THE PREVALENCE OF *BRUCELLA ABORTUS* AND MYCOTOXIN CONTAMINATION OF ANIMAL FEEDS AND IMPLICATIONS ON MILK SAFETY IN RURAL AND PERI-URBAN SMALL-HOLDER DAIRY SYSTEMS IN NAKURU COUNTY, KENYA

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A thesis submitted to Graduate School in partial fulfilment for the requirements of the Degree of Master of Science in Food Science of Egerton University.

EGERTON UNIVERSITY

May 2017

DECLARATION AND APPROVAL

Declaration

I declare that this thesis is my original work and has not been presented in this or any other Universityfor any degree.

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DEDICATION

To my son Jacob Mwendwa Makau, any dream is possible through God's favour, grace and hard work. To my father, Mr. Raphael Makau Kinyanzui and mother Mrs. Scholastica Nduku Makau and siblings Martin Makuu Makau and Michael Nzioka Makau, thanks for your unconditional support and prayers.

ACKNOWLEDGEMENTS

I wish to acknowledge God for the gift of life and health. I also wish to acknowledge Egerton University for the opportunity to pursue my studies and the entire staff of the Department of Dairy and Food Science and Technology for their support since I enrolled for my studies. I appreciate my supervisors; Prof. Joseph W. Matofari and Dr. Patrick S. Muliro for their technical advice, guidance, professional supervision, inspiration and limitless support during the entire study period. I sincerely appreciate Egerton University, Germany Ministry of Research and Education (BMBF) for awarding me scholarship and National Commission for Science, Technology and Innovation (NACOSTI) for partly funding my data collection and analysis. Special thanks also go to the entire staff and farmers of Olenguruone, Wanyororo and Wecha (Bahati) milk cooperatives for their assistance and cooperation during data collection and to the entire dairy farmers of Nakuru, County. I would like to express my sincere gratitude for the expert support I got from members of staff of the regional Veterinary Investigation Laboratory, Nakuru and State Department of Livestock Nakuru County headed by Dr Musabi. Special thanks also go to Dr. Paul Ayieko, Mr. Sawe and Mr. Robinson Kibet for their help in the serology laboratory. Special thanks to Mr. Mainga of Department of Public Health and Toxicology, University of Nairobi for assistance in mycotoxin analysis . I wish to appreciate Mr. Nobert Wafula and Mr. Johnson Mwove for their assistance in statistical analysis. I also thank my parents, siblings, 2013 Msc. Students class and friends for their moral support. I Above all, praise, honour and gratitude go to the almighty God for His grace, favour, love, care, and guidance during the study.

ABSTRACT

Mycotoxin and Brucella abortus contamination of milk along the dairy value chain is of public health concern and contributes to food insecurity. Animal feeds that are contaminated with mycotoxin causing fungi pass the contaminationtion to the milk. Inhygienic handling of milk can lead to Brucella contamination of milk. This study aimed at determining the on-farm risk factors associated with Mycotoxins and *B. abortus* occurrence and their prevalence on smallholder farms at rural and peri-urban dairy sub-value chains in Nakuru County. A cross-sectional survey using semi-structured questionnaires was administered randomly to 280 farmers and raw milk market players. Alongside, milk samples (317), blood for serum ((n=245) and animal feed (74) samples were collected. Feed samples were analyzed for Aflatoxin (AFs) concentration and Deoxynivalenol (DON) and milk samples for Aflatoxin M1 (AFM1) using commercial Enzyme Linked Immune Sorbent Assay (ELISA). Serum samples were first screened for antibodies against B. abortus using milk ring test (MRT) and Rose Bengal Plate test (RBPT). Positive reactors were further confirmed by Complementary Fixation Test (CFT) and competitive enzyme linked immunoassay (c-ELISA) test. Data was analyzed using SPSS version 20 for qualitative data and SAS version 9 for the quantitative data and Means were compared using least significant differences (LSD) at 95% confidence level. The study identified three risk factors for mycotoxin contamination of animal feeds at the farm level; 1) the type of feeds, 2) condition of feed and 3) storage method. Type and condition of animal feeds were found to be significant $P<0.05$ risk factors for infestation of mycotoxigenic mold. The feed samples had 0 - 147.86 5µg/kg AFs concentration with 56% above the European Union (EU) limits of 5µg/kg and Deoxynivalenol concentration ranged between 0 and 179.89 µg/kg and positive in 63% of the samples. All milk samples from the rural dairy value chain were below the EU limits of 0.05µg/L. Milk samples, 68% from peri-urban dairy farms, 29% from transporters, 40% from cooperatives and 17% milk bars had a concentration of 0-0.083µg/L and exceeded the EU limits of 0.05µg/L. The study identified the risk factors for brucellosis at the farm level; method of breeding, thus use of a bull was significant $P<0.05$ as a risk factor for brucellosis. The prevalence of brucellosis was 1.3% and 1.7% in peri-urban and rural dairy systems respectively. Milk safety in rural and peri-urban dairy systems is a concern for public health. Mycotoxins from animal feeds and brucellosis due to usage of bulls for breeding are the major risks

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

AFs	Aflatoxins
AFB_1	Aflatoxin B_1
AFM_1	Aflatoxin M_1
CFR	Case fatality rate
DON	Deoxynivalenol
ELISA	Enzyme linked immune sorbent assay
FAO	Food and Agriculture Organization
GDP	Gross Domestic Product
GIS	Geographic Information System
GLM	General linear model
GOK	Government of Kenya
IFPRI	International Food Policy Research Institute
ILRI	International Livestock Research Institute
KARI	Kenya Agricultural Research Institute
KDB	Kenya Dairy Board
KEMRI	Kenya Medical Research Institute
MOA	Ministry of Agriculture
MRT	Milk ring test
RBPT	Rose Bengal Plate Test
NACOSTI	National Commission for Science, Technology and Innovation
BMBF	Federal Ministry of Education and Research (Germany)
RELOAD	Reduction of Post-harvest Losses and Value Addition in East African Food Value Chains
C-ELISA	Complement-enzyme linked immuno sorbent assay

LIST OF APPENDICES

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Kenya is the leading milk producer in East Africa, producing an estimated 4 billion litres per year (GOK, 2010). This production is from 1 million smallholder farmers who generate 80% of Kenya's milk supply (Muriuki, 2011). The dairy industry contributes 14 % of agricultural GDP and 3.5 % of total GDP (GOK, 2008). Rural and peri-urban dairy farming in Kenya has grown extensively during the past decade as a result of liberalization of milk trade in the mid-1990s resulting in new entrants of private investors attracted by the new opportunities. In addition, 70% of milk produced by these systems is marketed through informal suppliers due to inadequate milk marketing infrastructure and high pasteurized milk prices in major urban areas (Muriuki, 2011).

Milk produced by these systems creates employment and food security in Kenya. The rural and peri-urban dairy production systems are characterized by semi-intensive and intensive zero-grazing regimes respectively. Milk production levels in rural and peri-urban dairy units range from 5.7– 17 litres/cow/day (Gillah *et al.,* 2012). However, these small holder dairy farmers in both production systems face a lot of constraints that lead to milk losses. Seasonal quantitative and qualitative feed shortages especially during the dry season forces these farmers to store forage during rainy seasons or buy forage from neighbours, make on-farm silage or buy feeds from agrovet shops that are not quality certified because of low pricing as animal feed. Most farmers also have low or inadequate technical knowledge on on-farm feed formulations. There is also lack of genetic improvement where indigenous cattle genotypes are selected for adaptation to the environment rather than milk productivity. In addition, high prevalence of diseases that affect milk productivity such as sub-clinical than clinical mastitis that ranges from 25 to 95% (Mdegela *et al.*, 2005; Almaw *et al.*, 2009)

Milk losses of the Kenyan dairy value chain are estimated at 6% and are based on quality and quantity. Milk loss in the small holder dairy production systems is estimated to be 1-5%, but rises up to 10% during the wet season when delivery rejections due to spoilage are common (Katuku, 2009; Muriuki, 2011). Spoilage of raw milk is a result of naturally occurring mixed microflora inherent in the milk and environmental factors such as temperature and pH. These provide the necessary favourable factors for *Lactobacilli* spp, *Enterococci* spp, and *Streptococcus faecium* to grow (Leroy and De Vuyst, 2004). They cause clotting due to secretion of enzymes during growth followed by proteolysis and lipolysis which cause syneresis which is the whey separation induced by gel contraction, resulting in rearranging or

restructuring of casein matrix formed during enzymatic coagulation (Jovanović *et al.,* 2004) in milk. *Enterococci* in milk are indicators of hygiene in the production area and are of faecal origin. Biochemical contamination of milk by mycotoxins and antibiotics also lead to milk spoilage (Chelule *et al.,* 2010)

Animals fed on AFB_1 and B_2 contaminated feeds excrete into their milk the toxic AFM_1 and M_2. These are metabolized in the liver and AFM_1 is hydroxylated metabolite of the AFB_1 parent compound. AFs are highly carcinogenic causing liver cancer in humans (Zinedine *et al.,* 2007). Deoxynivalenol (DON) is associated primarily with *Fusarium graminearum* and *F. culmorum*, both of which are important plant pathogens which cause fusarium head blight in wheat and fusarium ear blight in maize (Bottalico and Perrone, 2002). DON is a mycotoxin belonging to the group of trichothecenes, which contaminates grains and cereal-based foods and feeds (Korosteleva *et al.,* 2009). It is known to cause acute gastrointestinal adverse effects such as vomiting (emesis) both in animals and humans (Vincelli *et al.,* 2002).

AFs pose food safety concerns in intensive livestock systems (Unnevehr and Delia, 2013) and therefore relevant in Kenya where about 80% of the milk is produced by smallholder farmers in rural and peri-urban dairy farming, mostly under intensive zero grazing units (Muriuki, 2011). Some of the practices used by the dairy farmers that contribute to the occurrence of mycotoxins in animal feeds and result in the carry over effect of AFM1 in milk in the dairy value chain in Kenya. Animals are frequently fed on farm crop residue and discarded cereals from mold spoilage and uncertified commercial feeds sold by the agro-vet dealers. This practice points to lack of knowledge of feed safety especially in feed formulation. Rural and peri-urban dairy farmers lack knowledge on proper feed formulation and storage such as silage making and lack properly constructed feed stores (Lukuyu *et al.,* 2011).

Bovine brucellosis is a zoonosis commonly caused by *Brucella abortus* (McDermottm and Arimi, 2002). The disease is an occupational risk for farmers, veterinary surgeons, and workers within the meat industry. The sources of infection for animals include aborted materials, vaginal discharges, milk and semen from infected animals (WHO, 2016). Brucellosis is an infectious zoonotic disease that is associated with chronic debilitating infections in humans and reproductive failure in domestic animals . Apart from zoonotic concerns of brucellosis, the economic losses to small scale dairy farmers are massive. This includes abortion leading to loss of calves in cows and loss in milk yield in aborting heifers and cows (Mangen *et al*., 2002).

Brucellosis has increased in sero-prevalence in humans in Kenyan urban areas to 5.6% (Ogola *et al*., 2014).The driving factors for epidemiology of the disease on small holder dairy farms is not known and available data are inadequate. Practices likely to cause transmission

and sustain *Brucella spp* in herds and milk include: Breeding using bull, replacement and purchase of infected animals, un-controlled grazing pattern with proximity to infected herds No vaccination of animals and utilization of un-pasteurized milk (McDermottm and Arimi, 2002). There is no analysis on practices used by dairy actors in Kenya in relation to the extent of mycotoxin and *Brucella spp* contamination in milk and contribution to milk loss.

However, scarce data exists on the occurrence of mycotoxins and *Brucella abortus* in milk on rural and intensive small-scale dairy farms. This study assessed the risk factors for mycotoxin and prevalence of *Brucella abortus* occurrence in animal feeds and milk at the farm, transportation, processing and market outlets from where milk is channeled to urban consumers in Nakuru town mostly through informal market agents.

1.2 Statement of the problem

The rural and peri-urban dairy subsector in Kenya is characterized by limited potential for milk production due to post-harvest milk losses that account for 6 %. The major cause for the low potential is loss through spoilage of milk by microbial contamination and biochemicals like mycotoxins. Animal feed handling practices including animals being frequently fed on farm crop residue and discarded cereals from mold spoilage and uncertified commercial feeds sold by the agro-vet dealers lead to mycotoxin contamniation of milk.Utilization of un-pasteurized milk is a potential threat to public health due to incidence of pathogenic microorganisms including *Brucella* spp that cause milk borne brucellosis. There has not been a systematic assessment of the risks leading to occurrence of mycotoxins and *B. abortus* in the smallholder dairy systems and how these impact on milk loss. This study aimed at documenting the risk factors for mycotoxin and prevalence of *Brucella abortus* occurrence in animal feeds and milk at the farm, transportation, processing and market outlets from where milk is channeled to urban consumers in Nakuru town mostly through informal market agents.

1.3 Objectives

1.3.1 General objective

To contribute to food security by reducing post-harvest milk losses in the rural and peri-urban production systems in Kenya by documenting risks for mycotoxin and Brucella occurrence along the informal chain.

1.3.2 Specific objectives

1. To characterize the risk factors associated with prevalence of Aflatoxin (AFs), Deoxynivalenol (DON) and *Brucella abortus* in rural and peri-urban milk sub-value chains in Nakuru County.
2. To determine the prevalence of aflatoxins (AFs), Deoxynivalenol (DON) and Aflatoxin M_1 (AFM_1) in rural and peri-urban milk sub-value chains in Nakuru County
3. To determine the prevalence of *Brucella abortus* in rural and peri-urban milk sub-value chains in Nakuru County.

1.4 Hypotheses

1. There were no risks associated with the prevalence of Aflatoxin (AFs), DON and *Brucella abortus* in rural and peri-urban milk sub-value chains in Nakuru County
2. There was no difference in prevalence of aflatoxins (AFs), Deoxynivalenol (DON) and Aflatoxin M_1 (AFM_1) in rural and peri-urban milk sub-value chains in Nakuru County
3. There was no difference in prevalence of *Brucella abortus* between in rural and peri-urban milk sub-value chains in Nakuru County

1.5 Justification

Milk post-harvest losses are experienced by small holder farmers in rural and peri-urban dairy systems. Currently, the amount lost in these systems is estimated to be 1-5%. But rises up to 10% during the wet season when delivery rejections are common. The losses are in form of spoilage as a result of mycotoxins and pathogenic microbiological contamination of the milk along these sub-value chains leading to safety concerns, quality, nutritive and volume losses. Management practices of small holder farmers and post-harvest handling provide favourable conditions for the proliferation of pathogenic bacteria. Milk borne diseases like brucellosis have increased in sero-prevalence in humans in Kenyan urban areas to 5.6% (Ogola et al., 2014).The concentration of aflatoxin B_1 (AFB_1) in animal feeds used in peri-urban systems has reached 1,000 ppb above the acceptable local draft standards of 20ppb (Muture and Ogana, 2005).This is risk factor for aflatoxin M_1 (AFM_1) in milk. The resultant reduction in product availability and lost income value that would otherwise have accrued from sale of the lost product could have been used to provide food security to the Kenyan population. Therefore, there is need apportion the volume lost to the real cause. Knowledge on prevalence of mycotoxins and pathogenic microbial causes of milk loss will inform on the mitigation measures to be taken to reduce these safety concerns and milk losses.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Rural and peri-urban dairy production systems in Kenya

There are two major types of smallholder dairy farming in East Africa: intensive and semi-intensive. Small holder intensive dairing includes urban and peri-urban production systems that exclusively stall-feed their dairy cattle (Orodho, 2005). Rural small holder farmers in Kenyan highlands practise semi-intensive dairing . Semi-intensive systems use both the indigenous Small East African (SEA) zebu and crosses of indigenous and exotic breeds, and are mostly found in high rainfall areas, which are suitable for exotic breeds (Orodho, 2005).

Rural production systems comprise the production and marketing of milk and milk products that are channeled to rural consumers or cooperative societies located in the rural areas. The characteristics of semi intensive rural small holder dairy farmers intensify their farming systems by integrating dairying with crop production and shifting from free grazing, semi-zero and zero-grazing (Bebe , 2003). Producers own less than 10 cows and about 2-4 ha of land. Cattle are paddocked, tethered or herded on roadsides. Few concentrates or mineral supplements are used (Orodho, 2005). Rural dairy farmers practicing zero-grazing produce forage such as napier grass in a cut and carry system Rural dairy farms have low performance of dairy herds attributed to type of breeds kept, low numbers of lactating cows and replacement heifers, short lactation period and utilization of low quality feed resources (Ibrahim *et al.*, 2000). This contributes to reduced productivity in the rural production systems.

Peri-urban dairy production systems comprise the production, processing and marketing of milk and milk products that are channeled to consumers in urban centres. These peri-urban dairy production systems arose to meet the increasing demand for milk in urban centres as a result of increasing urbanization (Omiti *et al.*, 2009). The characteristics of peri-urban dairy production systems include labour and management intensive and use of special inputs that are linked to the type of genotype of the cow and involve artificial insemination and supplementary feeds to stall-fed roughages. Conserved hay, agro-industrial by-products and commercial concentrate rations are the major feed resources used by urban and peri-urban dairy farmers. Hay makes up almost the entire basal diet of the peri-urban dairy farms. Agro-industrial by-products such as sunflower and cotton seed cakes are fed as supplement to roughage based diets, due to the fact that most of the by-product processing industries are located around cities and towns where the demand for the edible major products is high (Azage *et al.*, 2000). In Kenya, these peri-urban units located within or close to major cities, have herd sizes of about 10 cows, kept under an intensive zero-grazing regime. Milk production levels in rural and peri

urban dairy units range from 5.7– 17 litres/cow/day (Gillah *et al.*, 2012). Financial inputs go towards the purchase of improved crossbred animals, production of farm- grown fodder where land is available, purchase of supplementary feeds and payment for veterinary services and hired labour. Milk is marketed through informal channels to local traders and individual consumers (Olaloku *et al.*, 1998)

Peri-urban dairy farmers face quite a number of challenges which limit their ability to produce quality safe milk in sufficient quantities. They rely on rain-fed agriculture which is not sufficient and supplement with water sourced from municipal water system, boreholes, rivers and raw sewage. There is shortage of animal feed forcing farmers to source feeds from industrial and market wastes, residues and other commercial feeds. Some of these feeds are substandard and likely to be contaminated and are unsafe (M.O.A., 2010). Some of the practices used by the dairy farmers that contribute to the occurrence of mycotoxins in animal feeds and result in the carry over effect of AFM_1 in milk in the dairy value chain in Kenya. Animals are frequently fed on farm crop residue and discarded cereals from mold spoilage and uncertified commercial feeds sold by the agro-vet dealers. This practice points to lack of knowledge of feed safety especially in feed formulation. Rural and peri-urban dairy farmers lack knowledge on proper feed formulation and storage such as silage making and lack properly constructed feed stores (Lukuyu *et al.*, 2011).

Although small holder dairy farming in Kenya can make a significant contribution to household income and food security for the urban population, unfortunately, these benefits are undermined by potential health hazards (M.O.A., 2010). Technical constraints responsible for such low productivity in peri-urban production system revolve around three factors: lack of genetic improvement, seasonal quantitative and qualitative feed shortages, and lack of management skills and health care (Olaloku *et al.*, 1998). Other constrains include land tenure issue, water scarcity and institutional support services in terms of credit facilities, health delivery, input supply and distribution, and technical advisory services are of crucial importance to the successful management of peri-urban dairy units, but are often not adequately provided (FAO, 2011).

2.2 Bovine brucellosis in Kenya

Bovine brucellosis is a zoonosis commonly caused by *Brucella abortus*. Brucellosis is an infectious zoonotic disease that is associated with chronic debilitating infections in humans and reproductive failure in domestic animals.The disease is prevalent in Kenya and Africa (Muriuki *et al.*, 1997). *Brucella abortus* are gram-negative, catalase-positive, oxidase-positive, short oval rods (0.3 mm 0.4 mm) which are non-motile and usually occur singly or in pairs. It grows

optimally around 37 °C and is killed by heating at 63°C for 7-10 minutes (Mangen *et al.*, 2002). Transmission of bovine brucellosis in cattle occurs when large quantities of the bacteria are excreted with the foetus, the placenta and the uterine fluid, mainly at the time of calving. After abortion or parturition, the organism continues to be excreted mainly via the milk of infected cows (Charters, 1980; DFRA, 2002). According to DFRA, (2002), infected breeding bulls can transmit the infection to cows at the time of service via the semen. Apart from direct contact between animals, other sources of infection within and between herds are contaminated water and feed supplies (Morgan and MacKinnon, 1979). When shed in the milk of an infected animal it can survive for many days provided the acidity remains low. Brucellosis is transmitted from animals to humans by ingestion of raw milk, milk products, raw liver, and close contact with animals through breeding, birth, slaughtering and contaminated dust (Cooper, 1992). Brucellosis in animals is caused by five recognized species of the genus *Brucella*. Four species commonly infect man: *B. abortus, B. melitensis, B. suis and B. canis.* The disease in cattle causes abortions and is mainly spread by material contaminated by body fluids. In humans, brucellosis presents as a febrile flu-like illness (Wanjohi *et al*., 2012).

Consumption of raw milk or cream is the principal food vehicles. *Brucella* is readily killed by normal milk pasteurization conditions so there is no risk from pasteurized milk or products processed from it (Wanjohi *et al*., 2012). The transmission of *Brucella* from infected animals to humans occurs either by occupational contact or the consumption (Mangen *et al.*, 2002). Other than drinking raw milk, consumption of traditionally fermented milk may be another way of contracting the disease. *Brucella* organisms have been isolated from cheese and can persist in cheddar for about 6 months and are resistant to low pH achieved when making these products (Farrell, 1996). Some of the practices used by the dairy farmers that contribute to the occurrence of *Brucella abortus* in milk in the dairy value chain in Kenya are: Breeding using bulls, replacement and purchase of infected animals, un-controlled grazing pattern with proximity to infected herds, no vaccination of animals and utilization of un-pasteurized milk (Namanda *et al*, 2009).

2.3 Losses of milk in Kenya.

Losses in the dairy industry can be described as losses at the farm level after milking and through the market chain up to the consumption. This is the milk, either raw, fresh or in its various products forms that gets spoilt due to poor handling and lack of cooling facilities. Losses can either be through spillage and/or spoilage. The spillage losses are most likely on the minimum side. Most of the milk is lost through spoilage. In most places, farms are only

able to sell their milk in the morning. The afternoon or evening milk has to be used by the family, the calves, if there are any, and sold or given to the neighbour. In most cases, the family may be consuming more than it would normally require hence the forced consumption (Muriuki, 2003). Post-harvest losses of milk and dairy products are significant not only because of the resultant reduction in product availability but also due to the foregone income that would otherwise have accrued from sale of the lost product. When viewed at the wider national level, these losses have far-reaching economic implications. Based on the dry season rapid appraisal data, the total value of post-harvest milk losses per year amounted to 17.8 million US dollars for Kenya (Lore *et al.*, 2005). A FAO study on post-harvest milk losses in Kenya noted that they are highest at the farm level (Lore *et al.*, 2005). Losses at the farm level are a result of spoilage, lack of market and contamination with biochemical residues.

In Europe and America, fresh milk is regularly checked for aflatoxin M_1. Concentrations of AFM_1 above 0.05 μg/kg in the EU, or 0.5 μg/kg in the US, are considered undesirable and such milk cannot be used for products that go into the human food chain. Contaminated milk must be discarded, and apart from the cost of lost milk revenue, the dairy producer must also suffer the cost of properly disposing of the contaminated milk (Whitlow *et al.*, 2000). This is not a practice in Kenya under the current local regulations raw and pasteurized milk specifications KS EAS 67:2007 and KS EAS 69:2007 respectively. Mycotoxins in feedstuffs which have the potential of affecting the health and productivity of farm animals. In previous studies, the association of mycotoxins in grain with milk production loss in dairy cows was documented (Zain, 2011; Van Egmond *et al.*, 1997).

Bovine brucellosis is a serious disease of livestock that has significant animal health, public health, and international trade consequences. Considering the damage done by the infection in animals — decreased milk production, weight loss, loss of young, infertility and lameness — this disease is a formidable threat to livestock. From literature, milk losses through decreased milk production has been directly linked to *brucella.* However, it not possible to find any estimate on the reduction of milk production attributable to *brucella* infection. (Mangen *et al.*, 2002)

Total farm-level losses were quantified as 4.5 per cent of milk value available at the farm; this includes physical loss of milk through spillage and spoilage (3.8 % of milk production) and economic loss through "forced consumption" of evening milk and surplus milk above normal household requirements (2.4 %) (Lore *et al.*, 2005). Along the market chain, almost all the milk lost is due to spillage during transport and within premises. Other causes of loss are adulteration and spoilage. Proportions of milk lost by the 3 major groups of market agents

were relatively lower than farm losses: co-operatives and self-help groups (2.8 % of milk handled), small- and large-scale traders (1.3 %) and milk bars, kiosks and shops (2.3 %) (Lore *et al.*, 2005).

2.4 Mycotoxin contamination of animal feeds and Aflatoxin M_1 Contamination in milk

Mycotoxins are a diverse group of secondary fungal metabolites that are harmful to animals and humans. These toxins are produced by saprophytic fungi during storage or by endophytic fungi during plant growth. Among mycotoxins, aflatoxins (AFs) are produced mainly by *Aspergillus flavus* and *A. parasiticus* (Caloni *et al*., 2006). They have carcinogenic, immunotoxic, teratoxic, neurotoxic, nephrotoxic and hepatotoxic causing great harm to human health and high economic damage to the revenue derived from domestic animals (Makun *et al.*, 2012). Cancerogenity of AFM_1 has been observed in vivo, although lower cancerogenity than those of AFB_1 and its cytotoxicity has been definitively demonstrated (Caloni *et al*., 2006). In 1981, Kenya experienced its first recorded outbreak of aflatoxicosis with confirmed 20 patients. The most severe aflatoxicosis outbreak ever reported in Kenya occurred in Eastern Province in 2004. This outbreak resulted in 317 cases and claimed 125 lives, a case fatality rate (CFR) of 39% after consumption of AFB_1 contaminated maize (IFPRI, 2013). The economic losses caused by aflatoxins are many and multi-component. Toxigenic fungi cause plant yield loss. Feed mycotoxin contamination reduces animal productivity due to health problems. The contamination of crops and animal products (e.g., milk) is costly from a human health perspective. Additional losses associated with aflatoxins include the cost of prevention, sampling, mitigation, litigation, and research.

Aflatoxins occur in many animal feed concentrates including cereal grains, soybean products, oil cakes (from groundnuts, cottonseed, sunflower, palm, and copra), and fishmeal. Brewers grains (a byproduct from the production of cereal-based alcoholic drinks) can have high levels. In general, livestock in intensive systems are at higher risk of dietary exposure than animals in extensive systems. Worldwide, a high and increasing proportion of dairy cattle kept in intensive systems; aflatoxins are thus likely to be an increasing problem (Unnevehr and Delia, 2013). In countries where regulation for aflatoxins in animal feeds exists, the total permissible aflatoxin levels in animal feeds range from 0 to 50 parts per billion (ppb) with an average of 20 ppb (FAO 2004) (Standards for individual feed components may be higher.) Studies find that in developing countries around 25–50 percent of samples have levels above 20 ppb and contaminations of 100 to 1,000 ppb are not uncommon (Binder *et al.*, 2007; Rodrigues and Naehrer, 2012).

Deoxynivalenol is a *Fusarium* produced mycotoxin, commonly detected in feed. It is sometimes called vomitoxin because it was associated with vomiting in swine. Surveys have shown DON to be associated with swine disorders including: feed refusals, diarrhea, emesis, reproductive failure, and deaths. The impact of DON on dairy cattle is not established, but clinical data show an association between DON and poor performance in dairy herds (Whitlow *et al.,* 1994).

Mycotoxins have various acute and chronic effects on humans and animals (especially monogastrics) depending on species and susceptibility of an animal within a species.

The economic impact of mycotoxins includes loss of human and animal life, increased health care and veterinary care costs, reduced livestock production, disposal of contaminated foods and feeds, and investment in research and applications to reduce severity of the mycotoxin problem

Animals fed on $AFB_1$and B_2 contaminated feeds excrete into their milk the less toxic $AFM_1$and M_2, respectively which are metabolized in the liver. AFM_1 is of particular interest being the hydroxylated metabolite of AFB_1 and is known to have 2-10% of the carcinogenic potency of the parent compound (Zinedine *et al.,* 2007). Its stability to heat, cold storage, freezing and drying (Yousef and Marth, 1985) during processing makes dairy products another important source of AFM_1 exposure. Milk and milk products are traditionally staple food commodities for the African communities. They are recognized by the elites as natural balanced diet and so are increasingly consumed by the urban populace in the continent. Therefore, they can no longer be ignored as they are among the main entry routes of AFM_1 into the human dietary system in Africa (Makun *et al.,* 2012). Urban dairy farmers in Kenya have been shown to spend nine times more money to purchase commercial feeds than their rural counterparts (Staal *et al.,* 2003) and are at a higher risk of feeding AFB_1 contaminated animal feeds (Kang'ethe and Lang'a, 2009).

Considering that milk and milk derivatives are consumed daily and, moreover, that they are of primary importance in the diet of children who are most vulnerable, many African countries have adopted the set up maximum admissible levels of (0.05µg/L) by the European Union (European Commission, 2003). Kenyan farmers lack knowledge on safe formulation of feed rations and as result feed their dairy animals mostly on farm crop residues, cereals that are discarded due to mould spoilage and commercially prepared concentrates from uncertified agrovet dealers. Rural and peri-urban dairy farmers lack training on proper feed storage such as silage making and lack of properly constructed feed stores (Lukuyu *et al.,* 2011). There is also no monitoring and evaluation (MandE) system and inadequate enforcement of regulation

in Kenya to evaluate the standards of market animal feeds. This presents a risk of dairy animal toxicity with mycotoxins. The economic impact of mycotoxins includes loss of human and animal life, increased health care and veterinary care costs, reduced livestock production, disposal of contaminated foods and feeds, and investment in research and applications to reduce severity of the mycotoxin problem (Zain, 2011). There is no "effective" legislated chemical decontamination process to remove mycotoxins from food and feed. The food and feed industry controls moulds and mycotoxins by implementing a suite of food safety management procedures by feed manufacturers (KMDP, 2013)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

Field survey was conducted in three divisions namely; Bahati, Olenguruone, and Dundori of Nakuru County, Kenya. There area had twelve locations and thirty sub-locations. Olenguruone division lies at about 35° 40’60”E and 0° 34’60”S in DMS (degree minute seconds). Bahati and Dundori divisions lie at about 36° 16′ 12″ E and 0° 12′ 0″ S. The climate of the region varies seasonally and with location; varying from hot and wet to hot and dry. The annual rainfall is between 760-1270mm distributed throughout the year with long rains between April and May and short rains between October and December. Nakuru County is located 160km North West of Nairobi and is the fourth largest City in Kenya.

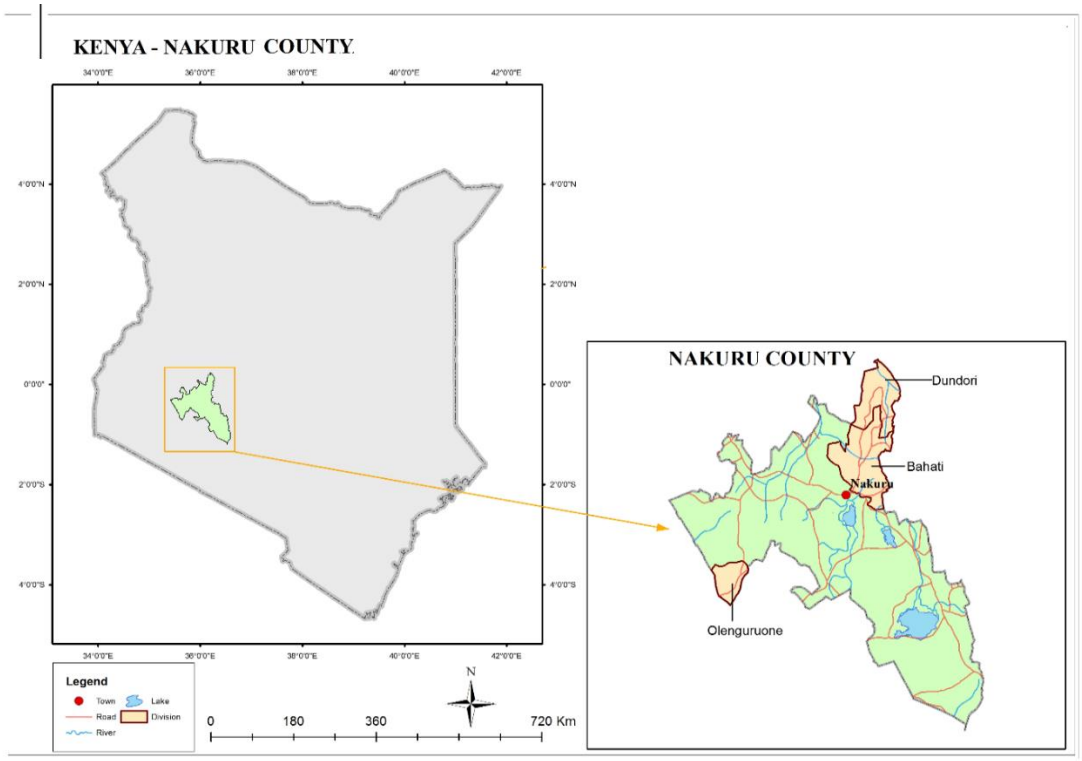


Figure 1:**Map showing Bahati, Dundori and Olenguruone Districts**

Source: DIVA-GIS and International Livestock Research Institute (ILRI)

3.2 Survey of the on-farm and market level management practices associated as risks

The approximate sample size for dairy farms was determined from the formula $n=Z^2 P_{exp} (1 – P_{exp})/L^2$, where Z is confidence level of 95 %, L is desired precision of 10 %. Structured pre-tested two hundred and eighty questionnaires were administered in both the rural and peri-urban areas to the randomly selected smallholder dairy farmers a (Appendix I), milk transporters (Appendix I), milk shops (Appendix I) and milk collection centers (Appendix I)

to obtain qualitative and/or quantitative data. The data collected included feeding practices such as the type of feed used and feed storage practices that predispose feed to fungi growth. There was data on farm and market level risk factors associated with *Brucella abortus* to the cow such as type of breeding and risks to humans such as ingestion of raw milk, milk products, and close contact.

3.3 Study design

A cross-sectional study was conducted in the Nakuru County dairy value chain between March 2015 and October 2015. A value chain approach was used to investigate contamination of animal feeds and milk at on-farm production stage. Emphasis on carryover effect of contamination at processing and marketing of milk that is channeled to urban Nakuru consumers mostly through informal market agents was evaluated.

3.4 Sampling design

The technique of sampling raw milk was random stratified design. A completely randomized design in nested arrangement was used for analysis of the bacterial loads for animal feeds. In the nested arrangement, there were two factors each with different levels: dairy system (rural and peri-urban), dairy market players (farmers, transporters, shops and collection centers). For milk samples, there were five sets of milk sampling units; individual cow composite from four udder quarters, transporters bulk, milk vendors' bulk and collection centers tank bulk were used. A sample of 10ml of milk was poured into sterile labelled screw cap tubes and stored in a cool box maintained at 8°C - 10°C by cooling elements. The collected field samples of milk were transported to microbiology laboratory at Egerton University, Department of Food Science and Technology within 6 hours. Physico-chemical analysis of the milk was done within 12 hours of collecting the milk sample.

For each cow that milk sample was taken, approximately 10mL blood sample was also obtained from the jugular vein venopancture using a vacutainer tubes without EDTA (Bencton Dickson, UK). Samples were labeled using codes describing the specific animal and farm. Then, the tubes were set tilted overnight at room temperature to allow clotting. Next morning, sera were separated from the colt after being centrifuged at 3000 for 10 min to obtain clear serum and siphoned into cryovials and stored at -20°C until analyzed. Blood samples were collected handled according to OIE protocols (OIE, 2000). Blood assay was done at Veterinary Investigation Laboratory, Nakuru. State Department of Livestock, Ministry of Agriculture Livestock and Fisheries

For animal feed samples, a representative sample of 500 grams taken was after mixing from into sterile plastic sampling bags and transported to the laboratory for analysis. Drying of samples was done by keeping them in an oven with the temperature set between 50˚C and 60˚C for 2 days. The samples were then ground and stored at 20˚C under dry cool conditions for analysis. Milk samples from lactating cows on the same farm were collected. A total of 120 milk samples were collected from the individual lactating cows on small holder farms (n=69), milk transporters bringing milk to dairy cooperative outlets (n=30), cooperatives (n=12) and milk bars (n=19) in sterile 60 ml tubes. Samples were transported in cool boxes to the Egerton university laboratory under ice and frozen at -20˚C using a freezer until analyzed for AFM_1 within 3 months of collection.

3.5 Physico-chemical analysis of milk samples

The pH was determined by a pH meter (PHS-3B) after a short but vigorous shaking. The pH meter was calibrated with buffers 4.0 and 7.0. Acid development was measured in percent lactic acid (% LA) of the milk samples. Nine ml of the milk sample was pipetted into a conical flask, 1 ml of 0.5% alcoholic phenolphthalein indicator was added and then titrated against the 0.1N sodium hydroxide (NaOH) until a faint pink colour appeared The number of ml of NaOH solution was divided by ten and expressed as % lactic acid.

3.6Analysis of animal feeds

3.6.1 Determination of water activity in animal feed samples

This was done according to Mapesa, (2004). A durotherm (Aw Messer- Germany) was calibrated using saturated solution of barium chloride and left to stand for 3 hours until water activity reading was at 0.900. Approximately 10g of feed sample was finely chopped into small pieces and placed in the durotherm. The water activity levels were recorded after 3 hours.

3.6.2 Analysis of pH of animal feed samples

The animal feed samples were subjected to pH analysis of the glass electrode (Hinga *et al*., 1980). 20g of air dried feed samples was transferred into 100ml shaking bottle. 50ml of distilled water was added and shaken for 2hours in reciprocal shaker. The pH was determined by a pH meter (PHS-3B) after a short but vigorous shaking. The pH meter was calibrated with buffers 4.0 and 7.0.

3.6.3 Isolation of mycotoxigenic fungal microorganisms from animal feed samples

Five-fold serial dilution of 1gram of feed with distilled water then 0.1ml of the dilution was cultured by spread plate technique onto Potato dextrose agar (PDA) supplemented with chloramphenicol at 40 µg/ml and Gentamycin at 500 µg/ml and incubated for 5 to 14 days at room temperature. Pure culture of the different colonies (based on morphology) was

obtained by sub-culture of the isolates on potato dextrose agar plates and sabouraud's dextrose agar plates. The fungal isolates was identified to the genus level based on macroscopic, microscopic and biochemical characteristics of the isolates obtained from pure cultures (Islam *et al*.,2014).

3.6.4 Determination of moisture content of animal feed samples

The moisture content was determined according the procedure provided by the Association of Official Analytical Chemists (AOAC) International (AOAC, 2000). Samples weighing 2 g were dried in triplicates an oven at 105 °C for 3 h. cooling of the dried samples was done in a desiccator for 10 min. Moisture content was calculated as the loss in weight expressed as a percent of the original weight of the animal feed. The amount of moisture was reported in terms of loss in weight.

3.7 Screening of *Brucella abortus*

3.7.1 Milk ring test

Screening for *Brucella abortus* was done using milk ring test (Alton *et al.,* 1988) for the milk samples from individual cow and the market samples. The test was performed by adding 30 µl (0.03 ml) of *B. abortus* Bang Ring Antigen (hematoxylin-stained antigen).The height of the milk column in the tube was kept up to 25 mm. The milk (antigen) mixtures were incubated at 37°C for 1 hour, together with positive and negative control samples. Agglutinated *Brucella* cells was picked up by fat globules as they rise, forming a dark cream layer on the top of the sample. A strongly positive reaction was indicated by formation of a dark blue-ring above a white milk column. The test was considered negative if the color of the underlying milk exceeded that of the cream layer and when the cream layer was normal.

3.7.2 Rose-Bengal plate test.

Serum samples and antigen were warmed to room temperature (22 ± 4°C); only sufficient antigen for the day's tests was removed from the refrigerator. Each serum sample 25–30 µl was placed on a white tile, enamel or plastic plate, or in a WHO haemagglutination plate. Antigen bottle was shaken well, but gently, and placed an equal volume of antigen near each serum spot. Immediately after the last drop of antigen was added to the plate, the serum and antigen was thoroughly mixed (using a clean glass or plastic rod for each test) to produce a circular or oval zone approximately 2 cm in diameter. The mixture was agitated gently for 4 minutes at ambient temperature on a rocker or three-directional agitator. Agglutination was read immediately after the 4-minute period was completed. Any visible reaction was considered to be positive. A control serum that gives a minimum positive reaction was be tested before each day's tests were begun to verify the sensitivity of test conditions.

3.8 Confirmatory tests for *Brucella abortus*

3.8.1 Complement Fixation Test (CFT)

The Complement Fixation Test (CFT) was done following the protocol described by (McMillan, 1990) using a Complement fixation test antigen (ID vet Innovative Diagnostics, Product code Ch.-B: AG-BRU-002, Grabels, France), VCM buffer complement (ID vet Innovative Diagnostics, Grabels, France), Amboceptor (ID vet Innovative Diagnostics, Grabels, France), and 2% sheep serum.

3.8.2 The competitive Enzyme Linked Immunosorbent Assay Test (c-ELISA)

The Svanova c-ELISA (SVANOVIR® Biotech Ab, Product code: A27658, Uppsala, Sweden) kits were used for confirmation of RBPT-positive cases. The c-ELISA was analyzed according to the manufacturer's instructions (Muma *et al*. 2006). The c-ELISA kit contained the antigen, control sera, buffers and conjugates. The Brucella Ab ELISA kit accurately detected weak and strong positive OIE reference according to EU Directives using microplate reader (SENOVA, China). In this procedure, the samples were exposed to *Brucella abortus* smooth lipopolysaccaride (S-LPS) coated wells on microtiter plates together with mouse monoclonal antibody (mAb) specific for an epitope on the o-poly-saccharide portion of the S-LPS antigen. After an incubation period the microplate was washed and goat anti mouse IgG antibody conjugate with hoseradish peroxidise (HRP) was added which binds to any mAb's bound to S-LPS on the plate. Unbound materials were removed by rinsing before the addition of the substrate solution. Color development was due to the conversion of the substrate by the conjugate. Stop solution was added and the optical density was measured by microplate photometer at 450nm. In the absence of anti-Brucella antibody in the test serum (negative), the mAb binds to the o-polysaccharide epitope of the S-LPS antigen and was indicated by color development. If the test serum contains Brucella specific antibodies (Positive) they competed with the mAb for the epitope sites and inhibit the mAb binding to the o-poly- saccharide portion of S-LPS antigen and subsequent color development.

3.9 Detection of mycotoxin in animal feeds and milk

3.9.1 Quantitative analysis of aflatoxinB_1 in feed sample by Enzyme immunoassay

Preparation of the sample

A representative sample was triturated and thoroughly mixed in a mixer. Accurately, 2 grams of the ground sample was weighed into a screw cap glass vial. The sample was mixed with 10ml of methanol/distilled water (70/30; v/v) and mixed for 10 minutes at room temperature using a shaker. The entire extract was filtered. 100 µL of the filtrate was diluted

with 600 µL of the sample dilution buffer. The 50 µL of sample per well was employed in the assay.

Enzyme immunoassay for Aflatoxin (AFs) total in animal feeds

The total AFs concentration was analyzed using a commercial ELISA kit (Ridascreen, Aflatoxin Total R-Biopharm, Darmstadt, and Germany. Product code R4701). Detection limit for feed samples were 1.75 ppb with recovery rate of 85%. AFs concentration in animal samples was measured according to the instructions of the manufacturer using standards of 0, 0.5, 1.5, 4.5, 13.5, and 40.5 µg/Kg. A standard of 50 µL solutions or prepared sample were added to separate duplicate well. 50 µL of diluted enzyme conjugate was added to each well. The antibody binding sites were occupied proportionally to the AFs concentration during incubation of 30 min at room temperature (20–25°C) in the dark. The liquid was poured out of the wells and the wells were washed 3 times using 250 µL of distilled water per well. 50 µL of substrate solution and 50 µL of chromogen was added to each well, the plate was mixed and incubated for 30 minute at room temperature in the dark. At the end, 100 µL of the stop solution was added to each well and was mixed. The AFs concentration was measured photometrically at 450 nm (Readwell strip, ROBONIK, India).

3.9.2 Quantitative analysis of Deoxynivalenol in feed sample by Enzyme immunoassay

Preparation of the sample

Five grams of the ground sample was weighed and added into a suitable container with 25ml of distilled water and shaken vigorously for 3 minutes. The extract was filtered through Whatman No. 1 filter paper. 50 µL of sample per well was employed in the assay.

Enzyme immunoassay for Deoxynivalenol in animal feeds

The Deoxynivalenol (DON) was analyzed using a commercial ELISA kit (Ridascreen, DON R-Biopharm, Darmstadt, Germany. Product code R5906.). Detection limit for feed samples were 18.5ppb with recovery rate of 85%. DON in animal samples was measured according to manufacturer's instructions using standards of0, 3.7, 11.1, 33.3, and 100µg/Kg. 50 µL of standard solutions or prepared sample were added to separate duplicate well. 50 µL of diluted enzyme conjugate was added to each well. The antibody binding sites were occupied proportionally to the DON concentration during incubation of 30 min at room temperature (20–25°C) in the dark. The liquid was poured out of the wells and the wells were washed 3 times using 250 µL of distilled water per well. 50 µL of substrate solution and 50 µL of chromogen was added to each well, the plate was mixed and incubated for 30 minutes at room temperature

in the dark. At the end, 100 µL of the stop solution was added to each well and the plate was mixed. The DON was measured photometrically at 450 nm (Readwell strip, ROBONIK, India).

3.9.3 Quantitative analysis of AFM_1 in milk sample by Enzyme immunoassay

Sample preparation

Milk samples that had been stored at -20ºCwere thawed and centrifuged before analysis using an ELISA kit. 20 mL volume was measured for each sample and analyzed for M_1.

Determination of Aflatoxin M_1in milk using Enzyme immunoassay.

The AFM_1 was analyzed using a commercial ELISA kit (Ridascreen, DON R-Biopharm, Darmstadt, Germany. Product code R1121.) Detection limit for milk samples were 5ng/L with recovery rate of 95% as stated by manufacturer. AFM_1 in skimmed milk samples was measured according to the instructions using standards of (0, 0.01, 0.02, 0.04, and 0.08 µg/L). Skimmed milk of 100 µ L samples plus 10 µL of standard solutions (0.08 µg/L was used due to the detection limit of 0.05µg/Kg) were added to each well. The antibody binding sites were occupied proportionally to the AFM1 concentration during incubation of 60 min at room temperature (20–25°C) in the dark. The liquid was poured out of the wells and the wells were washed 3 times using 250 µL of washing buffer per well. 100 µL of the enzyme conjugate was added and incubated for 60 minutes at 25ºC in dark. Washing of the wells by the buffer was done twice then 50 µL of substrate solution and 50 µL of chromogen was added to each well. The plate was shaken to mix the substrate and the chromogen before incubation for 30 minute at 25ºC in the dark. To stop the reaction, 100 µL of the stop solution was added to each well and the plate was shaken to mix. The AFM_1 was measured photometrically (Readwell strip, ROBONIK, India) at 450 nm.

3.9.4 ELISA Method Validation

The validation of the ELISA method was carried out by determining the recoveries and the coefficient of variation (% CV). The mean absorbance values obtained for the standards and the samples were divided by the absorbance value of the zero standards and multiplied by 100. The zero standards were made equal to 100% and the absorbance values of other standards and samples were quoted in percentages of these values. The values calculated for the standards were entered in a system of coordinates semi- logarithmically and analyzed against the mycotoxin concentration using Excel (Microsoft, Inc. USA). The mycotoxin concentration in µg/L or µg/Kg corresponding to the absorbance of each sample was read from the calibration

curve. Calibration curves were prepared for each mycotoxin AFM_1, AFB_1, DON and coefficients of determination (r^2) were calculated respectively. In milk, AFM_1 curve was prepared from standard solutions in range 0.005 - 0.08 µg/L with r^2 =0.988. In animal feeds, AFB_1 curve was obtained from the standard solutions in range 0.05 – 4.05 µg/Kg with r^2 = 0.982. In animal feeds, DON curve was prepared from standard solutions in range of 3.7 - 100 µg/Kg with r^2 =0.987 (APPENDIX VI). In milk, recovery of ELISA was evaluated by analyzing spiked uncontaminated milk samples in triplicates at the level of 0.01µg/L and 0.05 µg/L corresponding to the maximum value allowed by the European Commission as a bench mark. Animal feeds were spiked with three different concentrations (Table1). The validation experiments were performed as described for the samples above. Both the recovery and % CV were in compliance with Commission Regulation (EC, 2006b).

Table 1: Validation of ELISA data for AFM_1 AFB_1 and DON as per EU standards

Spiked sample	Spiked level[a]	Recovery (%)	Coefficient of variation
Milk (AFM_1)	0.01	101	1.1
Milk (AFM_1)	0.05	98	3.2
Animal feed (DON)	11.1	97	2.7
Animal feed (DON)	33.3	97	3.8
Animal feed (DON)	100	98	4.4
Animal feed (AFB_1)	4.5	98	2.1
Animal feed (AFB_1)	13.5	98	4.7
Animal feed (AFB_I)	40.5	98	3.6

[a]µg/L for AFM_1 and µg/Kg for AFB_1/DON with three replicates at each level.

3.10 Statistical Data Analysis

For risks factors, the data obtained from the structured questionnaires were analyzed using SPSS version 20 software. Chi-square test was used to test independence of the nominal variables at $P<0.05$. The quantitative data was analyzed by use of the SAS statistical analysis package (SAS version 9) (SAS, 2006) to obtain analysis of variance (ANOVA) test at the 95% confidence level. The relationship of the occurrences of *Brucella* and its risk factors was done through logistic regression. The relationship of the occurrences of abiotic factors and its mycotoxin contamination was done through logistic regression. Means separation was done using least significant differences (LSD). Hypothesis testing of two means was done to compare means of aflatoxin M_1, AFs and DON concentrations at 95% confidence level.

CHAPTER FOUR

4.0 RESULTS

4.1 To characterize the risk factors associated with prevalence of Aflatoxins (AFs), DON and *Brucella abortus* in rural and peri-urban milk sub-value chains in Nakuru County.

4.1.1 Risk factors associated with prevalence of Aflatoxin (AFs) and DON contamination in animal feeds.

At the farm level, the type of feeds storage facility and the type and condition of animal feeds were found to be significant $P<0.05$ risk factors for infestation of mycotoxigenic mold.

4.1.2 Environmental factors and physico-chemical factors affecting fungal growth and mycotoxin contamination of animal feeds animal feeds in rural and peri-urban dairy systems.

The mean moisture content of animal feeds was significantly different at $P\leq0.05$ between the dairy systems as shown in Table 3. Water activity (a_w) and pH of the animal feeds were significantly different at $P\leq0.05$ between intensification types, classification and the condition they were found either wet or dry as shown in Table 3.

There exists a very strong positive correlation between moisture content in animal feeds and the water activity ($r=0.935$, $P<0.001$) as shown in Table 4, between environmental temperature and the storage bag temperature ($r=0.999$, $P<0.001$) (Table 4 and Figure 2) There There exists a very strong positive correlation between environmental humidity and the storage bag humidity which were closely correlated ($r=0.799$, $P<0.001$) (Table 4 and Figure 3.)

Table 2: Animal feed handling practices influencing mold infestation in animal feeds

Factors		Mold infested feeds (n)	Mold free feeds (n)	Prevalence (%)	Prevalence Ratio	P-Value
Dairy system	Peri-urban	30	10	75.0	0.95	0.650
	Rural	45	12	78.9		
Storage	Enclosed stores	24	28	53.8	1.78	0.040
	On open racks	43	2	95.6		
Source of feeds	On-farm formulation	55	25	68.8	1.28	0.144
	Bought from agrovets	15	2	88.2		
Types of animal feed	Concentrate	50	7	87.7	2.34	0.032
	Forages	15	25	37.5		
Any training on feed	Yes	27	10	72.9	1.23	0.087
formulation and handling	No	52	6	89.7		
Condition of animal feeds	Dry	50	7	87.7	2.34	0.011
	Wet	15	25	37.5		

Table 3: **Physico-chemical parameters of the animal feeds**

Factors		Physico-chemical properties		
		Water activity ($a_{w)}$	Moisture content	pH
Dairy system	Peri Urban	0.76±0.12[a]	33.41±29.5[a]	6.28±0.8[a]
	Rural	0.76±0.17[a]	43.45±31.8[b]	6.61±.06[a]
Intensification	Zero	0.74±0.17[b]	35.67±29.5[b]	6.03±0.9[c]
	Semi	0.78±0.17[a]	46.10±32.4[a]	6.58±0.6[b]
	Free	0.74±0.16[b]	37.87±31.1[b]	6.84±0.2[a]
Classification	forage	0.79±0.2[a]	47.70±31.7[a]	6.65±0.7[a]
	Concentrate	0.65±0.1[b]	19.45±17.3[b]	6.20±0.3[a]
Condition	Dry	0.62±0.08[b]	15.16±3.5[b]	6.64±0.4[a]
	Wet	0.94±0.03[a]	77.60±4.9[a]	6.45±0.9[a]

Mean values not followed by the same letter in a column on table 3 were significantly different (P≤0.05).

Table 4: **Correlation coefficients of relationships between the physico-chemical and environmental factors affecting feeds**

	MC	Aw	pH	StoreTemp	EnvTemp	StoreHum	EnvHum
MC	----	0.935***	-0.062	-0.176	-0.176	0.106	0.100
Aw		___	-0.115	-0.185	-0.184	0.006	-0.039
pH		___	___	-0.007	-0.009	0.164	0.189
StoreTemp		___	___	___	0.999***	-0.618***	-0.179
EnvTem		___	___	___	___	-0.618***	-0.179
StoreHum		___	___	___	___	___	0.799***

MC= moisture content; StoTemp = storage bag temperature; Envtemp = environment temperature; Stohum = storage bag humidity; envhum = environmental humidity

* is significant at P = 0.05, ** is significant at P = 0.01 and *** is significant at P = 0.001

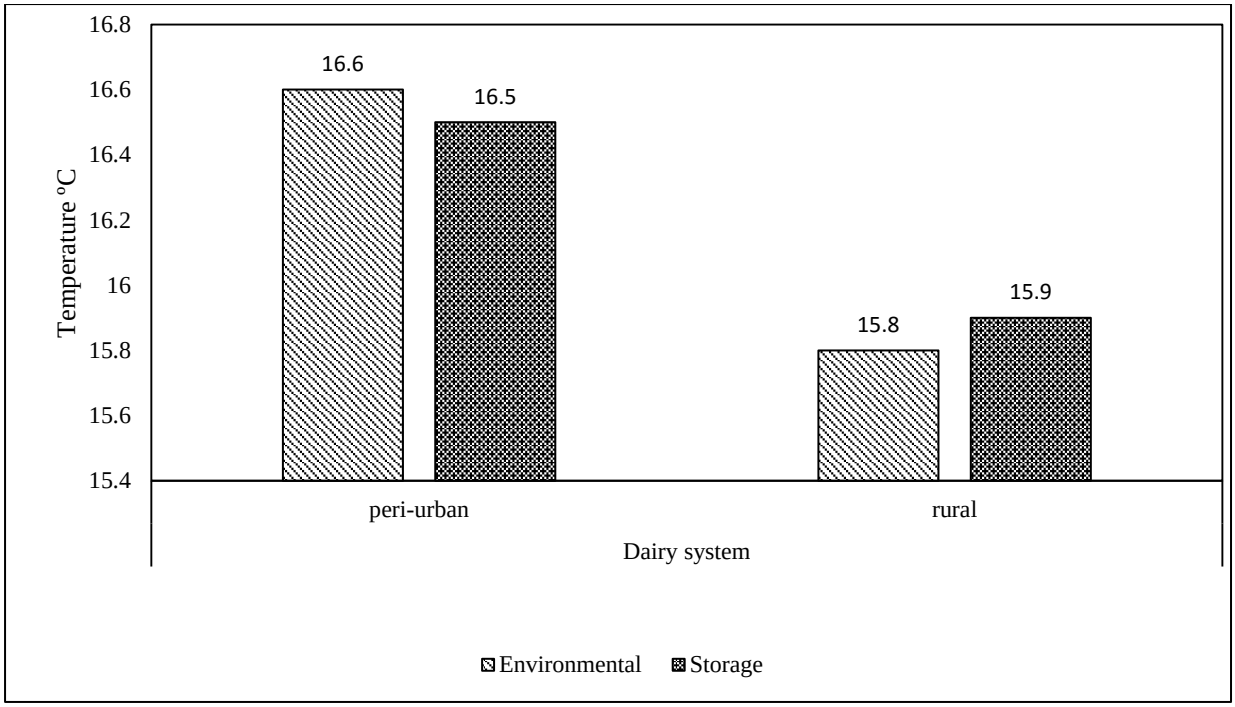


Figure 2: **Environmental and storage temperatures prevailing in the dairy systems**

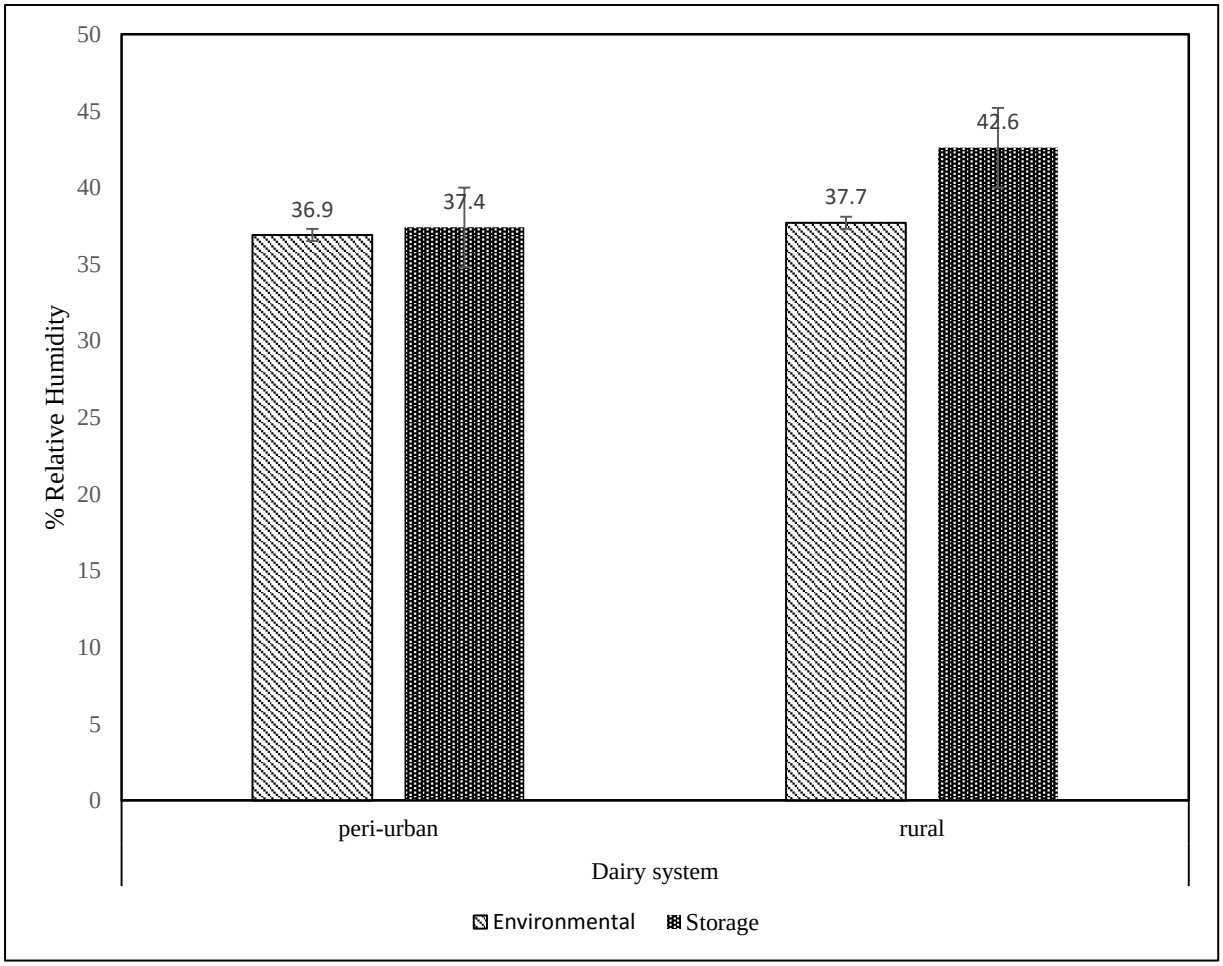


Figure 3: **Environmental and storage humidity prevailing in the dairy systems.**

Shredded maize stovers

Animal feed stored under humid conditions

Stored rotten maize combs for cows

Animal feed stored in the open exposed to extreme environmental conditions

Figure 4. Feed storage practices by farmers in both rural and peri-urban systems

4.1.3 Fungal enumeration, isolation and identification in animal feeds

The mean count of mold growth in animal feeds was significantly $P \leq 0.05$ different between the two dairy systems as shown in Figure 4. Animal feeds from peri-urban had the highest mold count of $\log_{10}4.92 \pm 0.4$cfu/g as compared to $\log_{10}3.99 \pm 0.9$cfu/g animal feeds from rural as presented in Figure 4.

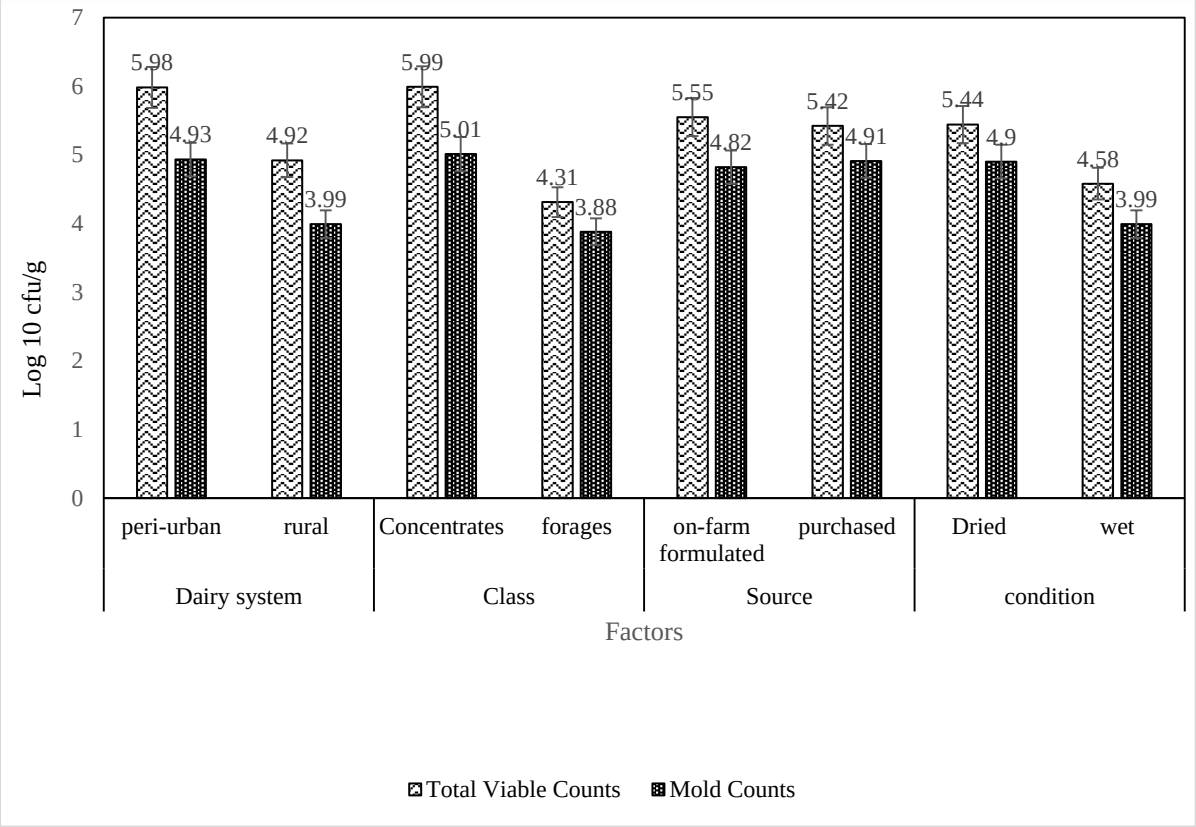
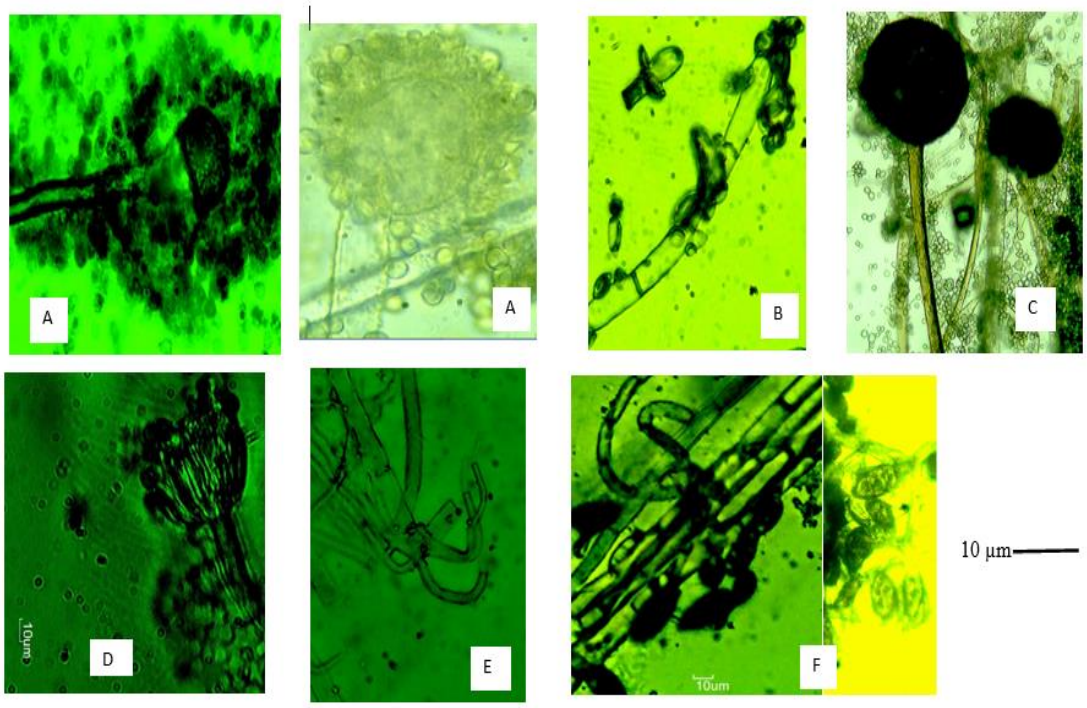


Figure 5: **The mean count of mold growth in animal feeds**

The dominant toxigenic fungi genera in both dairy systems were *Aspergillus* spp. 77%, *and fusarium spp*. 70% respectively as shown in Table 5. Microscopic monographs showing different fungal genus were used for identification (figure 5)

Table 5: **Prevalence of molds in animal feeds**

Fungal Genera	Number of positive samples			Frequency (%)		
	Rural (n= 57)	Peri-urban (n= 40)	N	Rural	Peri-urban	Overall
Aspergillus spp.	45	30	75	79	75	77
Fusarium spp.	42	26	68	74	65	70
Cladosporium spp.	13	12	25	23	40	26
Mucor spp.	16	13	29	28	33	29
Penicillium spp.	1	2	3	2	15	3
Alternaria spp.	3	2	5	5	5	5

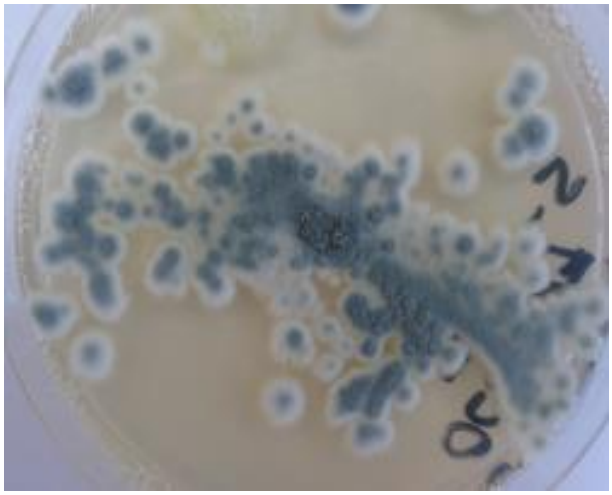


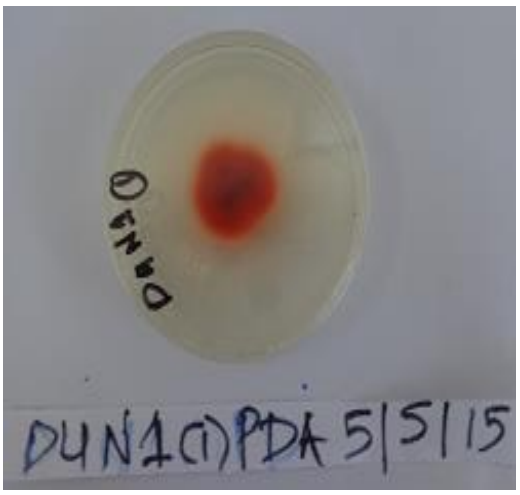
A= *Aspergillus*; B=*Cladosporium*; C=*Mucor*; D= *penicillium*; E= *Fusarium* and F= *Alternaria*

Figure 6 Microscopic monographs showing different fungal genus

Micro-morphological characteristics of different molds

Fungal Genera	Colony characteristics	Microscopic characteristics
Aspergillus (A)	Bluish green with sulphur yellow areas on the surface colonies	Flask shaped phialides on apical vesicle
Fusarium (E)	Orange colonies	Sickle – shaped macroconidia
Cladosporium (B)	Greenish- grey colonies	Blastoconidia
Mucor (C)	Grey colonies	Zygomycetes and sporangia
Penicillium (D)	Bluish green colonies	Brush arrangement of phiospores
Alternaria (F)	Dark green- black with grey periphery colonies	Chains of macroconidia; transverse septa conidia





Aspergillus genus *Fusarium genus*

Figure 7. Pictures showing colony characteristics of mycotoxigenic molds

4.1.4 Risk Factors associated with prevalence of brucellosis on the farm

At the farm level, the type of the cow breed, method of breeding and history of abortion were found to be significant P<0.05 risk factors for *brucellosis* as shown in Table 6. The history of vaccination, bought-in-cattle and type of intensification were found not to be significant at P>0.05 risk factors or preventative factors for *brucellosis* as shown in Table 6.

Table 6: Risk factors associated with the spread *brucellosis* at the farm level

Factors		Infected cows	Healthy cows	Prevalence (%)	Prevalence ratio	P-Value
Dairy system	Peri-urban	1	75	1.3	0.74	0.634
	Rural	3	166	1.8		
Intensification	Free grazing	3	166	1.8	1.38	0.257
	Restricted	1	75	1.3		
Breed	Indigenous	1	10	10.0	7.69	0.001
	Improved and Cross	3	231	1.3		
Breeding	Bull	3	127	2.4	2.67	0.03
	AI	1	114	0.9		
Vaccination	Vaccinated	0	0	0	-	-
	Not vaccinated	4	241	1.7		
Abortions	Aborted	3	64	4.7	7.83	0.001
	Not aborted	1	177	0.6		
Bought-in-cattle	Yes	2	96	2.1	1.50	0.12
	No	2	145	1.4		

4.1.5 Risk factors for brucellosis at the market level

At the market level, fermentation of milk and pasteurization of milk were found in this study to be significant P<0.05 preventative factors for *brucellosis* as shown in Table 7. However, boiling of milk was found to be a risk factor for the *brucella* zoonosis.

Table 7: Risk factors for brucellosis at the market level

Factors		MRT positive	MRT negative	Prevalence (%)	Prevalence ratio	P-Value
Boiling	Boiled	2	19	9.0	0.42	0.452
	Not boiled	5	23	21.0		
Fermentation	Fermented	2	16	12.5	0.46	0.021
	Not fermented	7	26	26.9		
Pasteurization	Pasteurized	0	2	0.0	-	0.001
	Not pasteurized	7	40	17.5		

4.2 Determination of Aflatoxin (AFs) and DON contamination in animal feeds and quantification aflatoxin M_1

4.2.1 The prevalence of Aflatoxin (AFs) in animal feeds

Animal feed contamination with Aflatoxins (AFs) was more frequent in rural (60%) than in peri-urban (53%) dairy system as illustrated in Figure 1, but the median concentration was higher in peri-urban (60.43 µg/kg) than in the rural (12.25 µg/kg) system from the estimates in Table 8. The analysis of variance (ANOVA) showed concentrates had significantly higher total AFs concentration levels compared to forage at $P<0.001$ (Table 9). The lowest observed level of total AFs concentration contamination of 2.31µg/kg was from a hay feed sample from rural while the highest total AFs concentration level of 147.86µg/kg was observed in an on-farm formulated concentrate feed sample that contained maize germ from peri-urban as shown in Table 8. Association of water activity (a_w) and moisture content of the feeds with levels OF total AFs contamination of the feeds was significant at $P<0.05$ and $P<0.01$ respectively (Table 10).

Table 8: **Aflatoxin (AFs) and DON contamination of animal feeds on small holder dairy systems in Nakuru**

Factor	Level	Statistic	AFs	DON
Dairy system	Rural	Median	12.25	21.62
		Range	2.31 - 84.41	0.00 - 82.79
	Peri-urban	Median	60.43	60.61
		Range	0.00-174.86	0.00-179.89
Type of animal feeds	Forage	Median	7.52	1.33
		Range	2.31-29.52	0.00-96.20
	Concentrates	Median	42.07	66.25
		Range	21.33-147.86	0.00 -179.89

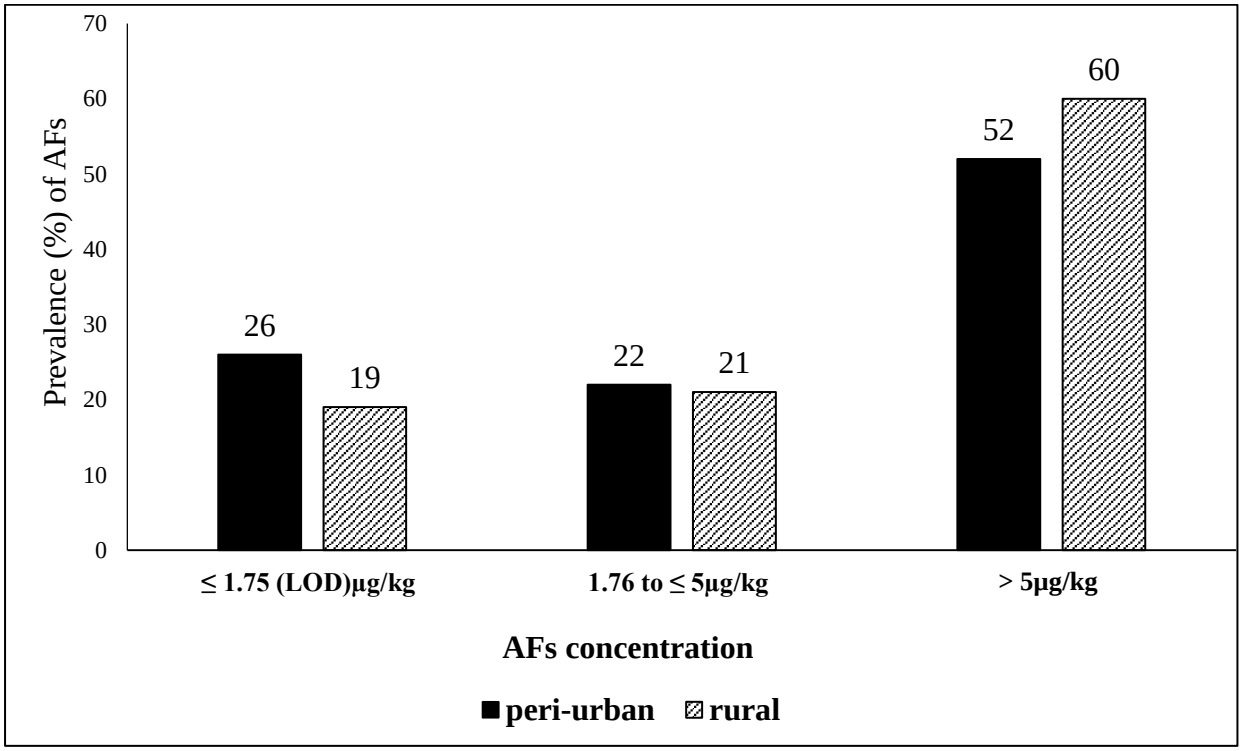


Figure 8: Prevalence of contamination of animal feeds with AFs concentration in the peri-urban (N= 27) and rural (N= 47) dairy systems in Nakuru, Kenya.

Table 9: The analysis of variance of Aflatoxin (AFs) and DON contamination in the animal feeds from the two dairy systems and type of the feeds

Source of Variation	DF	MS for AFs	MS for DON
Dairy system	1	2029.670**	6362.286*
Type of Feed	1	33623.769***	51336.681***
Dairy system*Type of Feed	1	1354.295^{ns}	3275.537^{ns}
Error	70	730.152	1629.592

DF=Degree of Freedom, MS=Mean Square, AFs = Total Aflatoxins, DON= Deoxynivalenol, ns= not significant, * is significant at P=0.05, ** is significant at P=0.01 and *** is significant at P=0.001

Table 10: **Linear regression model showing association of abiotic factors with level of Aflatoxin (AFs) and DON contamination in animal Feeds**

	AFs			**DON**		
Variable	Coefficients	95% confidence interval	P-value	Coefficients	95% confidence interval	P-value
Constant	50.10	32.26 - 67.94	0.006	330.67	234.82-426.52	0.001
a_w	-3.04	-0.67- -5.41	0.024	125.06	46.33- 203.79	0.012
pH	-1.39	-1.90- -0.88	0.610	-36.62	-46.66- -26.58	0.008
Moisture Content	-0.41	-0.26- -.056	0.007	0.92	0.57- 1.27	0.004

AFs = Total Aflatoxins, DON= Deoxynivalenol , a_w=Water activity

4.2.2 The prevalence of DON in animal feeds from rural and peri-urban dairy systems

Figure 2 illustrates that the contamination of feeds with DON was more frequent at 71% with a higher concentration of median 60.61µg/kg in the peri-urban than the rural dairy system at frequency of 53% with concentration of median 21.62µg/kg (Table 8). The analysis of variance (ANOVA) (Table 9) showed the DON contamination was significantly higher in concentrates than in forage. The lowest level of DON contamination of 4.37µg/kg was obtained from hay sample from rural dairy system while the highest level of DON contamination of 179.89µg/kg was observed in silage feed sample from peri-urban system as shown in Table 2. Association of water activity, pH and moisture content of feeds with DON contamination levels was significant at P<0.01, P<0.01 and P<0.01 respectively (Table 10).

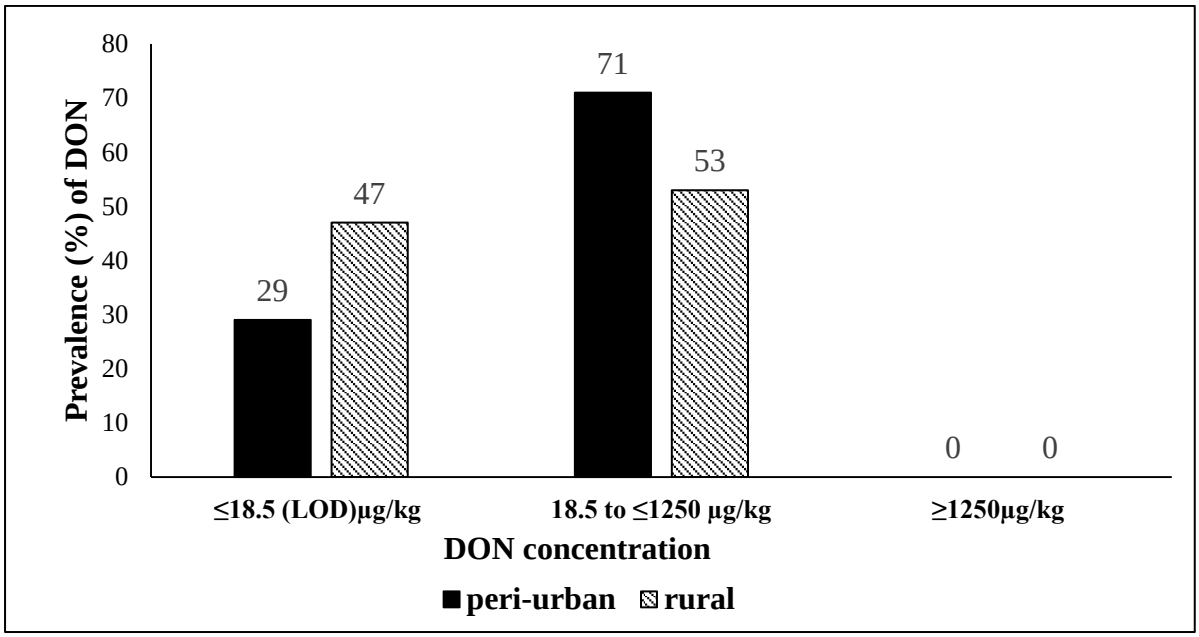


Figure 9: **Prevalence of contamination of animal feeds with DON in the peri-urban (N= 24) and rural (N= 19) dairy systems in Nakuru, Kenya**.

4.2.3 Prevalence of aflatoxin M_1 in milk samples along rural and peri-urban sub-value chains

In the peri-urban system, the prevalence of AFM_1 contamination ranged from 68% at production, 29% at transporters, 40% at cooperatives and 17% at milk bar outlets with a median value of 0.073 µg/L at production level as shown in Figure 7 and Table 11. All milk samples in the rural system were contaminated with AFM_1 concentration levels of less than 0.05 µg/L with a median value of 0.006 µg/L at production level as shown in Figure 8and Table 11. A

majority of the samples along the rural value chain were below the limit of quantification of 0.005 µg/L as shown in Figure 8.

Table 11: **Aflatoxin M_1 contamination in milk in the rural dairy system and peri-urban dairy systems**

Dairy System	Statistic	Production	Transporters	Cooperatives	Milk bars
Rural	Mean ±SD	0.011 ± 0.010[b]	0.007±0.006[b]	0.005±0.008[b]	0.006±0.004[b]
	Median	0.006	0.006	0.00	0.00
	Range	0.00-0.041	0.00-0.019	0.00-0.022	0.00-0.034
Peri-urban	Mean ±SD	0.062±0.019[a]	0.049±0.021[a]	0.043±0.025[a]	0.033±0.015[a]
	Median	0.073	0.048	0.042	0.029
	Range	0.022-0.083	0.020-0.083	0.019-0.082	0.017-0.069

Means with same letter along the column were significantly different at P≤0.05 and SD=Standard Deviation

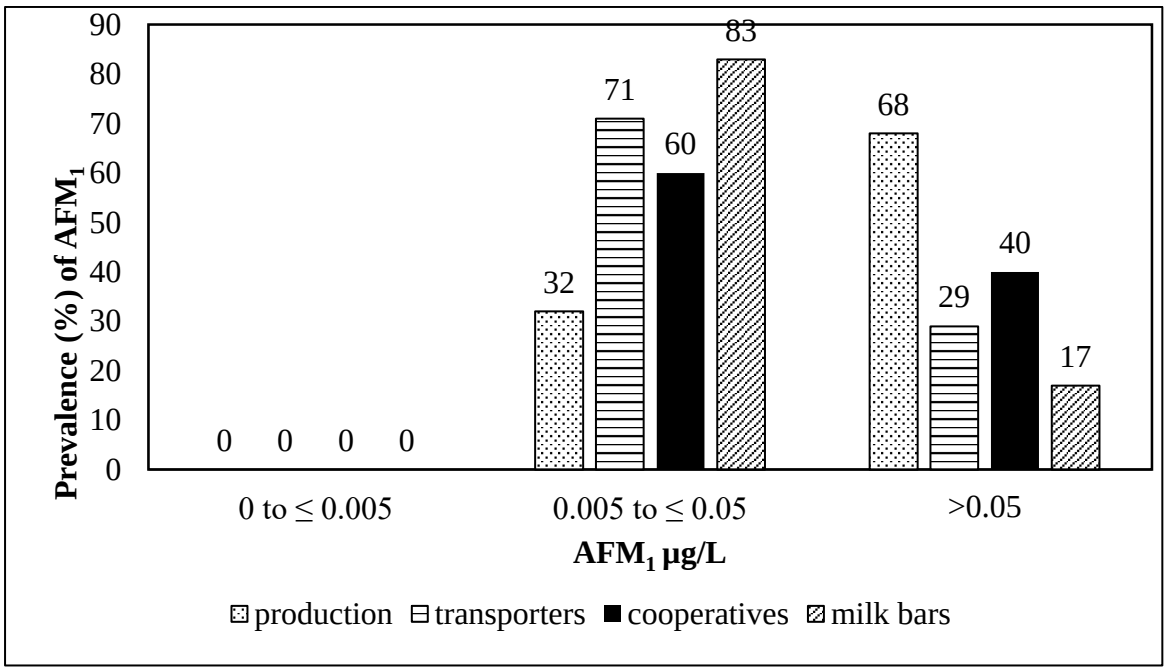


Figure 10: **Prevalence of contamination of milk samples with AFM_1 in the peri-urban dairy system (N= 68) in Nakuru, Kenya**.

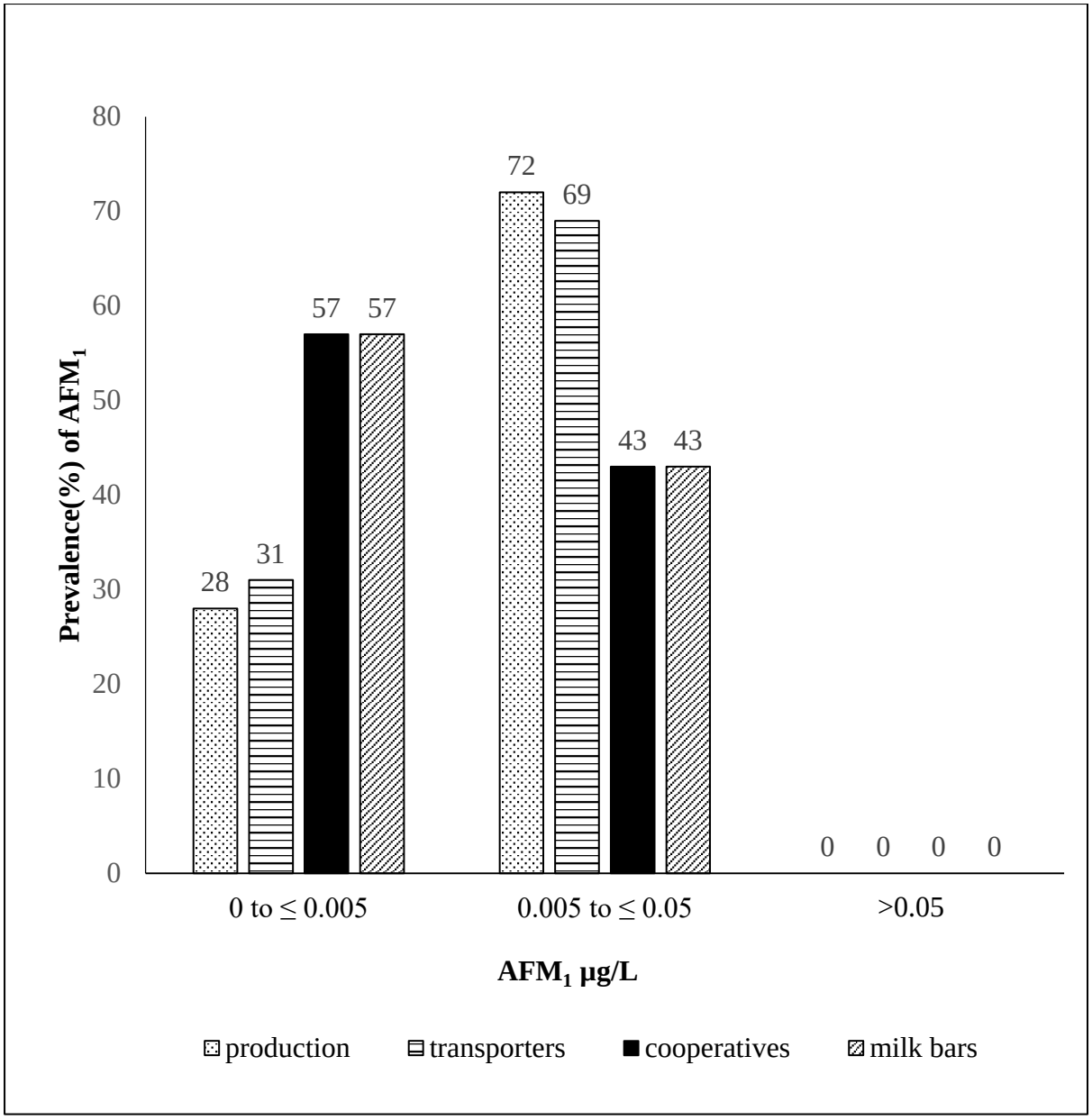


Figure 11: Prevalence of contamination of milk samples with AFM_1 in the rural dairy system (N=62) in Nakuru, Kenya.

The reducing trend of AFM_1 contamination along the value chain was observed with milk from cooperatives and milk bars having slighter range of contamination compared to milk from individual cows at production. Linear regression showed significant association between AFM_1 contamination in milk and use of concentrates in the peri-urban dairy system

There was a moderate correlation between Aflatoxin (AFs) in feed samples and AFM_1 in milk samples ($r=0.46$, $P<0.001$) collected from the same dairy farm.

4.3 Determination of the prevalence of *Brucella abortus* in rural and peri-urban milk sub-value chains in Nakuru County.

4.3.1 Prevalence of B. abortus antibodies in rural and peri-urban sub value chains

The prevalence of *B. abortus* antibodies based on screening tests (MRT and RBPT) at the production level and confirmatory tests and CFT were shown in Table 13. At production level, the prevalence of both the screening and the confirmatory results in the rural system was higher than in the peri-urban system as shown in Table 13.

Table 12: Prevalence of *B. abortus* antibodies in rural and peri-urban sub value chains

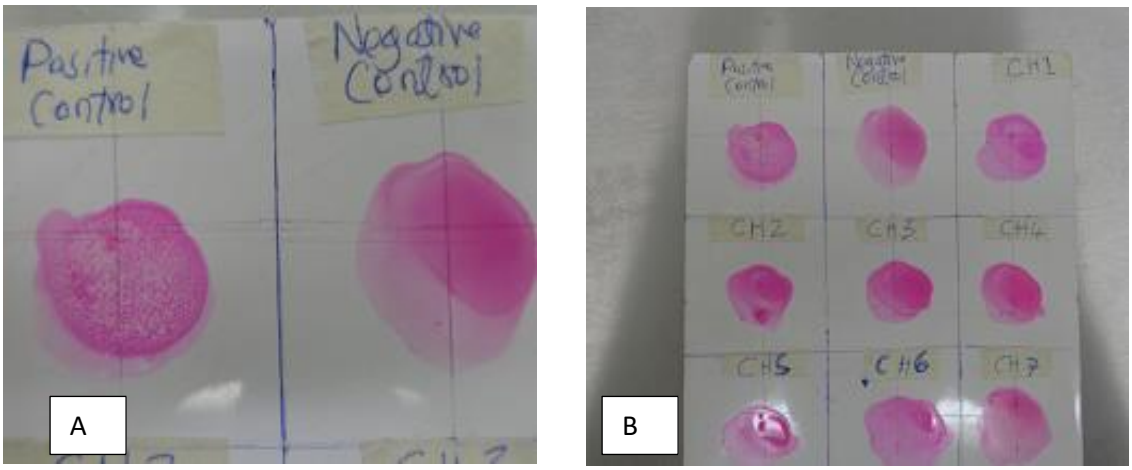
Dairy system	Level	Samples	Screening tests		Confirmatory tests			
			MRT (%)	RBPT (%)	CFT (%)	C-ELISA (%)	Mean pH	Mean TA (%)
Peri-urban	Production	76	43	5	1.3	1.3	6.29	0.17
	Market	21	9	-	-	-	6.11	0.19
Rural	Production	169	60	19	1.7	1.7	6.36	0.15
	Market	28	17	-	-	-	6.10	0.19

Key: MRT is milk ring test; RBPT is Rose Bengal Precipitation test; c-ELISA is competitive Enzyme Linked Immunosorbent Assay Test and CFT is Complement Fixation Test; TA is Titratable acidity

The peri-urban dairy system was found to have a low prevalent risk of 0.01 for the occurrence of *B. abortus* along the sub-value chain as compared to the rural dairy system which had a prevalent risk of 0.02 (Table 14).

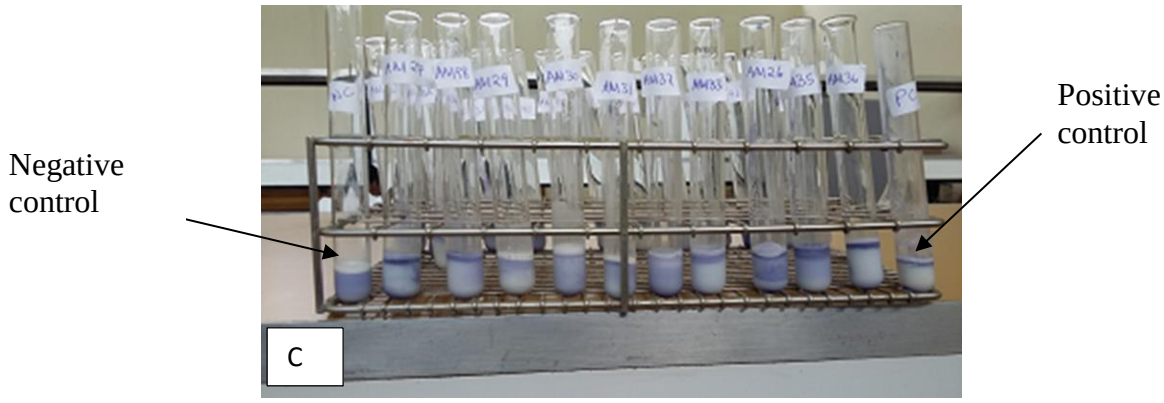
Table 13: Prevalent risk of *B. abortus* antibodies in rural and peri-urban sub value chains

Dairy system	Total samples	Positive samples	Negative samples	Prevalence risk
Peri-urban	76	1	75	0.01
Rural	169	3	166	0.02



A and B: Rose Bengal plate test results where negative control presents no agglutination and positive control presents complete agglutination of serum sample.

Figure 12.Pictures of Rose Bengal plate test results



C: Milk ring test results where the positive control forms a dark blue-ring above a white milk column

Figure 13. Pictures of Milk ring test results

CHAPTER FIVE

5.0 DISCUSSION

5.1 The risk factors associated with prevalence of Aflatoxin (AFs) and DON contamination in animal feeds.

The study identified three risk factors for mycotoxin contamination at the farm level; the type of feeds, type of storage facility and the type and condition of animal feeds were found to be significant $P<0.05$ risk factors for infestation of mycotoxigenic mold while the type of the dairy system, the source of animal feeds and any training on feed formulation and handling were found to present no significant risk factors $P>0.05$ for infestation of mycotoxigenic mold (Table 2).

The type of feeds was found to be significant $P<0.05$ risk factor for infestation of mycotoxigenic mold. Dry Concentrates had the highest mould count of 4.39±1.0 cfu/g as compared to 3.76±1.0 cfu/g in wet forages as shown in figure 4. The dominant toxigenic fungi genera in both dairy systems were *Aspergillus spp.* 77%, *and fusarium spp*. 70% respectively (Table 5). This was attributed by different extrinsic and intrinsic factors affecting the different types of animal feeds. Mycotoxin producing fungi establishment, development and subsequent mycotoxins production in feeds depended on extrinsic abiotic factors that were temperature, pH, water activity and gaseous composition of the surrounding atmosphere. Intrinsic factors were chemical composition of feed which had an influence on growth and mycotoxin biosynthesis. Wet forages had a significant $P<0.05$ high a_w with an average of 0.79±0.02 compared to dry concentrates that low a_w with an average of 0.65±0.01 (Table 8). There existed a very strong positive correlation between moisture content in animal feeds and the water activity ($r=0.935$, $P<0.001$) (Table 4). The moisture in feeds determined fungi colonization of concentrates or forages by enabling them to breakdown complex macromolecular compounds and utilize them for metabolism, growth and eventually mycotoxin production. *Penicillium* and *Aspergillus* grow best at a_W of 0.95, whereas *Fusarium* grow best at higher a_W of 0.98 (Moss,1996). The maximum *Aspergillus* growth rates have been obtained at 0.98 a_w and maximum aflatoxin produced at 0.98 a_w but at different temperatures, 15 and 25°C (Oviedo *et al.*, 2009; Oviedo *et al.*, 2011).

The level of acidity and alkalinity of the type of feeds also affected the growth of fungi in concentrates and forages which was related to its influence on enzyme activities in the fungi cells. The average pH of concentrates was 6.20±0.3 compared to forage which had a pH of 6.65±0.7 (Table 8). From previous studies optimum pH for Aflatoxin production by *Aspergillus*

spp. is between 3.5 and 8.0 and that for DON production by *Fusaruim spp.*is at pH of 7.5 (Comerio *et al.*, 1999).

The type of storage facility was found to be significant P<0.05 risk factors for infestation of mycotoxigenic mold. Farmers' mostly stored animal feeds under open structures (Table 2). Feeds stored in open structures had the mould count of 4.93±1.0 cfu/g as compared to 3.99±1.0 cfu/g in feeds stored in roofed stores as shown in figure 4. This is attributed to exposure of animal feeds to unpredictable environmental conditions temperature ranging between 15.8˚C to 16.6 ˚C and humidity ranging between 36.9% and 42.6% (Figure 3 and Figure 4). The role of temperature and humidity in the survival of fungi was related to its influence on the cell membrane structure as well as on enzyme activities within the cell as indicated by (Chin *et al.*, 2010**).**

5.2 The prevalence of Aflatoxin M_1 in rural and peri-urban milk sub-value chains in Nakuru County

5.2.1Aflatoxin (AFs) contamination in animal feeds

There was significant difference in Aflatoxin (AFs) contamination in animal feeds samples between the two dairy systems where the study was conducted. Feed samples from peri-urban had significantly higher levels of AFs ranging between 0 and 147.86 µg/kg compared to rural dairy system which was ranging between 2.31 and 84.41µg/kg (Table 8). This is because most of the peri-urban farmers practice stall feeding and feed their dairy animals on a basic diet comprising commercial and on-farm formulated concentrates which had higher AFs levels than forages which were mostly used by the rural farmers who practice free range grazing in the rural dairy system **.**

Higher Aflatoxin (AFs) contamination of was observed in concentrates commonly utilized in the peri-urban ranging between 0 and 147.86 µg/kg than forages commonly used in the rural dairy system with Aflatoxin (AFs) contamination levels of ranging between 0 and 29.52 µg/kg (Table 8)**.** This could be attributed to prolonged storage of animal feeds by peri-urban farmers because of animal feed deficits observed in the dairy system due to lack of grazing fields thus forcing them to formulate excess animal feeds. Prolonged storage conditions expose animal feeds to the environmental conditions like humidity and temperatures that favour the growth of *Aspergillus spp*. Besides, farmers lacked proper storage facilities for animal feeds with inadequate roofing leading to exposure of animal feeds to precipitation. These conditions contribute to mould growth leading to aflatoxin contamination. Peri-urban farmers also used low-quality ingredients in the formulation of on-farm formulated concentrates leading to aflatoxin contamination of animal feeds. Earlier studies had shown that dairy farmers in the

peri- urban wereas of Kenya mostly use maize grains milled to make on-farm formulated concentrates to feed their cattle. The grains used were usually those that were contaminated with moulds at harvesting time and were separated from the healthy grains which were meant for human consumption. The mould invaded grains have been associated with aflatoxin contamination (Muture and Ogana, 2005).

Extrinsic abiotic factors that affect growth of mycotoxin producing fungi measured in animal feeds included moisture content, water activity (a_w) and pH. Concentrates was identified moisture content, a_w and pH ranging between 11.20% -71.30%, 0.51 - 0.88 and 5.98 – 6.92 respectively that favour growth of mycotoxin producing fungi. Most storage fungi grow at aw below 0.75. The required aw for *Aspergillus spp*. growth is between 0.61 and 0.91 (Oviedo *et al*., 2009). Neutral pH ranging between 6 and 7 is also more suitable for mould growth which was exhibited in the study. From previous studies, optimum pH for aflatoxin production by *Aspergillus spp* is between 3.5 and 8.0 (Oviedo *et al*., 2011). The toxin-producing fungi such as *Aspergillums flavus* and *A. parasiticus* species show enormous growth under environmental moisture of between 50%-60%, temperature conditions of 25°C and 85–90% relative humidity (Bakirci, 2001).

Concentrates from both rural and peri-urban wereas had high Aflatoxin (AFs) contamination above the European Directives (Directive 2002/32/EC (EC, 2002) and amending Directive 2003/100/EC (EC, 2003) of 5µg/kg. This could be attributed to lack of quality assurance system in the animal feeds value chain in Kenya. Animal feed ingredients used in formulations were not guaranteed of quality and safety while local agro-vets lack specified regulatory guidelines for animal feed distribution and proper storage.

Overall, the prevalence of Aflatoxin (AFs) contamination of animal feeds was above the EU maximum limit of 5µg/kg in both rural dairy system and peri-urban system with 60% and 52% respectively. This condition presented a concern in the dairy industry in this region as the risk of Aflatoxin (AFs) toxicity in dairy cows was high in both dairy systems. This situation exposed cows to the risk of chronic intoxication with main target organ being the liver leading to hepatotoxicity, decreased weight gain, and decreased feed consumption, decreased reproductive performance and abortions (Haschek *et al.,* 2013). The reduced performance in dairy cows would cause farmers large milk and economic losses.

5.2.2 DON contamination in animal feeds

There was significant difference in DON contamination in animal feeds samples between the two dairy systems where the study was conducted. Feed samples from peri-urban had significantly higher levels of DON contamination ranging between 0 and 179.89 µg/kg compared to rural dairy system ranging between 0 and 89.79µg/kg (Table 8). This could be attributed to use of low-quality raw materials in feed formulation. This finding indicated that DON contamination may have occurred in the pre-storage period and probably the feed ingredients were contaminated before storage (Haschek *et al.,* 2013).

High levels of DON contamination was observed in commercial and on-farm formulated concentrates. This is attributed to the fact that local feed processors and on-farm formulations contain a great proportion of on-farm produced cereals. In corn, *Fusarium* moulds were associated with ear rot and stalk rot, and in small grains, they were associated with diseases such as head blight (scab). In wheat, excessive moisture at flowering and afterward is associated with increased incidence of mycotoxin formation. In corn, *Fusarium* diseases were more commonly associated with insect damage, warm conditions at silking, and wet conditions late in the growing season (Placinta *et al.,* 1999). The highest DON contamination of 179µg/kg was observed in silage feed sample from the peri-urban system. This could have been caused by the silage being exposed to oxygen, causing yeast to utilize lactic acid in silage as a substrate causing an elevation of pH above 4.5 and the silage becoming conducive for mould growth.

Silage is green forage preserved by lactic acid fermentation under anaerobic conditions. Silage with a terminal pH of less than 4.5 is ideal since it prevents fungal growth (Liu *et al*., 2005). Neutral pH ranging between 6 and 7 is suitable for mould growth than a low pH level and for this reason well-prepared silage is less susceptible to fungal spoilage. Silos should be properly sealed to prevent aerobic conditions that favour mould growth and further mycotoxin production

Linear regression model showed significant association of water activity, pH and moisture content of animal feeds with DON contamination of the animal feeds (Table 7). The maximum amount of DON is produced by *F. graminearum* at 0.98 a_w while Optimum DON production by *Fusarium spp* is at pH of 7.5 (Comerio *et al.,* 1999). The trichothecene DON persists in the animal feed at $\leq 0.90 a_w$ after it has already been produced (Hope *et al.,* 2005).

All samples in this study were below the maximum limits for DON in the feed of 1250 µg/kg set in EU regulation 1881/2006. This implied that risk of DON toxicity in dairy cows was low in both dairy systems. The impact of DON on dairy cattle was not established, but clinical data shows an association between DON and poor performance in dairy herds (Côté *et*

al., 1986). In previous studies, the DON-contaminated feed has caused a great economic loss in livestock, especially swine industry due to a well-documented reduction in feed consumption and weight gain. High dose acute DON exposure resulted in emesis, abdominal distress, increased salivation and listlessness (Haschek *et al.,* 2013).

5.2.3 The prevalence of AFM_1 in milk along the value chain of rural and peri-urban dairy systems

The study revealed that the peri-urban dairy system is contaminated with AFM_1 along the value chain ranging between 0.017 µg/L to 0.083 µg/L as shown in Table 14. The majority 48.5 % (33/68) of the milk samples from the peri-urban dairy system were above the EU regulation of 0**.**05 µg/L (EC, 2006a) as shown in figure 3. The study also revealed that all milk samples in the rural dairy system were below the EU regulation of 0**.**05 µg/L (EC, 2006a) as shown in Table 11. The cause for major differences in AFM_1 contamination levels of milk samples taken from rural and peri-urban farms can be explained by the different types of feeds that were provided to cows in these dairy systems. Peri-urban farms fed their cattle mainly on Aflatoxin (AFs) contaminated concentrates made of ingredients such as chicken feacal waste, maize germ, cotton and sunflower seed cake while most rural farms produced organic milk with lowest levels of AFM_1 by feeding their cows on a basic diet of pasture that comprised the tropical grass species *Pennisetum clandestinum* and *Pennisetum purpureum* also known as Napier grass. However, the concentrations of AFM_1 from the peri-urban dairy system in this study were lower compared to earlier studies in the urban Kenya reaching 0.68 µg/L (Kangethe and Lang'a, 2009).

Milk samples from consumption nodes which comprise milk bars and processors level in the value chain had a narrower range of contamination compared to milk from farms in both systems as shown in Table 11. This could be explained by the effect of diluting due to bulking milk during transportation and at the collection centers prior to processing.

The study also showed a moderate correlation between Aflatoxin (AFs) contamination in feed samples and AFM_1 contamination in milk samples ($r=0.46$ $P<0.001$) collected from the same dairy farm. AFM_1 is excreted in milk within twelve hours of consumption of contaminated animal feeds (Battacone *et al.,* 2003; Fink-Gremmels *et al.,* 2008). The occurrence of AFM_1 in milk and dairy products is a public health concern in the peri-urban dairy system which supplies milk to urban consumers. Milk is a primary part of the diet in Kenyan households and the effects of exposure to AFM_1 have been associated with poor growth in neonates and children (Haschek *et al.,* 2013).

Recent studies in Ethiopia show that 91.8 % of milk samples exceeded the maximum level set by EU regulations (Gizachew *et al*. 2016). Serbia (76%) of milk samples exceeded the maximum level set by EU regulations (Škrbić *et al*. 2014). In Brazil, 46% of ultra-high temperature milk samples were AFM_1 positive with AFM_1 (Iha *et al*., 2013). In Pakistan, 71% were positive with AFM_1 (Iqbal and Asi, 2013). In this study, AFM_1 levels in milk from both dairy systems were lower than those found in some other studies in different countries published recently. The difficulty of comparing results among different countries in the world is attributed to different investigative procedures used, sources of feed Aflatoxin (AFs) contamination, different on-farm feeding practices, climatic situations, animal feed handling and storage conditions, the sampling time and procedures.

5.3 The prevalence of *Brucella abortus* in rural and peri-urban milk sub-value chains in Nakuru County.

The prevalence of brucellosis was 1.3% and 1.7% (Table 13) in peri-urban and rural dairy systems respectively based on diagnosis using commercial kits of the competitive enzyme-linked immunosorbent assay (CELISA) and complement fixation test (CFT) for *Brucella abortus* antibodies. However, both the MRT and RBPT were less specific than the CELISA and CFT and the reported high prevalence(s) of as shown in Table13 that might be due to false-positive serum reactions (FPSR). False-positive serum reactions in *Brucella spp*. screening tests were known to be caused by unrelated *Enterobacteriaceae* and CELISA can eliminate such reactions. CELISA can eliminate this false-positive reaction only by approximately 50% (Alhajia *et al*., 2016).

The peri-urban dairy system was found to have a low prevalent risk of 0.01 for the occurrence of *B. abortus* along the sub-value chain as compared to the rural dairy system which had a prevalent risk of 0.02 as shown in Table 14. The infection suggests exposure to the bacteria because cow vaccination against brucellosis had not been carried out in the region. The reason for this could be due to susceptibility to *B. abortus*, which was the only *Brucella spp* identified in this study

The study identified two risk factors for brucellosis at the farm level; method of breeding and history of abortion were found to be significant $P<0.05$ risk factors for *brucellosis* as shown in Table 6. Use of bulls was shown to be a risk factor as shown in Table 6. Three cows using bull for breeding were found to be infected with brucellosis in this study. During natural breeding, cows come into contact with semen contaminated with *Brucella* leading to transmission of the disease. It was because of the low endemic status; however, one cow using artificial insemination was found to be infected with brucellosis in this study. This was

attributed to purchase of infected animal or contamination of frozen semen with *Brucella* could not be ruled out.

History of abortion was shown to be a risk factor as shown in Table 6. The result should be interpreted that *Brucella* sero-positive status of cows was a risk factor of abortion. Cows that have *brucella* were at very high risk of not carrying the pregnancy to the full term of gestation. Therefore, the occurrence of unexplained abortion on the farms can be due to the *brucella* disease and laboratory analysis should be done on fetus after abortion to ascertain the cause. Apart from the loss of cows' pregnancy which is an economic loss to the farmer, abortion also a significant predisposing risk factor to bovine *brucellosis* (Alhajia *et al*., 2016). The other possible explanation for the observed abortions on the farms could be malnutrition, deficiencies and other infections (Kebede *et al*., 2008).

The history of vaccination, bought-in-cattle and type of intensification was found not to be significant $P>0.05$ risk factors or preventative factors for *brucellosis* as shown in Table 6. In the study, a history of bought-in cattle was not a risk factor for brucellosis, suggesting the endemic status of brucellosis in and around Nakuru may be maintained indefinitely by low-level within herd transmission.

The level of intensification in this study was not a risk factor for brucellosis in Nakuru but three cows were identified seropositive with brucella were from large herds within free-grazing farming were located in rural dairy system. Control programmes in and around rural dairy system were recommended, with a special focus on large farms with free-grazing farming.

The history of vaccination in this study was not a risk factor for brucellosis. Cows were supposed to be vaccinated against the *brucella* disease as a preventive measure however in this study, none of the respondents was found to vaccinate their cows. Other reasons for the farm-level risk factors for *brucella* infection transmission have been categorized into between-farms e.g. replacement of animals, grazing pattern and proximity to infected herds and within-farms e.g. vaccination level, herd size and stocking density (Crawford *et al*, 1990).

At the market level, pasteurization of milk was found in this study to be significant $P<0.05$ preventative factors for *brucellosis* as shown in Table 7. However, boiling of milk and fermentation were found to be significant $P<0.05$ a risk factor for the *brucella* zoonosis. The milk that goes though informal markets is a predisposing risk factor for spread of the disease to humans since the form of heat treatment to the milk before consumption cannot be guaranteed to the by the consumers. It has been known that consumption of raw or not heat treated milk is a source of human zoonosis (Steele *et al*., 1997). Though similar studies in Kenya had found that over 77% of the population consumes raw milk because its affordable

compared to the processed, they however did not find *brucella* antibodies in milk marketed informally (Namanda *et al.,* 2009). The findings in this study were similar to the previous finding where it has found that in Nakuru 93% of milk consumers use it in tea where heat treatment is offered before consumption (Omore *et al.,* 2004) and therefore this practice reduces the risk of *brucella* zoonosis. However, the informal channel remains to be the risk factor the *brucella* zoonosis since

Another milk handling practice observed in this study that act as predisposing factor for human infection with *Brucella* organism include consumption of boiled milk. Though all farmers used boiled milk before consumption except the 3% in the rural system who used raw milk to make *mursik*, the guarantee of the boiling against *brucella* bacteria cannot be assured. This is because 12% of milk that had been boiled was positive *brucella* antibodies and therefore the boiling that was not adequately done. The *brucella* bacteria grow optimally at 37°C and were killed by heating at 63°C for 7-10 minutes. Boiling of raw milk achieves higher temperatures and duration than those attained during pasteurization destroy all zoonotic health hazards (Mangen *et al.,* 2002). MRT positive for milk suggests brucella infection, but mastitis and colostrum might have caused false positivity (OIE, 2009).

The consumption of the traditionally fermented non heat treated milk like *mursik* is also a risk of factor for the exposure to brucellosis. Fermentation causes the lowering of the milk pH and increase in lactic acid and other organic compound which were antimicrobial however studies have shown that *Br. abortus* were only mildly affected by acidity (Farrel, 1996). This would imply that homemade fermented milk could be a possible predisposing risk of milk-borne infection to humans. In this study the milk that had pH <4.0 i.e. the isoelectric point was negative for *brucella* antibodies. However, studies have shown that fermentation of unpasteurized milk to pH values below pH 4.0 has been shown to not inhibit the growth of *Brucella* strains (Estrada *et al.,* 2005).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

1. In the peri-urban dairy system, there was a risk of chronic exposure of AFM_1 to milk consumers.
2. There was a risk of chronic exposure to AFs by dairy animals in both dairy systems.
3. There was no risk of chronic exposure of DON by dairy animals in both dairy systems.
4. Milk safety in rural and peri-urban dairy systems is a concern for public health. Mycotoxins from animal feeds and brucellosis due to usage of bulls for breeding are the major risks

6.2 Recommendations

1. To reduce further milk losses because of AFM_I contamination, research on mycotoxin control in order to eliminate contamination of animal feeds is required.
2. The prevalence of bovine brucellosis together with high prevalence of abortion, calls for need of differential diagnosis of abortion from other diseases in this area.

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APPENDICES

Appendix 1. On-Farm questionnaire

EGERTON UNIVERSITY

FACULTY OF AGRICULTURE

DEPARTMENT OF DAIRY FOOD SCIENCE AND TECHNOLOGY

QUESTIONNAIRE FOR THE RESEACH ON ASSESSMENT OF PREVALENCE OF AFLATOXIN M_1 AND *BRUCELLA ABORTUS* IN MILK IN RURAL AND PERI-URBAN SUB-VALUE CHAINS IN NAKURU COUNTY, KENYA

FORM NUMBER……

INTRODUCTION

This survey is conducted by a postgraduate student in to Egerton university student in the partial fulfillment of a Master of Science in Food Science. Thus its purpose is purely academic. You were kindly requested to provide Information provide through interview with this questionnaire was confidential and shall only be used for the purpose of this study

Household consent obtained (yes) (No)

Thank you

1.0 **GENERAL INFORMATION**

Date of survey: _____ Time started: _____Study site: [_ _Rural, [_ _]

Peri-urban [_ _]

Name of the enumerator: _____Questionnaire Code: _____ HHID: _____

Sub County: _____ Ward: _____ Village: _____

GPS Coordinates:

Longitude [_ _] (1= North, 2= South): ____^{O} DEG1____ ''MIN1 ___ SEC1

Latitude (East): ____^{O} DEG2____ ''MIN2 ____ SEC2

2.0HOUSEHOLD CHARACTERISTICS

1.1. Name of the Respondent		
1.2. Name of the HHH		
1.3 Age of the Respondent	<30years [ ]30-40 years [ ]40-50years [ ]50-60years [ ] >60years [ ]	
1.3. Sex of the HHH	[ ]	1= Male, 2= Female
1.4. Relationship of the Respondent to HHH	[ ]	1= HHH, 2=Spouse, 3= Child, 4= Grandchild, 5= In-law, 6=Employee, 7=Neighbor
1.5. Education level of the HHH	[ ]	1= None, 2= Primary, 3= Secondary, 4= Tertiary
1.6. HH main occupation	[ ]	1= crop farming, 2= Livestock farming, 3= Mixed crop- livestock framing, 3= Salaried employment, 4= self employment, 5= Farm laborer in other farms, 6= Others (Specify)

1.0 FARM CHARACTERISTICS

3.1What is the type of intensification on the farm of keeping cattle in your farm? [____]

1= Zero grazing, 2= Semi zero grazing, 3=Free range grazing

4.0. FARM MANAGEMENT PRACTISES THAT INFLUENCE MYCOTOXIN CONTAMINATION OF FEED ON THE FARM

4.1 Source of feed on the farm	☐ 1=Industrial waste ☐ 2=Market waste ☐ 3= Crop residues ☐ 4= Commercial dairy meal ☐ 5= Pasture/Napier grass ☐ 6= Hay ☐ 7= Silage ☐ 8= Hydroponics ☐ 9=Any other, specify……..
4.2 Type of feed given during wet season	☐ 1= Pasture/Napier grass ☐ 2= Rotting crop residues ☐ 3=Any other, specify……..

4.3 Type of feed given during the dry season	☐ 1=Dried crop residues ☐ 2=Hay ☐ 3 = Silage ☐ 4 = crop residues ☐ 5=Any other, specify……..
4.2 What combination of feeds does the farmer give to the animals?	☐ 1=Crop residues alone ☐ 2=crop residues + Commercial dairy meal ☐ 3=Pasture/Napier grass/hay/silage+ Commercial dairy meal ☐ Any other, specify……..
4.3 The period taken to clean the feeding trough	☐ 1=Daily ☐ 2=Weekly ☐ 3=Never Specify…….
4.4 Has the farmer received any training from extension officer on feed storage?	☐ 1=Yes specify…….. ☐ 2=No
4.5 How does the farmer store his feed?	☐ 1=In a store ☐ 2= On the floor or ground ☐ 2=On a raised rack ☐ 3=Any other, specify……..
4.6 Does the feed storage /werea rack have a roof to prevent exposure to environmental conditions?	☐ 1= Yes ☐ 2= No (Take picture of feed storage site)
4.7Were crop residues/animal feed stored under humid conditions?	☐ 1=Yes specify…….. ☐ 2=No
4.8 Does the farmer have training on from extension officer on proper silage making?	☐ 1=Yes ☐ 2=No

4.9 Practices farmers take to ensure they obtain aerobic stable fermented forages from their silos?	☐ 1= Proper sealing once remove the feed from silo ☐ 2= Proper wall management ☐ 3= Other practice,specify……
4.10 Does the farmer have training on proper hydroponic cultivation?	☐ 1=Yes ☐ 2=No
4.11 Does the farmer buy commercial feeds from licensed agrovet?	☐ 1=Yes, specify…….. ☐ 2=No
4.12 Does the farmer check for mold growth in commercial animal feed before administration to cows?	☐ 1=Yes, specify…….. ☐ 2=No
4.13 Source of water for drinking and use on the farm	☐ 1=River ☐ 2=Borehole ☐ 3=Roof catchment ☐ 4= municipal tap water system ☐ 5=Anyother, specify……..

Observation checklist

1. Temperature inside feeds _____°C
2. Ambient temperature in feed storage werea _____°C
3. Humidity inside feeds_____
4. Humidity in feed storage werea_____

5. INFORMATION ON BRUCELLOSIS ON THE FARM

5.1 Has the milking person/farm owners have close contact with animals through breeding, birth and slaughtering	☐ 1=Yes ☐ 2=No

5.2Type of breeding on the farm	☐ 1= bull ☐ 2=AI ☐ 3= bull +AI
5.3Occurrence of abortion on the farm	☐ 1=Yes, specify…….. ☐ 2=No
5.4 Consumption of raw milk/raw milk products on farm	☐ 1=Yes, specify…….. ☐ 2=No
5.5 Type of milk used for making fermented products	☐ 1= Raw milk ☐ 2= Boiled milk Other…….
5.6 Cleanliness of the milking place (mud and dung on the floor, humidity and bad smell)	☐ 1=Very dirty (over 75% of the surface) ☐ 2=dirty (50 - 75%of the surface) ☐ 3=Average (25 – 50% of the surface0 ☐ 4=clean (10 - 30% surface) ☐ 5=very clean (less than 10% of surface)
5.7 Through which channel do you sell your milk	1=Formal (cooperative and processor) [ ] 2=Informal (trader, direct to consumer) [ ]

Appendix 2. Market actor's questionnaire

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DEPARTMENT OF DAIRY FOOD SCIENCE AND TECHNOLOGY

QUESTIONNAIRE FOR THE RESEACH ON ASSESSMENT OF PREVALENCE OF AFLATOXIN M_1 AND *BRUCELLA ABORTUS* IN MILK IN RURAL AND PERI-URBAN SUB-VALUE CHAINS IN NAKURU COUNTY, KENYA

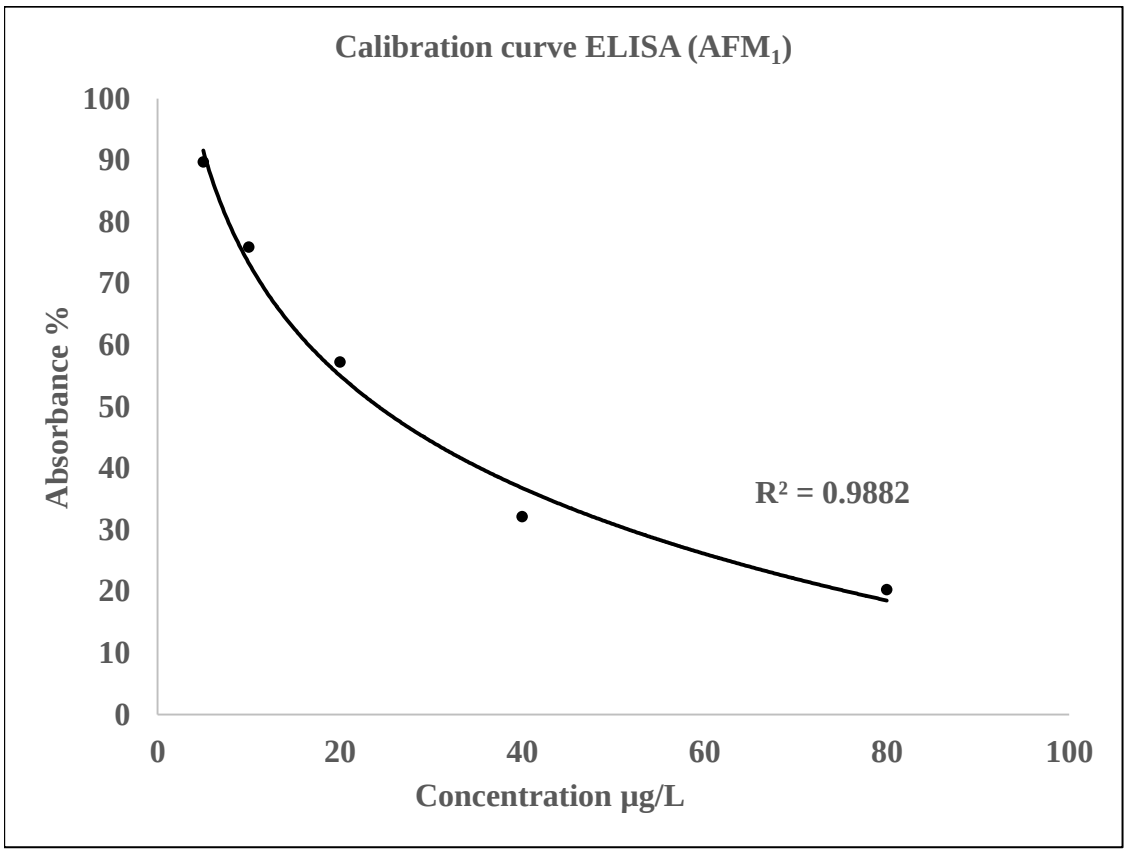
INFORMATION ON BRUCELLA IN INFORMAL MARKETS (milk bar/milk hawker)

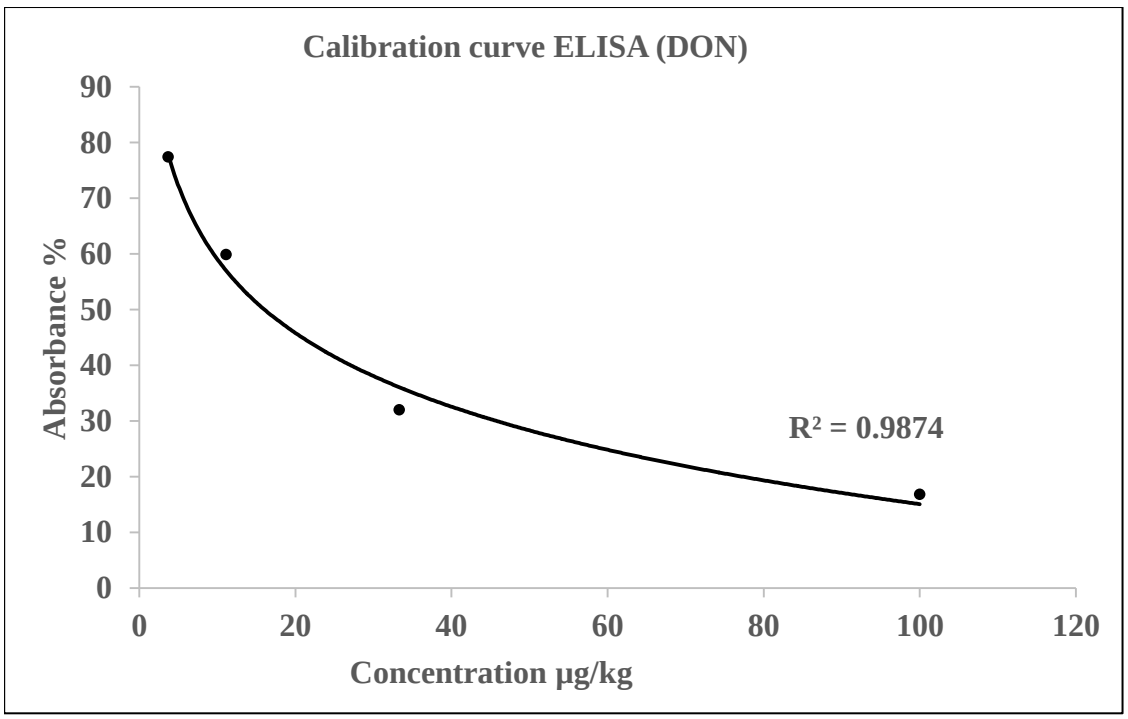
Informal actor

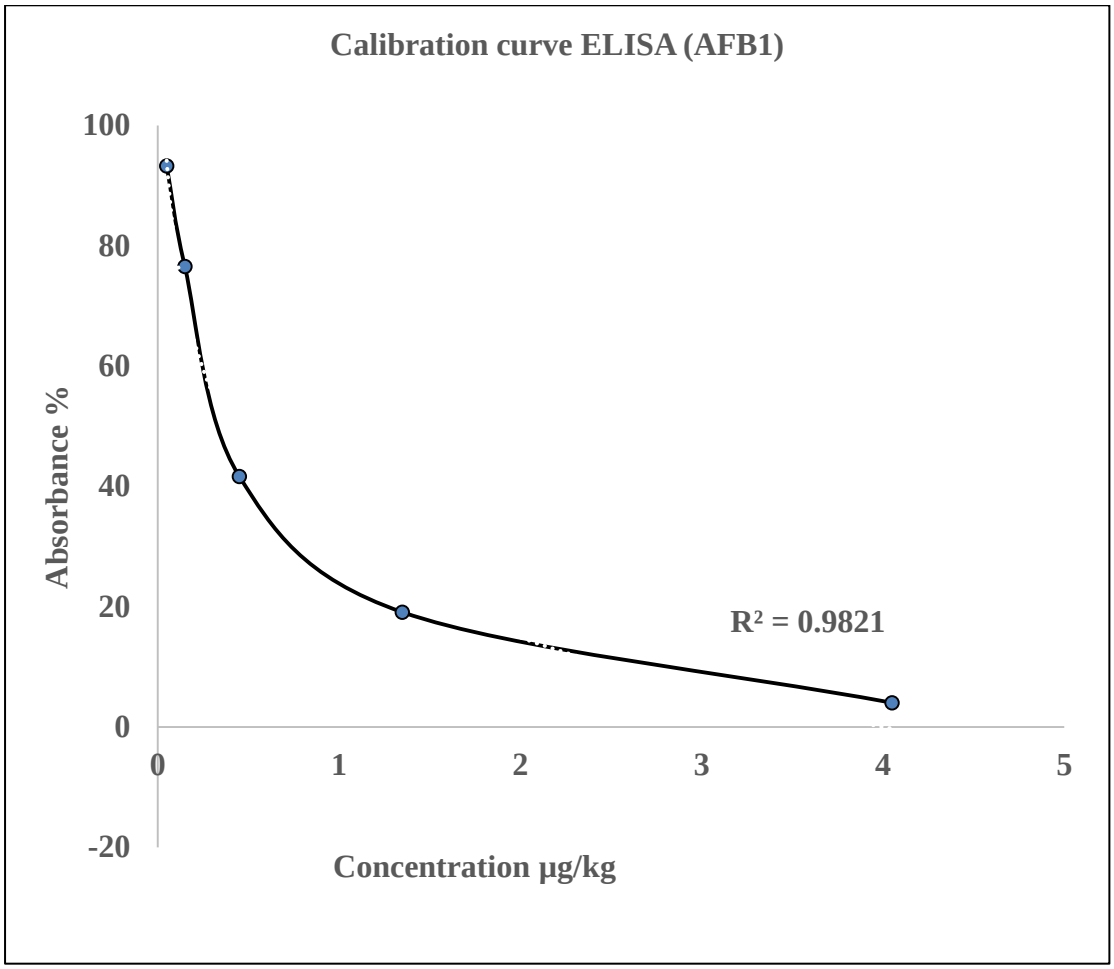
1= Milk hawker 2= Milk bar 3= Hotel, 4= Others (Specify) _____

5.4Methods of milk preservation?	☐ 1=Refrigeration ☐ 2=Addition of hydrogen peroxide ☐ 3=Immersion of storage containers in water baths ☐ 4=Boiling ☐ 5= Any other, specify……..
5.5 Milk subjected to quality tests before selling?	☐ 1=Yes, specify…….. ☐ 2=No
5.6 Do you use sanitizers to milk storage containers?	☐ 1=Yes ☐ 2=No
5.7 Cleanliness of milk storage containers. (presence of dirt, particles and milk residues in the container)	☐ 1=Very dirty (over 75% of the surface) ☐ 2=dirty (50 - 75%of the surface) ☐ 3=Average (25 – 50% of the surface ☐ 4=clean (10 - 30% surface) ☐ 5=very clean (less than 10% of surface)
5.8 Cleanliness of the milk bar	☐ 1=Very dirty (over 75% of the surface) ☐ 2 =dirty (50 - 75%of the surface) ☐ 3=Average (25 – 50% of the surface0 ☐ 4=clean (10 - 30% surface) ☐ 5=very clean (less than 10% of surface)
5.9 Type of milk used for making fermented products	☐ 1= Raw milk ☐ 2= Boiled milk Other…….

Appendix 3. Standard calibration curves for AFM1 and DON






Calibration curve ELISA (AFB1)
100
80
60
40
20
0
-20
Absorbance %
0
1
2
3
4
5
R² = 0.9821
Concentration µg/kg

Appendix 4. Research Paper 1

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

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Background: Mycotoxins are metabolites produced by phytopathogenic and spoilage fungi in animal feed as a result of poor storage. The mycotoxins can also originate in the field and are excreted in milk when dairy animals consume such feeds, posing a public health risk concern.
Methods: The aim of this study was to conduct a risk assessment in the informal sub-value chains of rural and peri-urban dairy systems in Nakuru County, by determining the prevalence and quantity levels of mycotoxins in animal feeds and milk. A total of 74 animal feed samples and 120 milk samples were simultaneously collected from individual cows and actors in the informal dairy value chain. Feed samples were analyzed for Aflatoxin B_1 (AFB_1) and Deoxynivalenol (DON) while milk samples were analyzed for Aflatoxin M_1 (AFM_1) using commercial Enzyme Linked Immune Sorbent Assay (ELISA) method.
Results: Aflatoxin B_1 contamination levels in 56 % (41/74) of the animal feeds exceeded the European Union (EU) limits of 5 µg/kg ranging between 0 and 147.86 µg/kg. Deoxynivalenol (DON) was identified in 63 % (27/43) of all the animal feeds ranging between 0 and 179.89 µg/kg. In the peri-urban dairy system, 48.5 % (33/68) of the milk samples were contaminated with the AFM_1 concentration above the EU regulation of 0.05 µg/L ranging between 0.017 and 0.083 µg/L. All milk samples from the rural dairy system had AFM_1 contamination levels below the EU limits of 0.05 µg/L ranging between 0 and 0.041 µg/L. Linear regression model showed significant association of abiotic factors; pH, water activity and moisture content of animal feed with AFB_1 and DON contamination of the animal feeds.

Conclusions: The results obtained from this study indicate that the peri-urban dairy farms, where intensive management predominate face the challenge of quality feeds, and one contributing factor is the on-farm production and handling of animal feeds.

DOI: 10.1186/s40550-016-0033-7
Source: International Journal of Food Contamination,
http://link.springer.com/article/10.1186/s40550-016-0033-7

Appendix 5. Research paper 2

Association of on-farm feeds handling practices with fungal growth and Mycotoxin production on feeds in smallholder dairy farms, Nakuru, Kenya

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Practices used by smallholder dairy farmers for handling of feeds at the farm pose a risk of mycotoxins to dairy animals and dairy products, hence a public health concern. The aim of the study was to document the on-farm practices of handling feeds used by these farmers and how they influence the growth of mycotoxin producing fungi together with prevailing extrinsic conditions. Study involved the use of structured questionnaire for interview of smallholder dairy farmers (n=120) for on-farm feed handling practices and collection of feed samples (n=97) for microbial analysis of the mycotoxin producing molds. The fungi counts were interrelated with the feed handling practice and therefore a measure of its impact. Results found out that rural dairy system was characterized by practice of free range grazing unlike peri-urban system practice that had semi-intensive stall feeding. At the farm level, the type feeds storage facility and the type and condition of feeds were found to be significant risk factors ($p<0.05$) for infestation of mycotoxic fungi. Feed contamination on farm at the sub-value chains with mycotoxic fungi is primarily due to poor storage facilities exposing feed to environmental conditions that favors fungi growth.

DOI: 10.5897/AJAR2016.11525

Source: African Journal of Agricultural Research,

http://www.academicjournals.org/journal/AJAR/article-full-text/C31B56560820

Appendix 6. Analysis of variance for mean squares of the water activity, moisture content, pH, animal feeds store temperature, environmental temperature, animal feeds store humidity, environmental humidity, total bacterial count and yeasts and molds counts.

		Physico-chemical			Environmental factors				Microbial count	
Source	**DF**	**AW**	**MC**	**pH**	**STOTEMP**	**ENVTEMP**	**STOREHUM**	**ENVHUM**	**TVC**	**YMC**
DSYSTEM	**1**	0.001^{ns}	780.593^{***}	0.837^{ns}	3.169^{ns}	3.187^{ns}	12.746^{***}	5.888^{ns}	8.646^{***}	6.742^{***}
SOURCE	**1**	0.191^{***}	8197.660^{***}	2.098^{*}	0.057^{ns}	0.054^{ns}	0.079^{ns}	0.307^{ns}	1.447^{ns}	0.525
INTENS	**2**	0.011^{*}	442.768^{***}	1.431^{*}	12.858^{ns}	12.614^{ns}	0.411^{ns}	0.306^{ns}	2.723^{ns}	1.714^{*}
CONDT	**1**	1.321^{***}	52442.240^{***}	1.617^{*}	2.065^{ns}	2.045^{ns}	0.008^{ns}	0.015^{ns}	10.696^{***}	5.760^{***}
INTENS(DSYSTEM)	**2**	0.014^{**}	4.911^{ns}	0.046^{ns}	0.778^{ns}	0.764^{ns}	0.076^{ns}	0.084^{ns}	0.002^{ns}	0.293
SOURC(DSYSTE*INTENS)	**3**	0.015^{**}	37.781^{sn}	0.485^{ns}	0.487^{ns}	0.475^{ns}	0.019^{ns}	0.707^{ns}	0.972^{ns}	0.593
COND(DSYS*SOUR*INTENS)	**4**	0.007^{ns}	5.950^{ns}	0.295^{ns}	0.115^{ns}	0.118^{ns}	0.247^{ns}	0.702^{ns}	0.806^{ns}	1.363
Error	**50**	0.003	16.139	0.365	1.928	1.937	0.416	0.623	0.990	0.563
R^2		0.9224	0.987	0.357	0.263	0.259	0.417	0.277	0.395	0.462
C.V		6.8711	9.552	9.216	8.706	8.673	0.902	2.096	19.639	18.229

A_W= water activity; MC= moisture content; STOTEMP= animal feeds store temperature; ENVTEMP=environmental temperature; STOHUM= animal feeds store humidity; ENHUM=environmental humidity; TVC=total bacterial count and YMC= yeasts and molds counts.

If * is significant at P≤0.05, ** is significant at P≤0.01, * is significant at P≤0.001 and ns= not significant at p≤0.05**

Appendix 7. SAS output for pearson correlation coefficients for biotic and abiotic factors

Pearson Correlation Coefficients, N = 65

Prob > |r| under H0: Rho=0

	AW	MC	PH	STOTEMP	ENVTEMP	STOHUM	ENVHUM
AW	1.00000	0.93480	-0.11489	-0.18548	-0.18440	0.00618	-0.03889
		<.0001	0.3621	0.1391	0.1414	0.9610	0.7584
MC	0.93480	1.00000	-0.06203	-0.17642	-0.17563	0.10562	0.10034
	<.0001		0.6235	0.1598	0.1617	0.4024	0.4264
PH	-0.11489	-0.06203	1.00000	-0.00674	-0.00892	0.18994	0.16361
	0.3621	0.6235		0.9575	0.9438	0.1297	0.1928
STOTEMP	-0.18548	-0.17642	-0.00674	1.00000	0.99989	-0.61787	-0.17852
	0.1391	0.1598	0.9575		<.0001	<.0001	0.1548
ENVTEMP	-0.18440	-0.17563	-0.00892	0.99989	1.00000	-0.61835	-0.17998
	0.1414	0.1617	0.9438	<.0001		<.0001	0.1514
STOHUM	0.00618	0.10562	0.18994	-0.61787	-0.61835	1.00000	0.79924
	0.9610	0.4024	0.1297	<.0001	<.0001		<.0001
ENVHUM	-0.03889	0.10034	0.16361	-0.17852	-0.17998	0.79924	1.00000
	0.7584	0.4264	0.1928	0.1548	0.1514	<.0001	

Appendix 8. Summary of abiotic factors measured in concentrates and forage types of animal feeds

Abiotic factors	Concentrates			Forages		
	Water activity	Moisture content	pH	Water activity	Moisture content	pH
Mean	0.64	15.84	6.33	0.78	42.73	6.59
Standard deviation	0.11	9.86	0.30	0.15	32.22	0.63
Median	0.60	13.70	6.25	0.80	19.50	6.89
Minimum	0.51	11.20	5.98	0.55	11.30	4.27
Maximum	0.88	71.30	6.92	0.96	81.30	6.98

Appendix 9. Linear regression model showing association of peri-urban system, class of feed and level of AFM_1 in milk

Variable	**Category**	coefficients	95% confidence interval	P-value
Dairy system	Peri-urban	48.897	36.630- 61.164	0.001
Feed	Forage*	1.000		
	Concentrates	5.970	39.37- 74.03	0.001
Constant		-31.889	-54.073- -0.9705	0.006

*the reference

Appendix 10. Descriptive Statistics for the Rural Dairy System

AFM_1 (µg/L)	Rural dairy system			
	Production (n=32)	**Transporters (n=16)**	**Cooperatives (n=7)**	**Milk bars (n=7)**
mean	10.93	7.49	5.05	6.01
Standard	13.35	6.39	8.71	12.76
Median	6.54	6.39	0.00	0.00
Minimum	0.00	0.00	0.00	0.00
Maximum	41.09	19.52	22.98	34.62

Appendix 11. Descriptive Statistics for the Peri-Urban Dairy System

AFM_1 (µg/L)	Peri-urban dairy system			
	Production (n=37)	**Transporters (n=14)**	**Cooperatives (n=5)**	**Milk bars (n=12)**
mean	62.50	48.60	43.09	33.57
Standard	19.06	21.18	25.55	15.37
Median	73.65	48.22	42.02	28.50
Minimum	22.52	20.08	19.69	17.66
Maximum	83.43	83.48	82.04	69.69

Appendix 12. Two sample T-Test SAS Output

Two-Sample T-Test and CI: B1, M1

Two-sample T for B1 vs M1

	N	Mean	StDev	SE Mean
B1	74	27.6	34.5	4.0
M1	69	33.0	30.8	3.7

Difference = mu (B1) - mu (M1) : (μ1=μ2)

Estimate for difference: -5.33754

95% CI for difference: (-16.18833, 5.51325)

T-Test of difference = 0 (vs not =): T-Value = -0.97 P-Value = 0.332 DF = 141

Both use Pooled StDev = 32.7976

Appendix 13. SAS output for logistic regression

The LOGISTIC Procedure

Testing Global Null Hypothesis: BETA=0

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	23.4106	1	<.0001
Score	20.6734	1	<.0001
Wald	15.5116	1	<.0001

Type III Analysis of Effects

Effect	DF	Wald Chi-Square	Pr > ChiSq
Disease	1	15.5116	<.0001

Analysis of Maximum Likelihood Estimates

Parameter		DF	Estimate	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept		1	-0.9666	0.2745	12.4020	0.0004
Disease	DSSNOT	1	1.0811	0.2745	15.5116	<.0001

Odds Ratio Estimates

Effect	Point Estimate	95% Wald Confidence Limits	
Disease DSSNOT vs DSSYES	8.689	2.963	25.484

Association of Predicted Probabilities and Observed Responses

Percent Concordant	23.0	Somers' D	0.204
Percent Discordant	2.6	Gamma	0.794
Percent Tied	74.3	Tau-a	0.102
Pairs	14950	c	0.602

Appendix 14. SAS output for means separation for DON in regard to the condition of feed

The GLM Procedure

t Tests (LSD) for don

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	34
Error Mean Square	1391.389
Critical Value of t	2.03224
Least Significant Difference	23.127
Harmonic Mean of Cell Sizes	21.48837

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	class
A	86.95	21	concentr
B	17.83	22	forage

t Tests (LSD) for don

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	34
Error Mean Square	1391.389
Critical Value of t	2.03224
Least Significant Difference	54.894
Harmonic Mean of Cell Sizes	3.813953

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	Condt
A	67.64	2	wet
A			
A	50.80	41	dried

Appendix 15. Means separation for DON in regard to the dairy systems

The GLM Procedure

t Tests (LSD) for don

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	34
Error Mean Square	1391.389
Critical Value of t	2.03224
Least Significant Difference	27.364
Harmonic Mean of Cell Sizes	15.34884

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	Source
A	75.53	10	purchase
B	44.33	33	Homegrow

t Tests (LSD) for don

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	34
Error Mean Square	1391.389
Critical Value of t	2.03224
Least Significant Difference	23.278
Harmonic Mean of Cell Sizes	21.2093

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	Dsystem
A	71.33	24	purban
B	26.65	19	Rural

Appendix 16. SAS output for pearson correlation coefficients for abiotic and biotic factors

Pearson Correlation Coefficients, N = 43
Prob > |r| under H0: Rho=0

	Aw	moisture	pH	don	ymc
Aw	1.00000	0.79820	-0.69552	0.46364	0.39221
		<.0001	<.0001	0.0017	0.0093
moisture	0.79820	1.00000	-0.74129	0.52656	0.44797
	<.0001		<.0001	0.0003	0.0026
pH	-0.69552	-0.74129	1.00000	-0.56699	-0.58877
	<.0001	<.0001		<.0001	<.0001
don	0.46364	0.52656	-0.56699	1.00000	0.91333
	0.0017	0.0003	<.0001		<.0001
ymc	0.39221	0.44797	-0.58877	0.91333	1.00000
	0.0093	0.0026	<.0001	<.0001	

The REG Procedure
Model: MODEL1
Dependent Variable: don

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	103878	103878	206.24	<.0001
Error	41	20650	503.66795		
Corrected Total	42	124529			

Root MSE	22.44255	R-Square	0.8342
Dependent Mean	51.58533	Adj R-Sq	0.8301
Coeff Var	43.50568		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	-42.22281	7.37435	-5.73	<.0001
ymc	1	27.76344	1.93323	14.36	<.0001

Appendix 17. SAS output for mean separation for aflatoxin B1 in regard to class and condition of feeds

AFLATOXIN B1
The GLM Procedure

t Tests (LSD) for AFB

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	66
Error Mean Square	559.5197
Critical Value of t	1.99656
Least Significant Difference	10.996
Harmonic Mean of Cell Sizes	36.89189

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	CLASS
A	47.837	39	concentr
B	5.143	35	forage

The GLM Procedure

t Tests (LSD) for AFB

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	66
Error Mean Square	559.5197
Critical Value of t	1.99656
Least Significant Difference	14.894
Harmonic Mean of Cell Sizes	20.10811

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	CONDTN
A	32.156	62	dried
B	4.333	12	wet

Appendix 18. SAS output for mean separation for aflatoxin B1 in regard to source of feeds and the dairy system

The GLM Procedure

t Tests (LSD) for AFB1

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	66
Error Mean Square	559.5197
Critical Value of t	1.99656
Least Significant Difference	11.182
Harmonic Mean of Cell Sizes	35.67568

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	SOURCE
A	41.927	30	purchase
B	17.905	44	Homegrow

The GLM Procedure

t Tests (LSD) for AFB

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	66
Error Mean Square	559.5197
Critical Value of t	1.99656
Least Significant Difference	11.404
Harmonic Mean of Cell Sizes	34.2973

NOTE: Cell sizes are not equal.

Means with the same letter are not significantly different.

t Grouping	Mean	N	DSYSTEM
A	30.609	27	purban
A			
A	25.941	47	Rural

Appendix 19. SAS output pearson correlation coefficients for biotic and abiotic factors

Pearson Correlation Coefficients, N = 74
Prob > |r| under H0: Rho=0

	Aw	MOISTURE	pH	AFB	YMC
Aw	1.00000	0.84659	-0.09688	-0.31973	-0.37944
		<.0001	0.4116	0.0055	0.0009
MOISTURE	0.84659	1.00000	-0.02954	-0.34745	-0.40243
	<.0001		0.8027	0.0024	0.0004
pH	-0.09688	-0.02954	1.00000	0.13664	0.16946
	0.4116	0.8027		0.2457	0.1489
AFB	-0.31973	-0.34745	0.13664	1.00000	0.92909
	0.0055	0.0024	0.2457		<.0001
YMC	-0.37944	-0.40243	0.16946	0.92909	1.00000
	0.0009	0.0004	0.1489	<.0001	

Appendix 20. The SAS output of the regression procedure for Aflatoxin B1

The REG Procedure
Model: MODEL1
Dependent Variable: AFB

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	75202	75202	454.36	<.0001
Error	72	11917	165.51142		
Corrected Total	73	87118			

Root MSE	12.86512	R-Square	0.8632
Dependent Mean	27.64405	Adj R-Sq	0.8613
Coeff Var	46.53849		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	-20.93829	2.72604	-7.68	<.0001
YMC	1	18.33764	0.86029	21.32	<.0001

Appendix 21. Correlation coefficients between Aflatoxin B1, Aflatoxin M1 and DON

Mycotoxins	Correlation coefficient	P-value
AFB_1 vs AFM_1	0.4556	0.001
AFB_1 vs DON	-0.1436	0.3583

Appendix 22. SAS output for descriptive statistics for AFB1

Analysis Variable: AFB1

DSYSTEM	Obs	Mean	Median	Minimum	Maximum
Rural	47	25.94	12.25	0.00	84.41
purban	27	30.61	6.43	0.00	147.86

Analysis Variable: don

Dsystem	Obs	Mean	Median	Minimum	Maximum
Rural	19	26.65	21.62	0.00	82.79
purban	24	71.33	60.61	0.00	179.89