Wageningen.*

Bwibo, N. O., & Neumann, C. G. (2003). The need for animal source foods by Kenyan children. *The Journal of nutrition, 133*(11), 3936S-3940S.

Byarugaba, D., Nakavuma, J., Vaarst, M., & Laker, C. (2008). Mastitis occurrence and constraints to mastitis control in smallholder dairy farming systems in Uganda. *Education, 32*, 53.53.

Carraro, A., De Giacomo, A., Giannossi, M., Medici, L., Muscarella, M., Palazzo, L., . . . Tateo, F. (2014). Clay minerals as adsorbents of aflatoxin M1 from contaminated milk and effects on milk quality. *Applied Clay Science, 88*, 92-99.

Coorevits, A., De Jonghe, V., Vandroemme, J., Reekmans, R., Heyrman, J., Messens, W., . . . Heyndrickx, M. (2008). Comparative analysis of the diversity of aerobic spore-forming bacteria in raw milk from organic and conventional dairy farms. *Systematic and Applied Microbiology, 31*(2), 126-140.

Dhanashekar, R., Akkinepalli, S., & Nellutla, A. (2012). Milk-borne infections. An analysis of their potential effect on the milk industry. *Germs, 2*(3), 101.

Diaz, D. E., Hagler, W. M., Jr., Blackwelder, J. T., Eve, J. A., Hopkins, B. A., Anderson, K. L., . . . Whitlow, L. W. (2004). Aflatoxin binders II: reduction of aflatoxin M1 in milk by sequestering agents of cows consuming aflatoxin in feed. *Mycopathologia, 157*(2), 233-241.

Duarte, S., Almeida, A., Teixeira, A., Pereira, A., Falcão, A., Pena, A., & Lino, C. (2013). Aflatoxin M1 in marketed milk in Portugal: Assessment of human and animal exposure. *Food Control, 30*(2), 411-417.

Duguma, B., & Janssens, G. P. (2015). Assessment of dairy farmers' hygienic milking practices and awareness of cattle and milk-borne zoonoses in Jimma, Ethiopia. *Assessment, 45*.

Duguma, B., & Janssens, G. P. J. (2016). Assessment of feed resources, feeding practices and coping strategies to feed scarcity by smallholder urban dairy producers in Jimma town, Ethiopia. *SpringerPlus, 5*(1), 717.

Elzupir, A. O., & Elhussein, A. M. (2010). Determination of aflatoxin M1 in dairy cattle milk in Khartoum State, Sudan. *Food Control, 21*(6), 945-946.

FĂT, A., Dan, S. D., TĂBĂRan, A., Reget, O., Mikle, D., Mihaiu, R., & Mihaiu, M. (2018). The Incidence of Aflatoxin M1 in Milk Produced in a Regional Surveillance System Between 2013 and 2017. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Veterinary Medicine, 75*(1), 111.

Fusco, V., Chieffi, D., Fanelli, F., Logrieco, A. F., Cho, G. S., Kabisch, J., . . . Franz, C. M. (2020). Microbial quality and safety of milk and milk products in the 21st century. *Comprehensive Reviews in Food Science and Food Safety*.

Galvano, F., Galofaro, V., & Galvano, G. (1996). Occurrence and stability of aflatoxin M1 in milk and milk products: a worldwide review. *Journal of Food protection, 59*(10), 1079-1090.

Gieseker, K. E., Control, C. f. D., & Prevention. (2004). Outbreak of aflatoxin poisoning-eastern and central provinces, Kenya, January-July 2004.

Girma, K., Tilahun, Z., & Haimanot, D. (2014). Review on milk safety with emphasis on its public health. *World J Dairy Food Sci, 9*(2), 166-183.

Gizachew, D., Szonyi, B., Tegegne, A., Hanson, J., & Grace, D. (2016). Aflatoxin contamination of milk and dairy feeds in the Greater Addis Ababa milk shed, Ethiopia. *Food Control, 59*, 773-779.

GonÇAlves, L., Dalla Rosa, A., Gonzales, S. L., Feltes, M. M. C., Badiale-Furlong, E., & Dors, G. C. (2017). Incidence of aflatoxin M1 in fresh milk from small farms. *Food Science and Technology, 37*(spe), 11-15.

Havelaar, A. H., Kirk, M. D., Torgerson, P. R., Gibb, H. J., Hald, T., Lake, R. J., . . . Gargouri, N. (2015). World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010. *PLoS medicine, 12*(12).

Ismail, A., Akhtar, S., Levin, R. E., Ismail, T., Riaz, M., & Amir, M. (2016). Aflatoxin M1: Prevalence and decontamination strategies in milk and milk products. *Crit Rev Microbiol, 42*(3), 418-427.

Jacela, J. Y., DeRouchey, J. M., Tokach, M. D., Goodband, R. D., Nelssen, J. L., Renter, D. G., & Dritz, S. S. (2010). Feed additives for swine: Fact sheets – flavors and mold inhibitors, mycotoxin binders, and antioxidants. *Kansas Agricultural Experiment Station Research Reports*(10), 27-32.

Jayarao, B., Pillai, S., Sawant, A., Wolfgang, D., & Hegde, N. (2004). Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. *Journal of Dairy Science, 87*(10), 3561-3573.

Kagera, I., Kahenya, P., Mutua, F., Anyango, G., Kyallo, F., Grace, D., & Lindahl, J. (2019). Status of aflatoxin contamination in cow milk produced in smallholder dairy farms in urban and peri-urban areas of Nairobi County: a case study of Kasarani sub county, Kenya. *Infection ecology & epidemiology, 9*(1), 1547095.

Kang'ethe, E. (2016). Aflatoxin B1 and M1 contamination of animal feeds and milk from urban centers in Kenya. *African Health Sciences, 9*(4).

Kang'ethe, E. K., & Lang'a, K. (2009). Aflatoxin B1 and M1 contamination of animal feeds and milk from urban centers in Kenya. *African Health Sciences, 9*(4).

Kebede, L. G., & Megerrsa, S. A. (2018). Assessment of dairy farmers hygienic milking practices and awareness on cattle milk-borne zoonoses in Bishoftu, Ethiopia. *Journal of Veterinary Medicine and Animal Health, 10*(2), 45-54.

Kiama, T., Lindahl, J. F., Sirma, A., Senerwa, D., Waithanji, E. M., Ochungo, P., . . . Grace, D. (2016). Kenya dairy farmer perception of moulds and mycotoxins and implications for exposure to aflatoxins: A gendered analysis. *African Journal of Food, Agriculture, Nutrition and Development, 16*(3), 11106-11125.

Kirino, Y., Makita, K., Grace, D., & Lindahl, J. (2016). Survey of informal milk retailers in Nairobi, Kenya and prevalence of aflatoxin M1 in marketed milk. *African Journal of Food, Agriculture, Nutrition and Development, 16*(3), 11022-11038.

Knight-Jones, T. J., Hang'ombe, M. B., Songe, M. M., Sinkala, Y., & Grace, D. (2016). Microbial contamination and hygiene of fresh cow's milk produced by smallholders in Western Zambia. *International journal of environmental research and public health, 13*(7), 737.

Kurwijila, L., Mboya, N., Laizer, M., & Omore, A. (2016). The efficacy of the Mazzican for milking, transportation and improving bacteriological quality of milk in the smallholder dairy value chain in Tanzania.

Kurwijila, L. R., Mboya, N., Laizer, M., & Omore, A. O. (2016). The efficacy of the Mazzican for milking, transportation and improving bacteriological quality of milk in the smallholder dairy value chain in Tanzania.

Kutz, R., Sampson, J., Pompeu, L., Ledoux, D., Spain, J., Vazquez-Anon, M., & Rottinghaus, G. (2009). Efficacy of Solis, NovasilPlus, and MTB-100 to reduce aflatoxin M1 levels in milk of early to mid lactation dairy cows fed aflatoxin B1. *Journal of Dairy Science, 92*(8), 3959-3963.

Langat, G., Tetsuhiro, M., Gonoi, T., Matiru, V., & Bii, C. (2016). Aflatoxin M1 Contamination of Milk and Its Products in Bomet County, Kenya. *Advances in Microbiology, 06*(07), 528-536.

Lawley, R. (2013). Aflatoxins. *Food Safety Watch*.

Liu, Y., & Wu, F. (2010). Global burden of aflatoxin-induced hepatocellular carcinoma: a risk assessment. *Environ Health Perspect, 118*(6), 818-824.

Makau, C. M., Matofari, J. W., Muliro, P. S., & Bebe, B. O. (2016). Aflatoxin B 1 and Deoxynivalenol contamination of dairy feeds and presence of Aflatoxin M 1 contamination in milk from smallholder dairy systems in Nakuru, Kenya. *International Journal of Food Contamination, 3*(1), 6.

Maki, C., Thomas, A., Elmore, S., Romoser, A., Harvey, R., Ramirez-Ramirez, H., & Phillips, T. (2016). Effects of calcium montmorillonite clay and aflatoxin exposure on dry matter intake, milk production, and milk composition. *Journal of Dairy Science, 99*(2), 1039-1046.

Maleki, F., Abdi, S., Davodian, E., Haghani, K., & Bakhtiyari, S. (2015). Exposure of infants to aflatoxin M1 from Mother's breast milk in Ilam, Western Iran. *Osong public health and research perspectives, 6*(5), 283-287.

Mallmann, C., Dilkin, P., Giacomini, L., Rauber, R., & Zaviezo, D. (2012). EVALUATION OF THE EFFICACY OF MYCO-AD IN PREVENTING FUMONISIN TOXICITY IN BROILER CHICKS. *Poultry Sci, 91*, 129.

Marroquin-Cardona, A., Deng, Y., Taylor, J., Hallmark, C., Johnson, N., & Phillips, T. (2009). In vitro and in vivo characterization of mycotoxin-binding additives used for animal feeds in Mexico. *Food Additives and Contaminants, 26*(5), 733-743.

McGuire, S. (2015). FAO, IFAD, and WFP. The state of food insecurity in the world 2015: meeting the 2015 international hunger targets: taking stock of uneven progress. Rome: FAO, 2015. *Advances in Nutrition, 6*(5), 623-624.

Min, W.-K., Kweon, D.-H., Park, K., Park, Y.-C., & Seo, J.-H. (2011). Characterisation of monoclonal antibody against aflatoxin B1 produced in hybridoma 2C12 and its single-chain variable fragment expressed in recombinant Escherichia coli. *Food Chemistry, 126*(3), 1316-1323.

Mpatswenumugabo, J., Bebora, L., Gitao, G., Mobegi, V., Iraguha, B., Kamana, O., & Shumbusho, B. (2017). Prevalence of subclinical mastitis and distribution of pathogens in dairy farms of Rubavu and Nyabihu districts, Rwanda. *Journal of veterinary medicine, 2017*.

Muia, J., Kariuki, J., Mbugua, P., Gachuiri, C., Lukibisi, L., Ayako, W., & Ngunjiri, W. (2011). Smallholder dairy production in high altitude Nyandarua milk-shed in Kenya: Status, challenges and opportunities. *Livestock Research for Rural Development, 23*(5), 2011.

Muraya, J., VanLeeuwen, J., Gitau, G., Wichtel, J., Makau, D., Crane, M., . . . Tsuma, V. (2018). Cross-sectional study of productive and reproductive traits of dairy cattle in smallholder farms in Meru, Kenya. *Livestock Research for Rural Development, 30*(171).

Mutua, F., Lindahl, J., & Grace, D. (2019). Availability and use of mycotoxin binders in selected urban and Peri-urban areas of Kenya. *Food Security, 11*(2), 359-369.

Ndungu, T. W., Omwamba, M., Muliro, P. S., & Oosterwijk, G. (2016). Hygienic practices and critical control points along the milk collection chains in smallholder collection and bulking enterprises in Nakuru and Nyandarua Counties, Kenya. *African Journal of Food Science, 10*(11), 327-339.

Nyachuba, D. G. (2010). Foodborne illness: is it on the rise? *Nutrition Reviews, 68*(5), 257-269.

Nyokabi, S., Luning, P. A., de Boer, I. J., Korir, L., Muunda, E., Bebe, B. O., . . . Oosting, S. J. (2021). Milk quality and hygiene: knowledge, attitudes and practices of smallholder dairy farmers in central Kenya. *Food Control*, 108303.

Obade, M., Andang'o, P., Obonyo, C., & Lusweti, F. (2015a). Aflatoxin exposure in pregnant women in Kisumu county, Kenya.

Obade, M., Andang'O, P., Obonyo, C., & Lusweti, F. (2015b). Aflatoxin exposure in pregnant women in Kisumu county, Kenya. *Current Research in Nutrition and Food Science Journal, 3*(2).

Obade, M. I., Andang'o, P., Obonyo, C., & Lusweti, F. (2015). Exposure of children 4 to 6 months of age to aflatoxin in Kisumu County, Kenya. *African Journal of Food, Agriculture, Nutrition and Development, 15*(2), 9949-9963.

Okoth, S. A., & Ohingo, M. (2004). Dietary aflatoxin exposure and impaired growth in young children from Kisumu District, Kenya: Cross sectional study. *African journal of health sciences, 11*(1), 43-54.

Oloo, J. (2010). Food safety and quality management in Kenya: An overview of the roles played by various stakeholders. *African Journal of Food, Agriculture, Nutrition and Development, 10*(11).

Omore, A. O., Arimi, S., Kang'ethe, E. K., McDermott, J. J., Staal, S. J., Ouma, E. A., . . . Koroti, E. (2002). Assessing and managing milk-borne health risks for the benefit of consumers in Kenya.

Orregård, M. (2013). Quality analysis of raw milk along the value chain of the informal milk market in Kiambu County, Kenya.

Oumer, E., Tsegaye, S., Damtew, A., & Feleke, A. (2017). Hygienic Practices and Bacteriological Quality of Cow Raw Milk from Selected Smallholder Dairy Farms of Mersa Town, North Wollo, Ethiopia. *Euro. J. Exp. Bio, 7*, 22.

Rademaker, C. J., Bebe, B. O., Lee, J., Kilelu, C., & Tonui, C. (2016). Sustainable growth of the Kenyan dairy sector. *A quick scan of robustness, reliability and resilience. Report 3R Kenya/WLR, 979*.

Reddy, K., & Salleh, B. (2010). A preliminary study on the occurrence of Aspergillus spp. and aflatoxin B 1 in imported wheat and barley in Penang, Malaysia. *Mycotoxin research, 26*(4), 267-271.

Reta, M. A., Bereda, T. W., & Alemu, A. N. (2016). Bacterial contaminations of raw cow's milk consumed at Jigjiga City of Somali Regional State, Eastern Ethiopia. *International Journal of Food Contamination, 3*(1), 4.

Salari, N., Kazeminia, M., Vaisi-Raygani, A., Jalali, R., & Mohammadi, M. (2020). Aflatoxin M1 in milk worldwide from 1988 to 2020: a systematic review and meta-analysis. *Journal of Food Quality, 2020*.

Sarkar, S. (2015). Microbiological considerations: pasteurized milk. *International Journal of Dairy Science, 10*(5), 206-218.

Senerwa, D., Sirma, A., Mtimet, N., Kang'ethe, E., Grace, D., & Lindahl, J. (2016). Prevalence of aflatoxin in feeds and cow milk from five counties in Kenya. *African Journal of Food, Agriculture, Nutrition and Development, 16*(3), 11004-11021.

Sirma, A. J., Makita, K., Grace Randolph, D., Senerwa, D., & Lindahl, J. F. (2019). Aflatoxin exposure from milk in rural Kenya and the contribution to the risk of liver cancer. *Toxins, 11*(8), 469.

Škrbić, B., Živančev, J., Antić, I., & Godula, M. (2014). Levels of aflatoxin M1 in different types of milk collected in Serbia: Assessment of human and animal exposure. *Food Control, 40*, 113-119.

Swai, E., & Schoonman, L. (2011). Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania. *Asian Pacific Journal of Tropical Biomedicine, 1*(3), 217.

Thornton, P. K. (2010). Livestock production: recent trends, future prospects. *Philosophical Transactions of the Royal Society B: Biological Sciences, 365*(1554), 2853-2867.

Torkar, K. G., & Teger, S. G. (2008). The microbiological quality of raw milk after introducing the two day's milk collecting system. *Acta agriculturae Slovenica, 92*(1), 61-74.

Unnevehr, L., & Grace, D. (2013). *Aflatoxins: Finding solutions for improved food safety* (Vol. 20): Intl Food Policy Res Inst.

Upadhaya, S. D., Park, M., & Ha, J.-K. (2010). Mycotoxins and their biotransformation in the rumen: a review. *Asian-Australasian Journal of Animal Sciences, 23*(9), 1250-1260.

Wang, P., Afriyie-Gyawu, E., Tang, Y., Johnson, N., Xu, L., Tang, L., . . . Ellis, W. (2008). NovaSil clay intervention in Ghanaians at high risk for aflatoxicosis: II. Reduction in biomarkers of aflatoxin exposure in blood and urine. *Food Additives and Contaminants, 25*(5), 622-634.

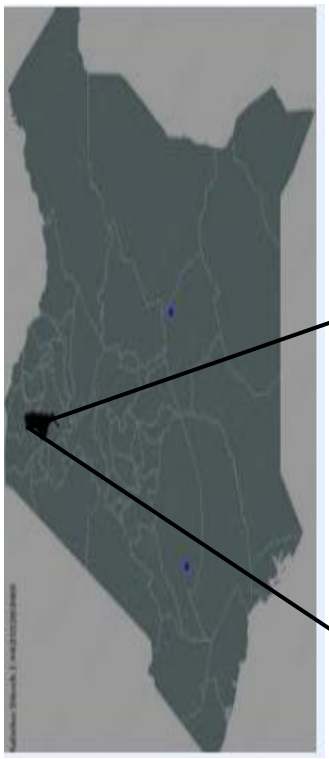
Wanjala, G., Mathooko, F., Kutima, P., & Mathara, J. (2017). Microbiological quality and safety of raw and pasteurized milk marketed in and around Nairobi Region. *African Journal of Food, Agriculture, Nutrition and Development, 17*(1), 11518-11532.

Welearegay, H., Yilma, Z., & Tekle-Giorgis, Y. (2012). Hygienic practices and microbiological quality of raw milk produced under different farm size in Hawassa, southern Ethiopia. *Agricultural Research and Review, 1*(4), 1132-1142.

Wild, C. P., Miller, J. D., & Groopman, J. D. (2015). Mycotoxin control in low-and middle-income countries.

APPENDICES

Appendix 1: Map of Kenya Showing Kisumu County



Latitude and longitude coordinates are: **-0.091702, 34.767956**.

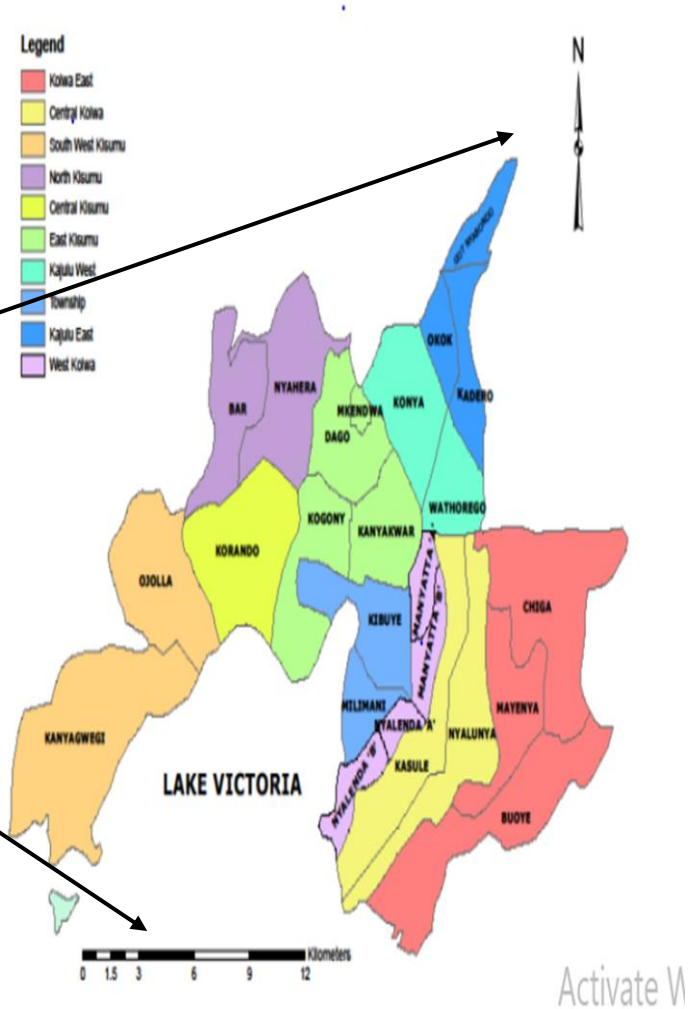


Figure 1: Map of Kisumu with sub locations (Maoulidi, 2010)

Appendix 2: QUESTIONNAIRE

Appendix 2.0: Baseline Survey

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

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 analyse 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.

We may also contact you again later to ask you to join in the field trial aimed at minimizing aflatoxins at your farm.

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

Gladys Anyango 0724956406

Dr Johanna Lindahl 0718-929937

milk

NAME OF THE ENUMERATOR _____ **SURVEY CODE:**

DATE: ___/___/___

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	0= respondent is household head 1= wife 2=husband	1=employed full time 2=employed casual 2= farming 3=other

	completed) 5= secondary school (completed) 6= college / university	3=son 4=daughter 5=farm worker 6=other, specify	
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 sale 5=Control some money from 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 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 :

e. Presence of children up to 5 years of age	How many are they _____

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*					

1.3.3 Animal Health

a. Have you ever encountered mastitis in your farm	[yes] [no] ***If no, skip to 1.3.3 e***
b. If yes, how often do you encounter mastitis in your herd	1= at least once in a week 2= at least once in a month 3= at least every two months 4= more rarely
c. What do you do when your cow has mastitis?	

d. What do you do to milk from a cow that has mastitis
e. If you treat with any medicine or drug, what do you use?
f. If you treat cow with any drug, what do you do with the milk from cow that is being treated
g. Do you check cows for mastitis: [ ] every milking [ ] Sometimes [ ] don't do this

1.3.4 What do you use to perform the following milking tasks

	Does not do this	Cold water	Warm water	Soap	Disinfectant	Other-specify
a. To clean hands before milking						
b. To wash udder and teats before milking						
c. To wash udder and teats after milking						
d. Daily cleaning of milking shed						
e. More thorough cleaning of milking shed						
f. Ensure milking equipment is clean after milking						

1.3.5. Do you dry the teats before milking? If yes, with what? _____

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	
c. How much, in a day, is produced by the other milking cows		

1.4.2 The estimated total amount of milk produced 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 liter	
How long is the milk stored for before being sold (hrs)	
How is the milk transported to market *0= Customer or trader come to farm to pick milk 1= walk to deliver the milk 2= use own bicycle 3= use public vehicle 4= use own vehicle*	

1.4.5 Of the total milk kept by the household how much is.

	Consumed raw	Consumed boiled	Processed e.g. fermented
Estimate of amounts in liters yesterday			

1.4.6 Describe how fresh milk is stored before being consumed within farm

1.5 Feeding

1.5.1 Feeding and feed storage practices

Feed type used by the farmer						
	Open grazing	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 molds	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= mold growth 5= dryness 6= pests / animal

Actions If stored feed had molds: 1= dispose 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 about molds and aflatoxins

1.6.1 Have you ever seen mold 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. Do you think the presence of aflatoxins in these foods pose any danger to humans? Which danger(s)?

SAMPLING OF MILK

Collect 2 x 40 ml in sterile falcon tubes from milk that is meant for household consumption or for sale	
Indicate the approximate time the sampled milk was milked	
Indicate if the sampled milk has been treated in any way, e.g. by boiling, 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 2.1 Trial Questionnaire

TRIAL

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 liters did they produce in total yesterday? _____ How many liters did you sell yesterday? ____	For yesterday's milk, how much did you sell the milk at, per liter? _____	In your own 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 in your farm since the last time we visited

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:

1.3 Since last time we visited, have you had any illness in your cows? (yes / no)

If yes, what disease (or symptoms) have you observed in your cows

_____

_____

1.4 What did you do to milk from the sick cow? (NA if the sick cow(s) was not producing milk)

_____

_____

1.5 Since last time we met, has anyone in the family had diarrhea, or vomiting or stomach pains?

IF so, how many people? _____ How many times each _____

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

_____

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

2.2 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 spoon provided to measure the binder				
Actual mixing of the binder with feed				
Cows eating feed mixed with binder				

2.3 Have you encountered any other challenge while using the binder in 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)

_____

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

_____

3.0 Using the Mazzicans®

3.1 Tell us what you are doing with the Mazzican®(**1**= using **2**=not using)

If **using**, describe how the Mazzican®is being used in your farm (1= milking 2=testing mastitis 3= transportation of milk 4= other use, describe _____

If **not using**, state why the Mazzican®is not being used in your farm

_____

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

3.2 How would you rate the Mazzican®compared to your former milking equipment in the context of the following:

	Very bad	bad	good	Very good	Reason for rating
(a) Storing milk					
(b) Transportation of milk to the market					
(c) Checking for mastitis					
(d) Milk hygiene					
(e) Cleaning of the equipment					

3.3 Assessment of the Mazzican® during the visit *(observe if available)*

Are the different Mazzican® parts present	a) Container (yes/ no)	b) Lid (yes / no)	c) Mastitis (black) part (yes / no)
What is the condition of the can?	a. Broken (yes/ no	b. Clean (yes / no)	c. Other – please describe

3.4 Since you started using the Mazzican® and the binders

	Worse	same	Better
How do you feel about drinking milk from your cows?			
How do you feel about selling milk from your cows?			

4.0 In your own view, how do you rate the following?

	Worse	same	Better
Feeding of your cows now			
Health of your cows Milk yield			

SAMPLING OF MILK

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

FOLLOW UP SURVEY

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

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 in 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`s Initials	signature
YES	Verbal		
	Written		
NO			

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. Our visit today is a follow up to research activities implemented in our previous visit to your farm. Two components of the study have already been implemented, namely 1) an initial baseline survey that helped us understand smallholder milk production systems in your area and 2) a field trial with a few of the farmers to analyze the effect of selected interventions on milk quality and safety. We are visiting you because your farm has been selected to participate in a follow up survey to help analyze the impact of what was done in the previous visits, with regards to improving milk quality and safety. If you accept to participate in the baseline study, we will ask you a few questions related to how you manage your dairy animals, their milk production, how the milk is used, and your own experiences during the trial months. We will also request you to provide us with a sample of milk for further laboratory testing, to assess its bacterial and aflatoxin contamination. We promise to respect privacy and confidentiality of what you tell usand wish to assure you that the information you give us will only be shared with our research team members, in the sharing, all names will be removed so that no one can be able to trace back the information to you.

We may also contact you again later to invite you to a workshop as a means of disseminating findings from this and other food safety research that we do..

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

Gladys Anyango 0724956406

Dr Florence Mutua 0733-546859

Dr Johanna Lindahl 0718-929937

SURVEY CODE:

NAME OF THE ENUMERATOR _____

DATE: ___/___/___

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

f. 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
g. If respondent is not household head	Gender of household head:	Age:	
h. 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 sale 5=Control some money from sale	1=Own cattle 2=Co-own cattle 3= involved with cattle 4=not involved cattle
i. **Presence of children up to 5 years of age in the family**	How many are they _____ How much milk does the youngest child take each day?…… *(The youngest child that is not breast feeding)*		

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*					

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

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

1.3.4 Animal Health

h. Have you ever encountered mastitis in your farm	[yes] [no] ***If no, skip to 1.3.3 e***
i. If yes, how often do you encounter mastitis in your herd	1= at least once in a week 2= at least once in a month 3= at least every two months 4= more rarely
j. Do you experience a change in mastitis episodes over the last 6 months?	0= No change 1= more mastitis 2= less mastitis
k. What do you do when your cow has mastitis?	
l. What do you do to milk from a cow that has mastitis	
m. If you treat with any medicine or drug, what do you use?	
n. If you treat cow with any drug, what do you do with the milk from cow that is being treated	
o. Do you check your cows for mastitis: [ ] every milking [ ] Sometimes [ ] don't do this	
p. How do you check this?	

1.3.4 What do you use to perform the following milking tasks

	Does not do this	Cold water	Warm water	Soap	Disinfectant	Other-specify

g. To clean hands before milking						
h. To wash udder and teats before milking						
i. To wash udder and teats after milking						
j. Daily cleaning of milking shed						
k. More thorough cleaning of milking shed						
l. Ensure milking equipment is clean after milking						

1.3.5. Do you dry the teats before milking? If yes, with what? _____

1.4 Milk production

1.4.1 Amounts of milk produced

d. How often are the cows milked in a day		
e. 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 liter	
How long is the milk stored for before being sold (hrs)	

1.4.5 Of the total milk kept by the household how much is.

	Consumed raw	Consumed boiled	Processed e.g. fermented
Estimate of amounts in liters yesterday			

1.4.6 Describe how fresh milk is stored before being consumed within farm

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

1.5.1 Feeding and feed storage practices

Feed type used by the farmer						
	Open grazing	Hay bales	Cut-carry-pasture	Concentrates/compound feed	Silage	Molasses
Does this feed option apply (check)						
What is the source of the feeds	NA					

CODES

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

Do you do any feed fermentation for any of your animals (feed fermentation = adding water and letting

1.6 Awareness about molds and aflatoxins

1.6.1 Have you ever seen mold 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 pose any danger to humans? Which danger(s)?

1.6.7 What can a farmer do to reduce moulds?

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

1.6.7 Do you do anything today to reduce mould in the feed?

1.6.8 Do you do anything today to reduce aflatoxins?

1.7 Mazzican Use

1.7.1 Have you ever used the Mazzican? Yes/No

1.7.2 Are you still using the Mazzican? Yes/No

If no, move to next question

	Problems encountered in the use of mazzican containers	Suggested changes
• *When used to milk*		•
• *When used to store milk* •		•
When used to transport milk •		•

1.7. 3. Are you willing to use the Mazzican in future? Yes/No

1.8 NovaSil Use

1.8.1 Have you ever fed your cows with aflatoxin binders? Yes/No

- If yes, were there any problems?

1.8.3. Are you still using the binder? Yes/No

1.8.4. Are you willing to use the binder in future? Yes/No

- How could the binders be made to work better?

Problems encountered in the use of binder	• Suggested changes to improve on binder use

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 _____ KES per day**
b. **If this reduces aflatoxins and the milk get safer but I can't sell more milk, I would be willing to pay _____ KES per day**
c. **If this reduces aflatoxins and I can sell milk to a higher price, I would be willing to pay _____ KES per day**
d. **Where would you suggest to source Mazzican and NovaSil binder from**

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 3.1: School of Graduate Studies Approval letter

MASENO UNIVERSITY

SCHOOL OF GRADUATE STUDIES

Office of the Dean

Our Ref: MPH/PH/00011/2016

Private Bag, MASENO, KENYA
Tel:(057)351 22/351008/351011
FAX: 254-057-351153/351221
Email: sgs@maseno.ac.ke

Date: 26th July 2019

TO WHOM IT MAY CONCERN

RE: PROPOSAL APPROVAL FOR GLADYS ANYANGO OWINO — MPH/PH/00011/2016

The above named is registered in the Master of Public Health Programme in the School of Public Health and Community Development, Maseno University. This is to confirm that her research proposal titled **"Effectiveness of a Milk Safety Intervention in Reducing Bacterial and Aflatoxin M1 Contamination in Milk Produced by Smallholder Dairy Farmers in Kisumu County, Kenya"** has been approved for conduct of research subject to obtaining all other permissions/clearances that may be required beforehand.

DEAN SCHOOL OF GRADUATE STUDIES * P.O. BOX PRIVATE BAG, MASENO * MASENO UNIVERSITY 29 JUL 2019

Prof. J.O. Agure
DEAN, SCHOOL OF GRADUATE STUDIES

Appendix 3.2: Ethics Review Letter

MASENO UNIVERSITY ETHICS REVIEW COMMITTEE

Tel: +254 057 351 622 Ext: 3050
Fax: +254 057 351 221

Private Bag – 40105, Maseno, Kenya
Email: muerc-secretariate@maseno.ac.ke

FROM: Secretary - MUERC

DATE: 5th February, 2020

TO: Gladys Anyango Owino
PG/MPH/PH/00011/2016
Department of Public Health
School of Public Health and Community Development
Maseno University
P. O. Box, Private Bag, Maseno, Kenya

REF: MSU/DRPI/MUERC/00762/19

RE: Effectiveness of a Milk Safety Training and Mazzican Milk Container and NovaSil Binder use by smallholder Dairy Farmers in Kisumu County, Kenya in Reducing Bacterial and Aflatoxin M_1 Contamination in Milk. Proposal Reference Number MSU/DRPI/MUERC/00762/19

This is to inform you that the Maseno University Ethics Review Committee (MUERC) determined that the ethics issues raised at the initial review were adequately addressed in the revised proposal. Consequently, the study is granted approval for implementation effective this 5th day of February, 2020 for a period of one (1) year. This is subject to getting approvals from NACOSTI and other relevant authorities.

Please note that authorization to conduct this study will automatically expire on 4th February, 2021. If you plan to continue with the study beyond this date, please submit an application for continuation approval to the MUERC Secretariat by 15th January, 2021.

Approval for continuation of the study will be subject to successful submission of an annual progress report that is to reach the MUERC Secretariat by 15th January, 2021.

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

Thank you.

MASENO UNIVERSITY SECRETARY 05 FEB 2020 ETHICS REVIEW COMMITTEE

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

Cc: Chairman,
Maseno University Ethics Review Committee.