TRADERS’ AWARENESS AND LEVEL OF AFLATOXINS IN HUMAN FOODS AND CATTLE FEEDS IN SELECTED MARKETS AND STORES IN NAIROBI COUNTY, KENYA

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REG. NO: 156/CE/11988/07

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (IMMUNOLOGY) IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY

OCTOBER, 2014
DECLARATION

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

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Signature-------------------------------- Date------------------------

Supervisors’ Approval

This thesis has been submitted for examination with our approval as university supervisors

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Signature-------------------------------- Date------------------------
DEDICATION

I dedicate this work to my husband, Dr James Moronge and my children, Lynn and Ryan whose support has been a source of encouragement and strength.
ACKNOWLEDGEMENTS

My heartfelt gratitude goes to my supervisors Dr. M. Gicheru and Professor James M. Mbaria for their professional guidance, supervision and patience as I carried out the study. I sincerely thank the staff of the department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, College of Agriculture and Veterinary Sciences, University of Nairobi for their technical support and allowing me to use the laboratory facilities during the entire period of the study.

I am grateful to my family that gave me moral support and encouragement during the study. I am indebted to every person who contributed in any way, however small; may God richly bless you! The oil that keeps the mantle burning was God's grace which is forever sufficient for me.
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<th>FULL FORM</th>
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<tr>
<td>AGROVET</td>
<td>Agricultural and Veterinary store</td>
</tr>
<tr>
<td>AOAC</td>
<td>Associate Official Analytical Chemistry</td>
</tr>
<tr>
<td>CAST</td>
<td>Council for Agricultural Science and Technology</td>
</tr>
<tr>
<td>CDC</td>
<td>Center for Disease Control and Prevention</td>
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<tr>
<td>CFR</td>
<td>Case Fatality Rate</td>
</tr>
<tr>
<td>DPF</td>
<td>Diamond Pet Food</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agricultural Organization</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
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<tr>
<td>IgA</td>
<td>Immunoglobulin Alpha</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin Gamma</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin Mu</td>
</tr>
<tr>
<td>MAFF</td>
<td>Ministry of Agriculture Fisheries and Foods</td>
</tr>
<tr>
<td>MRL</td>
<td>Maximum Residue Limit</td>
</tr>
<tr>
<td>OTA</td>
<td>Ochratoxin A</td>
</tr>
<tr>
<td>ppb</td>
<td>Parts per billion</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical package for social sciences</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<td>KEBS</td>
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ABSTRACT

Aflatoxins are a group of structurally related mycotoxin compounds produced by Aspergillus fungi that grow on wide variety of grains and groundnuts. Aflatoxins are naturally occurring carcinogenic substances. High level of aflatoxins exposure has been shown to cause acute aflatoxicosis in human and animals. Cases of aflatoxicosis have been reported among people living in Kenya’s Makueni, Kitui and Machakos counties due to aflatoxin contaminated maize. The main objective of this study was qualitative and quantitative analysis of aflatoxin in processed and non-processsed human foods and cattle feeds sold in open air markets and AGROVET stores in Nairobi County respectively. Twenty seven AGROVET stores in the eight divisions in Nairobi County were selected using simple random sampling procedure. Major open air food markets were selected based on size. A total of 54 dairy cattle feeds and 96 human food samples of each 250g were collected. The questionnaire was used to determine the traders’ awareness of aflatoxin contamination and effects of aflatoxins in food and feeds in relation to health. For detection and quantification of aflatoxins, ELISA technique was used. ELISA machine and Special software the RIDA® SOFT win (Art No. Z9999) was used for obtaining aflatoxin concentration in parts per billion (ppb). Data obtained was analyzed using Statistical packages for social sciences (SPSS). The data is presented in figures and tables. Descriptive statistics was used to obtain mean level of each sample type in parts per billion. T-test was used to compare aflatoxins levels in processed and non-processsed foods and feeds. The results show that 56.6 % of the traders were aware of aflatoxin contamination. Cattle feed traders were more aware of aflatoxin (40 %) than human food traders (17 %). Training on handling and storage of foods and feeds creates more awareness. Half of the traders were aware of proper storage foods and feeds. A very small portion of food traders (3.7 %) feed traders (8 %) were aware of health effects in human and animals respectively. The mean levels of aflatoxins in foods within open air markets and feeds in AGROVETS stores were above recommended maximum limit (20ppb). Nonprocessed maize (49.7±14.7), unpolished rice (38.2±10.5) and groundnuts (54.6±14.8) processed feeds; maize flour (101.2±21.3), polished rice (63.9±14.5) and groundnuts flour (120.9±27.2). Higher aflatoxin levels were detected in processed foods (95±12.7) than in non-processed foods (47.5±7.6). Grain related foods in open air market and cattle feeds in AGROVETS are contaminated. Therefore, there is need to create traders awareness of aflatoxin contamination, its effect and poor practices that contribute to aflatoxin contamination. Improve storage facilities and give guidelines to proper storage within open air market. To assess foods what enter the market in regard to aflatoxin contamination in order to curb spread of aflatoxin. Strengthen nationwide surveillance, increase food inspection in market areas and feeds in AGROVETS to ensure their safety. Research to be done on other foods sold in open air market other than maize, rice and groundnuts and also on the association of food related aflatoxin and raising cancer rate.
CHAPTER 1: INTRODUCTION

1.1 Background information

Aflatoxins are a group of mycotoxins produced as secondary metabolites by many species of genus *Aspergillus*, a fungus that grows in many substrates. Most notable species are *Aspergillus flavus* and *Aspergillus parasiticus* (Kang’ethe et al., 2007). Aflatoxins are produced most commonly in moist grains and nuts products stored under conditions that are favourable for growth of moulds. The favorable conditions include high moisture content (at least 70 %), range of temperature (10-40 °C), pH of 4-8, and capability of growing on dry surface. Stress such as drought, insect infestation, damage and broken grain kernels also contribute to mould colonization of the food and feed by *Aspergillus* (Jacques, 1988). *Aspergillus* species are wide spread in nature and can colonize and contaminate before harvest and during storage (Kang’ethe et al., 2007).

Aflatoxins exposure to human and animal is through consumption of fungal contaminated foods and feeds (Nelson et al., 1993). Chronic dietary exposure to aflatoxin is a major risk factor for hepatocellular carcinoma, especially in areas where hepatitis B virus infestation is endemic. High level of aflatoxin exposure has been shown to cause acute aflatoxitisosis which manifest as hepatotoxicity. Severe cases of acute aflatoxicosis in human and other species of animals cause fulminant liver failure (Fung and Clark, 2004). Human susceptibility to aflatoxicosis varies with age, health, level and duration of exposure. Children are
particularly affected by aflatoxin exposure which leads to stunted growth and
delayed development (Gong et al., 2002). An outbreak of human acute aflatoxin
poisoning involving 317 cases with 125 deaths was reported in Kenya in 2004.
The epidemiologic investigation attributed this to consumption of contaminated
maize (FAO, 2004). This brings to focus the potential danger of such
contaminated cereals getting into human and animal feeding industry in Kenya.
Many Kenyan farmers and traders may not be aware of the dangers of aflatoxins.
There are many open air markets trading in animal feeds and human foods stuff
in Nairobi.

Aflatoxins occur in a variety of animal feed stuffs which are routinely used by a
farmer to feed dairy animals (Lunyasurya, 2005). Aflatoxin B₁ and B₂ after
ingestion by lactating animals, are modified in the animals’ body to yield
aflatoxin M₁ and M₂. If cattle consume aflatoxin contaminated feeds, aflatoxins
M₁ and M₂ can appear in milk and occur in dairy products made with aflatoxin
contaminated milk. The aflatoxins are potent liver or hepato-carcinogens and
have been linked to effects on immuno-competence, growth and diseases
resistance in livestock and laboratory animals (Joanne, 2008).

1.2 Problem statement

Mycotoxins are fungal metabolites that can contaminate agricultural products and
threaten food safety. Food and Agricultural Organization (FAO) estimates that
Mycotoxins contaminate 25% of agricultural crops worldwide (FAO, 1998). Contamination of food supplies by aflatoxins and other naturally occurring toxins is of particular concern in rural communities of developing countries (Bhat et al., 1997). The problem of food and feed contamination with aflatoxins is of great concern as these toxins are known to be highly toxic, mutagenic, teratogenic and carcinogenic agents that have been implicated as causative agents in human hepatic and extra hepatic carcinogenesis (Massey et al., 1995).

There have been cases of aflatoxicosis outbreaks in tropical countries. For example in April 2004, there was an outbreak of human acute aflatoxicosis among people living in Kenya’s Makueni, Kitui, Machakos, and Kiambu Counties. Aflatoxin contaminated homegrown maize was reported to be the source of the outbreak (FAO, 2004). One of the largest outbreaks of aflatoxicosis documented took place in western India in 1975. This event occurred in the context of unreasonable rains during harvest which lead to contamination of homegrown maize stored under damp conditions (Krishnamachari et al., 1975).

A case study of aflatoxins animal feed contamination conducted in Dagoretti division in Nairobi County, Kenya showed that, 45.5% of milk was contaminated with aflatoxin M$_1$. It was presumed that aflatoxin M$_1$ contamination in milk must have originated from aflatoxin B$_1$ contaminated cattle feed of lactating cows (Kang’ethe et al., 2007). Smallhold farms in Kenya have little chance of selecting
feeds since farmers use what is available and consideration as to whether the feed is mould-infested or not is not made especially during the dry season when feeds are scarce. In addition to acute type aflatoxin toxicity, chronic type posses more serious risks when feeds with aflatoxin are used (Lunyasunya et al., 2005). Chronic toxicity occurs after long-term exposure of animals and human to low or moderate aflatoxin concentrations, chronic aflatoxicosis impact negatively on herd productivity and posses serious risks to human who consume contaminated milk or foods (Roben et al., 1992).

1.3 Justification

In order to prevent the occurrence of aflatoxin contamination, community members need to be educated on better methods of food and feed stuff storage. There are no documented studies on awareness of dangers of aflatoxin among animal feeds and food stuff traders in Nairobi, County, Kenya. Farmers, human food and animal feed traders should be made aware of the dangers of aflatoxins. Such knowledge will enable handlers of foods and feeds to participate in the programmes for minimizing human and animal exposure to aflatoxins.

Contaminated homegrown maize bought from the local farms enters the distribution system resulting in widespread aflatoxins contamination of market maize (Lewis et al., 2005). Therefore, there is need to investigate the levels of aflatoxins in human food cereals and cattle feed sold in stores in Nairobi County.
in order to reduce the risk of aflatoxicosis. Detection and quantification of aflatoxins levels in human foods in market stores and cattle feeds in AGROVET stores is important in order to compare levels of contamination with the recommended maximum residue limit (MRL) so that appropriate remedial action of aflatoxin contamination can be taken, and appropriate preventive practices of aflatoxin contamination during handling and storage of foods and feeds are implemented.

1.4 Research questions

i) What is the level of traders’ awareness on suitable conditions for storage of foods and feeds in regard to aflatoxin contamination?

ii) What is the level of traders’ awareness on dangers of aflatoxins in foods and feeds in Nairobi County?

iii) What is the level of aflatoxins in processed and non-processed human foods and cattle feeds sold in Nairobi County?

iv) What is the difference in levels of aflatoxin contamination between processed and non-processed cattle feeds and human foods sold in Nairobi County?

1.5 Hypotheses

i) There is no relationship between traders’ awareness of aflatoxin and the conditions under which the foods and feeds is stored.
ii) Traders are not aware of dangers of aflatoxins in foods and feeds.

iii) Human foods and cattle feeds both processed and non-processed sold in Nairobi County are not contaminated with aflatoxins.

iv) There is no difference in aflatoxins levels between processed and non-processed human foods and cattle feeds available in Nairobi County, Kenya.

1.6 Study objectives

1.6.1 General objective

To determine traders’ awareness and level of aflatoxins in non-processed and processed human foods and cattle feeds sold in markets within Nairobi, County.

1.6.2 Specific objectives

i) To determine traders’ awareness of aflatoxins and the conditions in which foods and feeds are stored in store outlets in Nairobi County.

ii) To determine traders’ awareness of effects of aflatoxins in foods and feeds relation to human and animal health.

iii) To determine the level of aflatoxins in non-processed (maize grains, unpolished rice and groundnuts) and processed (polished rice, maize flour and ground groundnuts) human foods.
iv) To determine level of aflatoxins in non-processed (maize grains) and processed cattle feeds (dairy meal).

1.7 Limitations

i) Unwillingness of traders to respond to the questionnaire and to sell their merchandise for aflatoxins testing.

ii) Language barrier; some traders were illiterate.

1.8 Significance of the study

The Cattle feeds and human foods that were selected are staple foods and widely used by human and animals. Therefore, it is important to establish their level of aflatoxins contamination. The results from the study can be used to get information on how aware and equipped the traders are in terms of aflatoxin contamination. The study area is relevant because it has major markets and AGROVET stores that serve large population. Limited research has been done on aflatoxin contamination in human foods and animal feeds in the study area.
CHAPTER 2: LITERATURE REVIEW

2.1 Mycotoxins

Mycotoxins are secondary metabolites of molds belonging essentially to the *Aspergillus*, *Penicillium* and *Fusarium* genera that colonize growing crops or harvested and stored feeds and foods (Pittet, 1998). Mycotoxins are therefore acutely toxic, carcinogenic, mutagenic and estrogenic secondary metabolites of moulds (Moss, 1991). Due to their toxic effects and their high stability to heat treatment, the presence of mycotoxins in foods and feeds is potentially hazardous to health of both human and animals. They have a significant impact on economics, causing losses in farm animals or giving rise to difficulties in their management or by rendering commodities unacceptable in national and international trade because they do not conform to the existing regulations (Moss, 1991).

Mycotoxin contamination of foods and feeds highly depends on environmental conditions that lead to mould growth and toxin production. Foodstuffs can be contaminated at any time from growth in the field through harvesting, processing storage and shipment (Pittet, 1998). Although there are many mycotoxins, the following groups are important from the agricultural point of view: the aflatoxins, ochratoxin A, patulin, fumonisins, deoxynivalenol (trichothecees) and zearalenone. Table 2.1 displays these important mycotoxins and the main fungi that produce them.
Table 2.1: Important groups of mycotoxins and the fungi that produce them

<table>
<thead>
<tr>
<th>Mycotoxins</th>
<th>Main producing fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B₁, B₂, G₁, G₂ and M₁</td>
<td><em>Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius.</em></td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td><em>Penicillium errucosum, Aspergillus alutaceus</em></td>
</tr>
<tr>
<td>Patulin</td>
<td><em>Penicillium expansum, Aspergillus clavatus, byssochlamys nivea.</em></td>
</tr>
<tr>
<td>Fumonisins</td>
<td><em>Fusarium moniliforme, Fusarium proliferatum</em></td>
</tr>
<tr>
<td>Dexynivalenol (trichotheccenes)</td>
<td><em>Fusarium graminearum, Fusarium culmorum, Fusarium crookwellense,</em></td>
</tr>
<tr>
<td></td>
<td><em>Fusarium sporotrichioides, Fusarium poae, Fusarium tricinctum,</em></td>
</tr>
<tr>
<td></td>
<td><em>Fusarium acuminatum</em></td>
</tr>
<tr>
<td>Zearalenone</td>
<td><em>Fusarium graminearum, Fusarium culmorum, Fusarium crookwellense</em></td>
</tr>
</tbody>
</table>

(Pittet, 1998)
2.1.1 Ochratoxin A

Ochratoxin A (OTA) is a nephrotoxic and nephrocarcinogenic mycotoxin produced by *Penicillium verrucosum* in temperate and cold climates and by a number of species in the genus *Aspergillus* in warm and tropical parts of the world (Madhyastha *et al.*, 1990). *Penicillium verrucosum* is associated with stored cereals and is very common in northern European countries and Canada. *A. alutaceus* is more common in beans and spices and is also more frequently isolated from cocoa beans, soybeans, peanuts, rice and corn (Kuiper-Goodman, 1989). The infestation with this fungi is known to occur both during pre-harvest and post–harvest. However, the post harvest formation of OTA is usually regarded as the predominant factor in the contamination of foods and feeds (Kuiper-Goodman, 1989). Ochratoxin A has been reported as naturally occurring in almost all cereals including corn, barley, wheat, sorghum, rye, oats, and rice (CAST, 1989). Beside cereals, OTA has been found to occur in many other food commodities including soybeans, beans, cowpeas, green coffee beans, cocoa beans, wine, grape juice beer, spices and herbs (MAFF, 1997).

2.1.2 Patulin

Patulin is a toxic secondary metabolite produced by a wide range of fungi belonging to the *Aspergillus* and *Penicillium* genera and *Byssochlamys nivea* (Moss, 1996). Among the patulin producing moulds, *Penicillium expansum*, a contaminant of apples and other fruits is the most important (Moss, 1996). The
growth of the mould and subsequent production of patulin normally occur only where the surface tissue of the fruit has been damaged. This surface damage may be caused by insect and/or storm damage during handling procedure (Sydenham et al., 1995). Occasionally, Patulin has been detected in fruits with spontaneous brown rot and the affected fruits include bananas, pineapples, grapes, peaches, apricots and tomatoes. Patulin reported in commodities other than apples and apples products is very low (Frank et al., 1976).

2.1.3 Fumonisins

Fumonisins are a group of recently characterized mycotoxins produced by a limited number of moulds in the genus Fusarium of which F. miniliforme and F. proliferatum are the most important of these moulds that frequently infect corn crops around the world (Shephard et al., 1996). There are three naturally occurring fumonisins known as B₁, B₂, and B₃. Fumonisins B₁ is the most abundant mycotoxin comprising of about 70% in naturally contaminated foods and feeds, followed by fumonisins B₂ and B₃ (Dutton, 1996). These mycotoxins produce a wide range of biological effects, including leukencephalomalacia in horses, pulmonary edema in pigs, and nephrotoxicity and liver cancer in rats (Dutton, 1996). In human, they have been associated with high incidence of esophageal cancer in certain areas of Transkei, South Africa (Rheeder et al., 1992). Fumonisins in combination with trichothecenes deoxynivalenol was reported to have played a role in the promotion of primary liver cancer in certain
endemic areas of China (Ueno et al., 1997). On the basis of available toxicological evidence, the International Agency for Research on Cancer (IARC) has declared *F. moniliforme* toxins as potentially carcinogenic to human and categorized it as a Class 2B carcinogen (IARC, 1993).

### 2.1.4 Deoxynivalenol (Trichothecenes)

Trichothecenes mycotoxins are a group of tetracyclic sesquiterpenoid fungal secondary metabolites produced essentially by a wide range of moulds in the genus *Fusarium* of which *F. graminearum* and *F. culmorum* are the most important (Moss, 1996). These toxins that contain an epoxy group in their chemical structure are also produced by various species of the genera *Cephalosporium, Verticimonosporium, Myrothecium*, and *Stachybotrys* (Morgan et al., 1990). These *Fusarium* fungi typically develop during prolonged cool, wet growing and harvest seasons to produce *Fusarium* head blight in cereal crops (CAST, 1989). Although there are 150 different trichothecenes that have been identified, information on their natural occurrence in foods and feeds is limited mainly to nivalenol, deoxynivalenol (vomitoxin), T-2 toxin, diacetoxyscirpenol, fusarenon-X, HT-2 toxin and neosolaniol (Scott, 1990). Significant concentration of deoxinivalenol are frequently detected in wheat, barley corns and oats in North America, Japan and Europe, while lower levels are found in rye, sorghum, and rice (CAST, 1989). The trichotheccenes that is most important and most toxic is T-
2 toxin which is produced primarily by *Fusarium sporotrichioides* and *F. poae* (Moss, 1996).

### 2.1.5 Zearalenone

Zearalenone also known as F-2 toxin is an estrogenic mycotoxin produced by numerous species of the genus *Fusarium* mainly *F. graminearum* and *F. culmorum* which colonize cereal crops worldwide (Kuiper-Goodman et al., 1987). The toxin has estrogenic effects on mammalian reproductive system, and has been associated in particular with reproductive problems in pigs (Morgan et al., 1990). Although *Fusarium* infected cereals in the field may accumulate zearalenone before harvest time, numerous experiments tend to indicate that high levels of toxin occur naturally in some samples of corn-based animal feeds result from improper storage rather than development in the field ((Kuiper-Goodman et al., 1987). Zearalenone contamination is however, not restricted to cereals, there are isolated reports of its presence in Job’s tears, beer, cassava, beans, walnuts, bananas, and soybeans (Gilbert, 1989). Although the toxicity of zearalenone may not be great, its action can result in considerable economic losses (Morgan et al., 1990).

### 2.1.6 Aflatoxins

Aflatoxins are a group of structurally related toxic compounds produced by *Aspergillus* species of fungi that grow on wide variety of grains and nuts (Patten,
Aflatoxins were discovered in 1960 when 100,000 turkey poultry died from eating fungus-infested peanut meal. *A. flavus* was found in the infested peanut meal together with alcohol extractable toxins termed aflatoxins (Joanne et al., 2008). The native habitat of *Aspergillus* is in soil, decaying vegetation, hay and grain undergoing microbiological deterioration. Fungi in the genus *Aspergillus* invade all types of organic substrates whenever conditions are favourable for its growth. Human foods which are frequently affected include cereals like maize, sorghum, pearl millet, rice and wheat. Oil seeds like peanuts, Soya bean, sunflower, and cotton seed, spices and tree nuts also support the growth of the fungus (Massey et al., 1995).

There are 18 different aflatoxins with aflatoxins B₁, B₂, G₁ and G₂ being the major ones. Aflatoxin B₁, which in its pure form is a pale-white to yellow crystalline and odorless solid, is found in large amount in cultures and food products and is considered the most toxic (CAST, 2003). Different species of *Aspergillus* may produce specific aflatoxins. *A. parasiticus* may produce aflatoxins B₁, B₂, G₁ and G₂, whereas *A. flavus* produces only B₁ and B₂ (Moss, 1989). Aflatoxins M₁ and M₂ are metabolic products of aflatoxin B₁ and B₂ produced by animals following ingestion of B₁ and B₂, and they are secreted in milk of both animal and human, and excreted in urine and faeces (Kang’ethe et al., 2007). Aflatoxin B₂ₐ and G₂ₐ which may be produced in minor amounts have been isolated from *A. flavus* and *A. parasiticus* (Reddy et al., 2000).
Aflatoxicol is a reductive metabolite of Aflatoxin B₁ and is normally secreted in milk and excreted in urine of dairy cattle and other mammalian species that have consumed aflatoxin B₁ (Paraica et al., 1999). Other compounds closely related to aflatoxins such as aflatoxin GM₁, and parasiticol are produced by Aspergillus flavus (Reddy et al., 2000). The mycotoxins produced by fungi, are not required for the growth or the development of the fungi, but serve as protective mechanism for the fungi. They weaken the receiving host and may use the host as a strategy to better their environment for further fungal proliferation (Fox et al., 2008).

2.1.6.1 Aflatoxin regulation

Many countries in the world have enacted legal regulation concerning the control of aflatoxins in food residues. Within the European Union (EU), harmonized maximum levels for some mycotoxins exist for feeds only, whereas in foods, maximum levels have been fixed (Rosner, 1998). A. flavus and A. parasiticus are thoroughly studied in both developed and developing countries, because of the nature of their toxicity and carcinogenicity (Chu, 2002). For reasons of preventive consumer protection, efforts are made to arrive at maximum levels for carcinogenic and genotoxic mycotoxins which are as low as possible but technologically feasible and analytically detectable in the food ready for consumption (Rosner, 1998).
To attest the seriousness of developed countries in the school of aflatoxicosis, the European Union has proposed the minimum possible levels of aflatoxins in foods and food products. Cereals should have a limit of 5 ppb, coffee 6-8 ppb, wine 1-2 ppb, dried fruits 5 ppb, spices 10 ppb and grape juice 3 ppb. The EU is not keen in the importation of foods from areas not observing these aflatoxins levels. The most pronounced aflatoxin contamination has been encountered in tree nuts, peanuts, and other oil seeds including corn and cottonseed (Van Egmond, 1987). Maximum acceptable levels of aflatoxins in human foods and animal feeds as established by Food and Drug Administration (FDA) are presented in Table 2.2.
Table 2.2 Action levels for aflatoxin present in foods and feeds to protect human and animal health.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Levels (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All products, except milk designated for human</td>
<td>20</td>
</tr>
<tr>
<td>Milk</td>
<td>0.5</td>
</tr>
<tr>
<td>For corn and other grains intended for immature animals and for dairy cattle.</td>
<td>20</td>
</tr>
<tr>
<td>For corn and other grains intended for breeding beef cattle, swine and poultry</td>
<td>100</td>
</tr>
<tr>
<td>For corn and other grains intended for finishing swine</td>
<td>200</td>
</tr>
<tr>
<td>For corn and other grains intended for finishing beef cattle</td>
<td>200</td>
</tr>
<tr>
<td>Cottonseed meal (as food ingredient) for cattle, swine and poultry</td>
<td>300</td>
</tr>
<tr>
<td>All feedstuffs other than the corn</td>
<td>20</td>
</tr>
</tbody>
</table>

Compliance Policy Guides 712026 / 710610 / 712633. 
Food and Drug Administration, 1997.
2.2 Conditions that favour aflatoxin contamination in foods and feeds

Mycotoxin contamination of foods and feeds depends on environmental conditions that lead to mould growth and toxin production (Pettet, 1998). Commodities can be contaminated at any time from growth in the field through harvesting, processing, storage and shipment (Pettet, 1998). *Aspergillus parasiticus* is well adapted to a soil environment and is prominent in peanuts, whereas *A. flavus* seems to be adapted to active development on the aerial parts of a plant (such as leaves and flowers) and is dominant on corn, cottonseed and tree nuts (Diener et al., 1987). *Aspergillus flavus* colonization in maize and oil seeds are encouraged by high humidity (80-89 %) and heat (10-40 °C) (Lunyasunya et al., 2005). Drought stress has been found to increase the number of *Aspergillus* spores in the air (Sorenson et al., 1984). Nitrogen stress (low soil fertility) and other stress that affect the plant growth during pollination can increase the level of aflatoxins production by *Aspergillus* fungi. Mature maize that remains in the field (as dry heaps) or maize that is stored without proper drying is susceptible to *Aspergillus* fungi growth and aflatoxin production (Lunyasunya et al., 2005). Poorly stored feeds and grains can indeed become contaminated with aflatoxin (Lillehoj et al., 1975).

Time of harvest has also been shown to have influence on aflatoxins level because *Aspergillus* does not compete well with other mould when maize is below 20 % moisture content. Thus harvesting maize with moisture content of above 20 % and
then drying down to at least a moisture content of 15 % within 24 to 48 hours of harvest will keep *Aspergillus* fungi growth and toxin production at minimum (Lunyasunya et al., 2005). Mold growth and toxin formation require a moisture content of the substrate greater than 14 % and a temperature of approximately 25 %. Reduced oxygen content diminishes aflatoxin formation (Diener et al., 1987). Damage of cereals by insects such as weevils and physical damage can greatly increase *Aspergillus* infection and the levels of aflatoxins. Protein supplements such as cotton seed cakes, sunflower cakes, fish meal and other oil seed by products which are often poorly stored are the primary source of the mould found in home made dairy concentrates on small hold farms (Lunyasunya et al., 2005).

Although maize is traditionally stored in granaries, storage inside homes occurs during periods of shortage. This may favour growth of moulds and subsequent contamination of maize with aflatoxins. The warm environment inside windowless homes and storage of maize on dirty floor may promote fungal growth in wet maize kernels (Eduardo et al., 2005). Traditional methods of drying and storing maize in elevated granaries are protective against aflatoxicosis. The granaries with elevated platform isolate the maize from the spores and insect on the ground (Eduardo et al., 2005). It is important to note that, although drying feeds and foods has been shown to reduce mould count, many moulds spores
remain in the feeds and foods after they have been dried. These spores can grow if conditions are right (Lunyasunya et al., 2005).

Majority of traders in the study area do not protect grains against the scorching sunshine, pests and mould colonization. This would lead to chronic poisoning of these foods due to contamination with mycotoxins. Therefore, to control Aspergillus fungi growth in foods and feeds, obvious sources of moisture in feeds and foods during handling and storage must be eliminated.

2.3 The biology of Aspergillus flavus and Aspergillus parasiticus

Aspergillus species are ubiquitous in the environment, growing in the soil, on plants and on decomposing organic matter. These moulds are often found in the outdoor and indoor air, water, on food items, and in dust (Joanne et al., 2008). A. flavus and A. parasiticus are closely related and grow as saprophytes on plant debris of many crop plants left on and in the soil. They are of worldwide distribution but are commonly found in tropical climates where there is extreme range in rainfall, temperature and humidity (Reddy et al., 2000). Members of genus Aspergillus are characterized by the production of non-septate conidiophore which is quite distinct from hyphae, they are swollen at the tip to form a vesicle on which specialized spore producing cells (phialides or sterigmata) are found. These specialized cells are either uniseriate or short growths known as metulae (biseriate) (Reddy et al., 2000). Colonies of Aspergillus flavus are green-yellow to
yellow-green or green on Czapek’s agar. They usually have biseriate sterigmata and reddish-brown sclerotia. The conidia are finely roughened and vary in size and are oval to spherical in shape. Colonies of A. parasiticus are dark-green on Czapak’s agar and remain green with age and their sterigmata are uniseriate. These colonies usually have no sclerotia, while the conidia are coarsely roughened. Unlike A. flavus, the colonies of A. parasiticus are uniform in size and shape (Reddy et al., 2000).

2.4 Chemical and physical properties of aflatoxins

Aflatoxins B₁, B₂, G₁, G₂, M₁, M₂, B₂A, and G₂A have melting points of 268-269, 286-289, 244-246, 237-240, 299, 293, 240, and 190 degrees centigrade respectively (Reddy et al., 2000). Aflatoxin B₁, B₂, G₁, and G₂ have closely similar structures and form a unique group of highly oxygenated, naturally occurring heterocyclic compounds. Their molecular formulas as established from elementary analysis and mass spectrometric determination are: B₁:C₁₇H₁₂O₆, B₂:C₁₇H₁₄O₆, G₁:C₁₇H₁₂O₇ and G₂:C₁₇H₁₄O₇. Aflatoxin B₂ and G₂ were established as the dihydroxy derivatives of B₁ and G₁ respectively. Whereas M₁ is tetrahydroxyl aflatoxin B₁ and aflatoxin M₂ is dihydroxyl aflatoxin B₂ (Heathcote et al., 1978). Extensive studies on the reactions of aflatoxins to various physical conditions and reagents have been conducted because of the possible application of such reactions in the detoxification of the materials contaminated with aflatoxins (Reddy et al., 2000). In the dry state, aflatoxins are
heat stable up to melting point. In the presence of moisture however, and at elevated temperature, aflatoxins are destroyed over a period of time. Such destructions of aflatoxins occur in oil seeds, meals, roasted peanuts, or in aqueous solution at pH 7. However, it seems likely that such treatments lead to the opening of the lactose ring, with possible destruction of decarboxylation at elevated temperature (Reddy et al., 2000).

In alkaline solution, hydrolysis of the lactose ring occurs, but this hydrolysis appears reversible, since it has been shown that recyclization occurs following acidification of basic solutions containing aflatoxins. At a temperature of 100°C, the ring opening is followed by decarboxylation. This reaction may proceed further and lead to loss of the methoxyl group from the aromatic ring. In the presence of the mineral acids, aflatoxins B₁ and G₁ are converted to aflatoxins B₂A and G₂A respectively due to acid-catalysed conditions of water across double bond in the furan ring. In the presence of acetic and hydrochloric acid, the reaction proceeds to give the acetoxy derivatives. Similar adducts of aflatoxin B₁ and G₁ are formed with formic acid-thionylchloride, acetic acid-thionylchloride and trifluoroacetic acid (Reddy et al., 2000).

Aflatoxin reactions with oxidizing agents such as sodium hypochlorite, potassium permanganate, chlorine, hydrogen peroxide, ozone and sodium perberate, changes the aflatoxin molecule in some way as indicated by loss of fluorescences. The
mechanisms of these reactions are uncertain and the reaction products remain unidentified in most cases (Reddy et al., 2000). Hydrogenation of aflatoxin B₁ and G₁ yields aflatoxin B₂ and G₂ respectively. If further reduced by three moles of hydrogen, aflatoxin B₁ yields tetrahydroxy aflatoxin, while reduction of aflatoxin B₁ and B₂ with sodium borohydride yields aflatoxins RB₁ and RB₂ respectively. The RB₁ and RB₂ arise because of the opening of the lactose ring followed by reduction of the acid group and the keto group in the cyclopentone ring. However, it should be noted that, breakdown of aflatoxins by various means does not guarantee safety of the contaminated substance. At times this breakdown is reversible or may lead to another form of aflatoxins. Besides, reaction products have not been subjected to detailed examination (Bankole et al., 2004).

2.5 Effects of food processing on mycotoxins

Several factors such as nature of the process, the food matrix, and moisture content of food stuff, presence or absence of additives, mode and level of contamination influence the degree of decomposition and loss of mycotoxin during processing (Scott, 1984). Mycotoxins may occur in processed feeds and foods since several of the mycotoxins including aflatoxin are relatively heat resistant so they persist in active form in pelleted feeds or canned foods processed from contaminated ingredients. Mycotoxins may develop in packaged feeds if they become wet in storage and the spores of a toxigenic mould are present (Scott, 1984). Aflatoxin is relatively heat stable and retains much of its biological
activities after exposure to dry heat up to 250 °C or after autoclaving for 30 minutes at 120 °C. Thus, many of the usual methods of feed and food processing including pelleting, canning and roasting are ineffective against aflatoxin or partially inactivate it (Scott, 1984). Physical cleaning methods such as dry cleaning, wet cleaning, density separation and preferential fragmentation were found to be generally ineffective in lowering the aflatoxin B₁ content of naturally contaminated corn (Brekke et al., 1975). The concentration of a flatoxin B₁ in artificially contaminated rice is greatly reduced by milling, with more than 95 % in bran and polish fraction (Scott, 1984). Normal cooking of rice destroys about 49 % of aflatoxin B₁ (Rahana et al., 1979). Pressure cooking and cooking with excess water destroys 73 % and 82 % of the aflatoxin B₁ in the rice respectively, providing further evidence of the influence of water on stability of aflatoxin during heating process (Scott, 1984).

Boiling contaminated maize flour in water to make *ugali*, a traditional African dish, destroys only 11.5 % and 17.6 % of aflatoxin B and G respectively (Seenappa et al., 1982). The actual bread baking process causes losses of 25 % (El-Banna et al., 1983). Aflatoxin B₁ is not completely removed during the process of beer making, for two levels of beer fermentation, contamination level of 18 % and 27 % remained respectively (Chu, 1975). Aflatoxin M₁ is stable during pasteurization; aflatoxin is more stable in naturally contaminated milk than in spiked milk during freeze drying (Wiseman, 1983). Greatest losses occur
during sterilization and spry drying of milk; concentrations remain about the same in yoghurt and buttermilk (Wiseman, 1983), while concentration increase while making cheese (Applebaum, 1982). Therefore researches above clearly indicate that aflatoxin is relatively resistant to most forms of processing especially in low moisture content.

Ochratoxin A is not eliminated from grain by cleaning, and on milling, it is distributed equally between flour and bran (Chelkowski, 1981). Ochratoxin A appears to be more readily destroyed in dry cereals than in the presence of water (unlike aflatoxin B1 and patulin). Oatmeal and rice cereals contaminated with Ochratoxin A and autoclaved for three hours with no added water, loses 87.5% and 86% respectively, whereas lesser amount of toxin (74% and 68.5%) are destroyed after autoclaving for three hours in the presence of 50% water (Trenk et al., 1971). Apples processed for juice may contain patulin if fruit rotten with Penicillium expansum is used (Lovett, et al., 1975).

Patulin and penicillic are unsaturated cyclic lactones that are less stable than either the aflatoxins or ochratoxin A in food systems, particularly in grains, grain foods, meat and cheese (Lieu, 1977). Stability of patulin in ground grains decreases with increasing moisture content. The rate of disappearance increases markedly on addition of vitamin C. On the other hand, sucrose protects patulin during heat treatment in berry jams (Scott, 1984). Almost complete destruction of
26

patulin occurs during alcoholic fermentation of apple juice. Wine making also destroys patulin (Stinson et al., 1978). More zearalenone is lost in wet-milling than in dry milling of corn. All dry-milled corn contained zearalenone and only 3% to 10% was removed by dry cleaning. Vomitoxin have proved to be more heat stable during food processing than any other mycotoxin tested (Scott, 1984). The commonly occurring ergot alkaloid toxins such as ergometrine, ergosine, ergotamine, ergocornine, and ergocristine are less stable than any of the mycotoxins (Scott, 1984). They do occur in flour but during baking, losses of up to 100% occur in all wheat bread and up to 85% in rye bread (Scott et al., 1982).

2.6 Aflatoxicosis

Aflatoxicosis is poisoning that result from ingestion of aflatoxins in contaminated foods in human and feeds in animals. It manifests as chronic or acute aflatoxicosis (Lunyasunya et al., 2005). Chronic aflatoxicosis results from ingestion of low to moderate levels of aflatoxins. Chronic dietary exposure to aflatoxins is a major factor for hepatocellular carcinoma (Fung and Clark, 2004). The effects are subclinical and are difficult to recognize. Common symptoms are impaired food conversion and slow rate of growth with or without the production of an overt aflatoxin syndrome. Ingestion of higher doses of aflatoxin can result in an acute aflatoxicosis which manifest as hepatotoxicity or in severe cases, fulminant liver failure (Fung and Clark, 2004). Acute symptoms include hemorrhage, acute liver damage, edema, alteration in digestion, absorption and/or metabolism of nutrients
and possibly death. No animal species is resistant to the acute toxic effect of aflatoxins (Lunyasunya et al., 2005).

The biological effects of aflatoxin can be grouped into four general categories: acute and chronic liver damage, reduced growth rate, impairment of immunologic and innate defense mechanisms and carcinogenic and tetragenic effects. Many of these effects of aflatoxins relate to their reaction with cellular protein and maintenance of cellular integrity (Patterson, 1976). Animal species respond differently in their susceptibility to chronic and acute toxicity of aflatoxins. This toxicity can be influenced by environmental factors, exposure level and duration of exposure, age health and nutritional status of diet (Finley et al., 1992). Aflatoxin B$_1$ is a very potent carcinogen in many species including non human primates, birds, fish and rodents. In each species, the liver is the primary target organ of aflatoxin toxicity and carcinogenicity in acute injury (Fung and Clark, 2004).

### 2.6.1 Human aflatoxicosis

Human exposure to levels of aflatoxins from nanogrames to micrograms per day occurs through consumption of contaminated maize, peanuts and other contaminated foodstuffs. Maize is the staple food in Kenya. It is milled into flour for various delicacies such as ‘Ugali’ (Lunyasunya et al., 2005). The human gastrointestinal tract rapidly absorbs aflatoxin after consumption of contaminated
food and the circulatory system transports aflatoxin to the liver (Fung and Clark, 2004). From 1-3% of ingested aflatoxin irreversibly bind to proteins and DNA bases to form adducts such as aflatoxin B\textsubscript{1} lysine in albumin. Disruption of protein and DNA bases in hepatocytes causes liver toxicity (Tandon et al., 1978).

Early symptoms of hepatotoxicity from aflatoxicosis can manifest as anorexia, malaise, and low grade fever. Aflatoxicosis can progress to potentially lethal acute hepatitis with vomiting, abdominal pain, hepatitis and death (Etzel, 2002). Symptoms of B\textsubscript{1} also include yellow eyes, swollen legs, vomiting, abdominal pain and bleeding. The health impact of aflatoxin exposure in animals mainly depends on dosage and response. Low dosages produce nutritional interference and immunological suppression, while high doses lead to acute illness and death. (Lunyasunya et al., 2005).

Aflatoxins have been detected in the blood of pregnant women, in neonatal umbilical cord blood, and in breast milk in African countries, with significant seasonal variations (Maxwell et al., 1989). Levels of aflatoxins detected in some umbilical cord blood at birth are among the highest levels ever recorded in human tissues and fluids (Coulter, 1984). Aflatoxins have been suggested as aetiological factor in encephalopathy and fatty tissue degeneration of viscera, similar to Reye syndrome, which is common in countries with a hot and humid climate (Turner, et al., 2002). The clinical picture includes enlarged pale liver and kidneys and severe
cerebral oedema. Aflatoxins have been found in blood during the acute phase of the disease, and in the liver of affected children (Turner et al., 2002).

The evaluation of epidemiological and laboratory results carried out in 1987 by the International Agency for Research on Cancer (IARC) found that there was sufficient evidence in humans for the carcinogenicity of naturally occurring mixtures of aflatoxins, which are therefore classified as Group 1 Carcinogens, except for aflatoxins M₁, which is possibly carcinogenic to humans (IARC, 1987). In recent studies, aflatoxins have been found in the brains and lungs of children who have died from kwashiorkor and those who had died from various other diseases (Oyelami et al., 1995; Oyelami et al., 1997).

2.6.2 Dairy cattle aflatoxicosis

Past studies have confirmed that most cases of animal poisoning by aflatoxins can be traced to the growth of fungi in poorly handled feeds (Smith, 1997; Jacques, 1988). Aflatoxins produce a wide range of harmful effects such as decreased feeds utilization and efficiency (reduced appetite) leading to low weight gain, liver and kidney damage, gastrointestinal dysfunction (hemorrhage and necrosis throughout the digestive tract), embryonic and early death of the newborn (interferes with conception, ovulation implantation, fetal development and viability of the newborn animals). Aflatoxins also cause teratogenicity (birth defects), tumors and suppressed immune system function (even when low levels
are consumed), reduced productivity (reduced milk production, productive efficiency), anemia, jaundice carcinogenesis and death (Pier, 1992). Depending on interaction with other factors, aflatoxins concentration of 100 ppb may be toxic to cattle (Garrett et al., 1968). It is clear that contaminated food and feeds can lead to health effects to human and animals and also cause economic loses to farmers and traders. Awareness of these among food and feed handlers will go along in reducing cases of contamination.

2.7 Aflatoxins and body immunity

Aflatoxin has been shown to have a marked suppressive effect on the development of acquired immunity through its action on the cell mediated immune system (Pier et al., 1980). The thymus and the thymus derived lymphocytes appeared to be exceptionally sensitive to the effects of B₁ and M₁ (Pier et al., 1979). The precise mode of action in this immuno-suppressive effect appears to involve T-cell population, antigen-cell interaction, phagocytosis and possibly lymphokine production ((Pier et al., 1979). Aflatoxin also exerts effects on innate defense mechanisms; it reduces phagocytic response of macrophages, reduces production of some portions of complement such as C₄ and cause a lag in interferon production. Generally, antibody production is not affected by aflatoxin, however, at extremely high level of intake, IgG and IgA but not IgM have been depressed (Pier et al., 1980).
2.8 Aflatoxin food poisoning in Africa and the world

The largest documented outbreak of aflatoxicosis took place in western India in 1972. This event also occurred in the contest of unreasonable rains during harvest leading to contamination of home grown maize stored under damp conditions (Krishnamachari et al., 1975). One of the most important accounts of aflatoxicosis occurred in more than 150 villages in adjacent districts of two neighboring states in northwest India in the fall of 1974. According to one of this outbreak, 397 persons were affected and 108 persons died. In this outbreak, contaminated corn was the major dietary constituent, and aflatoxin levels of 0.25 to 15mg/kg of corn were found.

In Philippines, aflatoxin B₁ was found in the lungs of one textile and two agricultural workers who died from the pulmonary and intestinal fibrosis. These individuals were probably occupationally exposed to aflatoxin B₁ via the respiratory route (Dvorackova et al., 1986). In the United Kingdom, it was found that intravenous heroin users can be exposed to aflatoxin B₁ from samples of heroin on sale (Hendrickse et al., 1989). In the United Kingdom and in the Netherlands, analysis of 121 urine samples obtained from heroin addicts revealed a higher proportion of samples contaminated with aflatoxins B₁, B₂, M₁ and M₂ and aflatoxicol (20%) than those from normal adult volunteers (Hendrickse et al., 1989).
2.9 Aflatoxin milk poisoning in Africa and the world

Aflatoxins have also been detected in milk. Dairy cattle that feed on aflatoxin contaminated feeds produce contaminated milk. Approximately 1-3% of the B1 initially present in the animal feedstuff appeared as aflatoxin M1 in milk, but its carryover varies from animal to animal (Pittet, 1998). Exposure of dairy cattle to low-moderate aflatoxins concentration passes serious risks to human that depend on it for milk (Robens et al., 1992). In Bogota Columbia, 241 retail milk samples were analyzed during the period of 2004 and 2005 for aflatoxins, 69.2% and 79.4% aflatoxin M1 contamination was found respectively (Diaz et al., 2006). In Southern Italy 44% of 252 samples of milk from cows, buffalo, sheep, and goats were found to be contaminated with aflatoxin M1. A prevalence of aflatoxin M1 at 51% raw and pasteurized milk was found in 110 samples (Diaz, 2006). Aflatoxin concentration of 100 ppb may be detrimental to the dairy stock and calves may be affected by exposure to aflatoxin metabolites in milk (Patternson et al., 1982). Aflatoxin M1 is frequently detected in human breast milk in many countries of the world, sometimes at alarmingly high levels in samples from tropical and subtropical regions (El-Nezami, 1995).

2.10 Aflatoxin food poisoning in Kenya

Outbreaks of acute aflatoxicosis from highly contaminated food have been documented in Kenya, India, and Thailand (CAST, 2003). In April 2004, an outbreak of an acute hepatotoxicity was identified among people living in Makueni,
Kitui, Machakos and Thika Counties. Epidemiologic investigation determined that the outbreak was the result of aflatoxins poisoning from ingestion of contaminated maize (Kang’ethe et al., 2007). As of July 2004, three hundred and seventeen cases and one hundred and twenty five deaths had occurred; making this one of the largest and most severe outbreak of acute aflatoxicosis documented worldwide (CDC, 2004). In 1981, an outbreak of aflatoxicosis from contaminated maize occurred in Makueni County. In both 1981 and 2004, drought and food shortages were followed by unreasonable rains during harvest which probably favored the growth of aflatoxigenic Aspergillus in household maize (Ngindu et al., 1982). From the above cases, it is clear that aflatoxin food poisoning is a common phenomenon in eastern parts of Kenya and occurs on cereals commonly used by many communities as staple food. These cereals can be stored in processed and non processed form.

2.11 Economic impact of aflatoxins

The economic impact of aflatoxins is derived directly from food and livestock losses as well as directly from cost of regulatory programmes designed to reduce risks to animal and human health (CAST, 1989). The Food and Agricultural Organization (FAO) estimates that 25% of world food crops are affected by mycotoxins of which the most notorious are aflatoxins (FAO, 1998). Aflatoxins losses to livestock and poultry producers from aflatoxin contaminated feeds include death and more subtle effects of immune system suppression, reduced
growth rate and loss in feeding efficiency. Other adverse economic effects of aflatoxin include lower yields for foods and fiber crops (CAST, 1989).

Aflatoxin contamination impacts on loss to farmers and traders, for instance, 32,000 bags of maize were condemned in Kenya in 2009. Aflatoxin leads to decreased production of animals, high cost of decontamination, loss of trade both locally and internationally. Human deaths could result in orphaned children creating a burden to society. The contamination also leads to reduced availability of both quantity and quality of food to people (Ngetich, 2011). In addition, the ability of aflatoxin to cause cancer and related diseases in human given their seemingly unavoidable occurrences in foods and feeds, make the prevention and detoxification of these mycotoxins one of the most challenging toxicology issues of the present time (Eaton et al., 1994).

2.12 Methods of detection and quantification of aflatoxins in food sample.

2.12.1 High performance liquid chromatography (HPLC)

High performance liquid chromatography is a method of choice for separating potentially carcinogenic substances. Detection normally is performed by fluorescence spectroscopic method on trifluoroacetic acid derivative. For analyzing aflatoxins, reverse phase liquid chromatography (RFLC) and normal phase liquid chromatography (NPLC) are used (Park, 1995). The RPLC for analysis of aflatoxins employs silica based HPLC columns bonded with C8 and
C18 groups which are used with mobile phase consisting of binary and ternary mixture of polar solvents. The commonly used solvents mixtures include deionized water, methanol and acetonitrile. In the reverse phase mode, the elusion order of the common aflatoxins is G₂, G₁ B₂ and B₁. Aflatoxins may be separated and detected by UV detection. However the sensitivity is not sufficient to detect these compounds at parts per billion (ppb) concentrations required for food analysis, with fluorescence detector (Nollet, 1992).

2.12.2 Lateral flow immunoassay for aflatoxins

Lateral flow immunoassay for aflatoxins applies same principle and reagents as in the micro-well type immunoassay, except for the fact that in lateral flow immunoassay (LFIA), the separation of bound and unbound antibody sites is obtained by means of lateral flow on suitable support (nitrocellulose membrane). The liquid flow transports immune reagents along the membrane where they encounter their partners in spatially confined zones of the membrane itself where immunoreactions take place. Lines (test and control lines) are traced on the nitrocellulose membrane by means of dedicated dispenser which enables dispensing of small volumes with high productivity. Interpretation of assay result depends on the presence and intensity of both test and control line. The indirect competitive format in which the antigen (protein conjugate of the target toxin) is coated on the membrane and the antibody is labeled is preferred for aflatoxins (Anfossi et al., 2011)
2.12.3 Competitive enzyme immunoassay

The bases of this test are the antigen-antibody reaction. The well in the microtiter strips are coated with capture antibodies directed against anti-aflatoxin antibodies. The standard or the sample solution, aflatoxin-enzyme conjugate and anti-aflatoxin antibodies are added. Free and enzyme conjugated aflatoxin compete for the aflatoxin antibody binding sites. At the same time the anti-aflatoxin antibodies are bound by the immobilized capture antibodies. Any unbound enzyme conjugate is then removed in a washing step. Enzyme substrate and chromogen are added to the wells and incubated. Bound enzyme conjugate converts the colourless chromogen into a blue product. The addition of the stop solution leads to colour change from blue to yellow. The measurement is made photometrically at 450nm; the absorption is inversely proportional to the aflatoxin concentration in the sample (R-Biopharm, 2003).

Competitive enzyme immunoassay is a preferred choice of testing aflatoxins because it is simple, sensitive, and cost effective and can be used to test over 100 samples unlike HPLC and lateral flow immunoassays which although sensitive are not cost effective and cannot be used on over 100. They are normally used as confirmatory tests (Waliyar et al., 2009). Immunochemical methods are quite specific and can be used to screen flatoxins in grains and grain products (Trucksess et al., 2007).
2.13 Traders’ awareness of aflatoxins contamination and level of aflatoxin in food and feeds in Nairobi County

The literature review apparently indicates that much research has been reported on conditions that favour aflatoxins contamination (Lillehoj et al., 1975; Lunyasunya et al., 2005; Strosnider et al., 2006) and health effects of aflatoxins in human and animals (Pier, 1992; Etzel, 2002; Fung and Clark, 2004; Lunyasunya et al., 2005). However, not much has been carried out to assess the level of awareness of aflatoxins contamination among foods and feeds handlers. Further, there are no documented studies on awareness of dangers associated with aflatoxin contamination among food and feed traders in Nairobi County.

Maize aflatoxin contamination has extensively been carried out in prone areas such as Kitui and Mukueni Counties in Kenya (Lewis et al. 2005; Muture et al. 2005; Eduardo 2005). However, limited work had been carried in Nairobi County especially in open air markets. Further, limited work has been carried out on processed foods and other food stuffs that are vulnerable to aflatoxin contamination. Further research was important aimed at determining level of awareness among food and feed traders who handle foods and feeds depended on by a large population of Nairobi County and prevalence of aflatoxins in foods that are common in most households and the cattle feed that is commonly used by urban farmers. This was essential for the purpose of comparing their level of contamination with the recommended maximum residue limit (MRL) and for
appropriate remedial action to be taken by the main stakeholders such as Ministry of Agriculture and Public Health.
CHAPTER 3: MATERIALS AND METHODS

3.1 Study area

The study was carried out in Nairobi County in the eight divisions namely Dagoretti, Kibera, Pumwani, Westlands, Makadara, Embakasi, Kasarani and Central Business District (CBD). The immediate environment of Nairobi consists of the productive highland area extending northwards and westwards. The strategic location of the study area enables open air markets as Toi, Kawangare, Kangemi, Uhuru market and Nyamakima to easily obtain inputs such as agro-based raw materials from the surrounding region. The open air market serves as a source of food materials for common Nairobian. The study area is the smallest administrative county by size in Kenya but the most important in terms of activities and functions, such as industries, trade and service provision. Most animal feeds are manufactured in Nairobi (CBD) and supplied to its suburbs such as Ngong, Rongai, Ruai and bordering counties such as Kiambu, Kajiado and Machakos County (Figure 3.1).
Figure 3.1: Study area; Nairobi County, Kenya
3.2 Study design

A standard questionnaire was developed, pre-tested and administered to 103 traders (53 food traders and 50 cattle feed traders) to gather their information on traders’ awareness of aflatoxin contamination of human food and cattle feeds, conditions that favour aflatoxin contamination during storage, measures taken to prevent contamination during storage and effects of aflatoxins on foods, feeds and health. Observation was also employed in assessing the market layout and storage (Appendix I). A total of 150 samples (96 food sample and 54 feed samples) were detected for aflatoxins.

3.3 Criteria

3.3.1 Inclusion criteria

i) The five major open air markets based on size and with large customer base in Nairobi County were chosen.

ii) AGROVET stores which deal with animal feeds were chosen.

iii) The human foods chosen were staple foods such as maize, rice and groundnuts.

iv) The cattle feeds (daily meal and maize) chosen are widely consumed by cattle.

3.3.2 Exclusion criteria

v) Supermarkets, small markets and retail shops were not chosen.
vi) Other types of foods and feeds were not chosen such as millet, sorghum beans, peas and spices.

3.4 Quantitative analysis of aflatoxins levels in foods and feeds

3.4.1 Samples

Human foods: Non-processed foods (maize grains, unpolished rice and groundnuts) and processed foods (polished rice, maize flour, and ground groundnuts). Cattle feeds: Non-processed (maize grains) and processed (cattle daily meal) brand A and B. Brand A and B are dairy meal products from different local companies purchased from traders.

3.4.2 Sample size determination

An average prevalence of 55% aflatoxin in foods was used which was reported in a study carried out in Kitui and Makueni Counties (Lewis et al., 2005). Sample size was determined using the method described by Fisher et al., 1998 as detailed below.

\[ N = \frac{Z^2 pqD}{d^2} \]

Where:

\[ N = \text{Sample size to be calculated.} \]

\[ Z = 1.96 \text{ at 90% standard error.} \]

\[ P = \text{Probability of occurrence 0.55} \]

\[ q = 1-p \]
D = design effect equals 1 (Nairobi city)

d^2 = Desired confidence of 0.1

Therefore N = 1.96^2 x 0.55 x0.45 x1/0.1^2

95.04.

Based on the calculations above a sample size of 96 food samples was established. For Sample size for animal feeds, a prevalence of 83% which was found in a study carried out in urban smallholders’ daily production in Dagoretti division, Nairobi (Kang’ethe et al., 2007).

N = Sample size to be calculated.

Z   = 1.96 at 90%

P   = Probability of occurrence 0.83

q   =1-p

Therefore N= 1.96^2 x 0.83 x 0.17 x 1/0.1^2

54 feed samples.

Total number of samples collected is 150 samples (that is 96 +54).

3.4.3  Sampling procedure

Simple random sampling procedure was used to select 54 samples of cattle dairy meal (250g each) from Agriculture and veterinary (AGROVET) stores in each Division of Nairobi. Six samples were picked randomly from AGROVET stores from each division (Dagoreti, Kibera, Pumwani, Westlands, Makadara, Embakasi, Kasarani), using the table of random numbers (Appendix IV). Twelve
samples were randomly picked from the Central Division. This is due to the high concentration of AGROVET stores within the Division.

Five major open air markets within Nairobi were selected based on size. The markets selected were Nyamakima Market, Toi Market, Kawangware Market, Kangemi Market and Uhuru Market. Open air markets are large markets where traders trade their merchandise in the open ground with minimal shade (Appendix IV). They receive their agro-based raw materials from neighbouring counties. These markets were chosen because they serve a large population of Nairobi County and are main source of commodities for even small markets. The proposed sample size for human foods was 96 samples. The human foods both processed and non-processed were drawn from these markets. Three sample quantities of 250 g of each food type were obtained from each market. A table for sampling human foods and cattle feeds from open air markets and various AGROVET stores respectively was used (Appendix II).

3.4.4 Sample storage

Collected foods and Cattle feed samples were transported to the laboratory at Department of Public Health, Pharmacology and Toxicology, Faculty of veterinary medicine, College of Agriculture and Veterinary Sciences, University of Nairobi. Samples were kept away in dark environment because aflatoxins are
light-sensitive and in absorbent paper bag in carton boxes away from dampness and at room temperature to prevent growth of fungi.

3.5. Laboratory analysis

3.5.1 Sample preparation
Laboratory analysis was carried out at the Department of Public Health, Pharmacology and Toxicology, Faculty of veterinary medicine, College of Agriculture and Veterinary Sciences, University of Nairobi. A representative sample was triturated and thoroughly mixed in a mixer. Two grams of the ground sample was weighed using an electrical balance into a screw cap glass vial. Ten milliliters of Methanol/distilled water (70%/30%), were weighed and added to the vial and mixed for 10 minutes at room temperature (20-25 °C; 68-77 °F) using a shaker. The entire extract was filtered using filter paper. Hundred microlitres of the filtrate were diluted with six hundred microlitres of the sample dilution buffer. Fifty microlitres were used per microtiter well on a microwell holder in the assay.

3.5.2 Reagents
The reagents provided in the Ridascreen® aflatoxin total kit were: microtiter plate with 96 wells coated with capture antibodies, aflatoxin standard concentrates were: 0 ppb (Zero standard), 0.5 ppb, 1.5ppb, 13.5ppb, 40.5ppb, aflatoxin enzyme conjugate (Peroxidase conjugated aflatoxin B1 concentrate), anti-aflatoxin antibodies (monoclonal concentrate), substrate (urea peroxide), chromogen
(tetramethylbenzidine), stop solution (1N sulfuric acid) and buffer (standard, sample, conjugate and antibody dilution buffer). All reagents were brought to room temperature before use. Then all reagents were returned to 2-8 °C immediately after use. Microtiter wells were not allowed to dry between working steps (R-Biopharm, 2003).

3.5.3 Aflatoxin analysis using competitive enzyme immunoassay

This test was a quantitative analysis of aflatoxin residues in cereals and feeds. The test procedure was carried out as described in Ridascreen aflatoxin total kit (R-Biopharm, 2003). Sufficient number of microtiter wells were inserted into the microwell holder for all standards and samples. These microtiter wells were coated with capture antibodies directed at anti-aflatoxin antibodies. Samples were run in duplicate. Standard and sample positions were recorded. Fifty microlitres of the standard solutions were added to standard microtiter wells and prepared sample were added to separate duplicate wells. Fifty microliters of a diluted enzyme conjugate (peroxidase conjugated aflatoxin B₁) were added. Fifty microliters of the diluted antibody (anti-aflatoxin antibody solution) were also added. They were mixed gently by rocking the plate with a shaker and incubated for 40 minutes at room temperature in the dark.

Free and enzyme conjugate competed for the aflatoxin antibody binding sites and at the same time the anti-aflatoxin antibodies were bound by immobilized capture
antibodies. After incubation, the reactants were poured out of the plate wells and the microwell holder was tapped upside down vigorously against absorbent paper to ensure complete removal of liquid from wells. All the wells were filled with two hundred microlitres of distilled water and the liquid poured out. This washing procedure was repeated three times. The purpose of the washing was to remove the unbound enzyme conjugate. After washing, fifty microlitres of the enzyme substrate and fifty microlitres of chromogen were added to each well. Mixing was then done gently by rocking the plate with a shaker before incubating the plates for 30 minutes at room temperature in the dark. The bound enzyme conjugate converts the colorless chromogen into a blue product as a result of hydrolysis of the substrate.

Hundred microlitres of stop solution (1N sulfuric acid) was added to each well, and then mixed gently by rocking the plate. The addition of the stop solution leads to color change from blue to yellow. The absorbance of the wells was measured photometrically at 450 nm within 30 minutes after addition of stop solution. The concentration of aflatoxin was calculated based on the fact that the absorbance is inversely proportional to the aflatoxin concentration in sample. An ELISA machine and Special software, the RIDA\textsuperscript{(R)} SOFT win (Art. No. Z9999) was used for obtaining aflatoxin concentration in each sample in parts per billion (ppb).
3.5.4 Calibration curve

The standards absorbance values were entered in a system of coordinates on semilogarithmic graph paper against the aflatoxin concentration in ppb. The calibration curve was generated that contains aflatoxin concentration in ppb corresponding to the absorbance of each sample. In order to obtain the actual aflatoxin concentration in ppb contained in a sample, the concentration obtained was further multiplied by the corresponding dilution factor. For cereals or feed the dilution factor is 35 (Figure 3.2).
Figure 3.2: Absorbance observed and the calibration curve obtained during analysis of aflatoxin
3.6 Statistical data analysis and presentation

The data obtained using questionnaires were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS) computer programme. For each sample type, general descriptive statistics were used to obtain mean aflatoxins concentration of each food type and feeds in parts per billion (ppb). To compare aflatoxin levels in non-processed and processed foods, a paired T-test was used. Two T-tests was used to compare aflatoxin levels in feeds. One-way ANOVA was used to compare aflatoxins level in the three types of foods (maize, rice, and groundnuts). Data were presented using tables and figures. A $P$ value $\leq 0.05$ was considered significant.
CHAPTER 4: RESULTS

4.1 Traders awareness of aflatoxin and conditions under which foods and feeds were stored by traders in Nairobi County, Kenya

4.1.1 Demographic information, awareness of aflatoxin, and level of education of traders

A total of 103 traders participated in the study and their views were received through structured questionnaires. Averagely a good proportion of the traders (56.3 %) were aware of aflatoxin. Awareness of aflatoxin was significantly high among the traders of cattle feeds than among the food traders ($\chi^2 = 25.112, P < 0.05$). A large proportion of cattle feed traders 40 %(40) were aware of aflatoxin as compared to food traders 17 % (18). The level of education of the respondents ranged from primary to tertiary. 42.7 % of the traders were secondary school leavers, 35.0 % (36) had tertiary education, and 19.4 % (20) of the traders had primary education, while 2.9 % (3) were illiterate. When considering awareness based on traders’ education levels, awareness of aflatoxin was significantly different in the traders’ levels of education ($\chi^2 = 34.901, P < 0.05$). Traders having tertiary education were more aware of aflatoxin than those having primary education as shown in table 4.1.
Table 4.1 Traders’ education level and awareness of aflatoxin in Nairobi County, Kenya

<table>
<thead>
<tr>
<th>Education level of traders</th>
<th>Awareness of aflatoxin</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>Aware of aflatoxin</td>
<td>N (%)</td>
<td>Not aware of aflatoxin</td>
</tr>
<tr>
<td>Primary</td>
<td>20 (19.4)</td>
<td>4 (20)</td>
<td></td>
<td>16 (80)</td>
</tr>
<tr>
<td>Secondary</td>
<td>44 (42.7)</td>
<td>19 (45.2)</td>
<td></td>
<td>25 (56.8)</td>
</tr>
<tr>
<td>Tertiary</td>
<td>36 (35)</td>
<td>34 (94.4)</td>
<td></td>
<td>2 (5.6)</td>
</tr>
<tr>
<td>Not attended School</td>
<td>3 (2.9)</td>
<td>1 (33.3)</td>
<td></td>
<td>2 (66.7)</td>
</tr>
<tr>
<td>Total</td>
<td>103 (100)</td>
<td>58 (56.3)</td>
<td></td>
<td>45 (43.7)</td>
</tr>
</tbody>
</table>
4.1.2 Level of aflatoxin awareness of traders who had trained on handling and storage of foods and feeds

Only 24 % (24) of the traders had attended organized workshops and seminars on training related to handling and storage of foods and feeds. A good proportion of the respondents (76.7 %) (79) had not attended any training on handling and storage of foods and feeds. It was established that training related to handling and storage of foods and feeds significantly influenced awareness by the traders on aflatoxin ($\chi^2 = 11.646, P < 0.05$) Table 4.2. Large proportion (87.5 %) (21) of those who had attended trainings related to handling and storage of foods and feeds were aware of aflatoxin as shown in table 4.2.

<table>
<thead>
<tr>
<th>Attended training</th>
<th>Aware of Aflatoxin</th>
<th>Not aware of aflatoxin</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attended</td>
<td>87.5 % (21)</td>
<td>12.5 % (3)</td>
<td>100 % (24)</td>
</tr>
<tr>
<td>Not attended</td>
<td>48.1 % (37)</td>
<td>51.9 % (40)</td>
<td>100 % (77)</td>
</tr>
</tbody>
</table>

4.2 Traders’ level of awareness on proper storage of foods and feeds

The traders’ awareness on proper storage of foods and feeds was assessed based on their ability to state suitable conditions for storage of foods and feeds. A small proportion of the traders reported cool dry place; food traders (28.3 %) (15), feed
traders (28 %) (14) and well ventilated store; food traders (33.9 %) (18), feed traders (18 %) (9), while a large proportion reported storage in bags; food traders (52.8 %) (28), feed traders (84 %) (42) and on raised ground; food traders (47.1 %) (25), feed traders (70 %) (35) as suitable storage conditions.

It was also observed that majority of the traders packed their food or feeds in nylon sacks. Some of the open air market traders (11.3 %) (6) stored their foods in nylon sacks under a polythene paper overnight. Food traders displayed their unpacked food products in the open during the day as a way of attracting customers. Unlike food, feed traders stored their feed in a much organized manner. The traders’ awareness on conditions that encourage aflatoxin contamination was assessed based on their ability to state factors that encourage growth of fungi. A good proportion of the traders reported high temperature; food traders (86.7 %) (46), feed traders (36 %) (18) and moisture; food traders (49 %) (26), feed traders (90 %) (45) as conditions that encourage growth of fungi during storage. Measures reported by some traders that control the growth of Aspergillus fungi during storage included buying small quantities that can sell quickly, storage of dry foods, putting pesticides, faster delivery of feeds and foods to the customers, spreading on open ground and not overstocking the stores (Table 4.3; Appendix IV, V and VI). The hypothesis was rejected; feed traders who were more aware of aflatoxin, stored their feeds in better conditions.
4.3 Traders’ level of awareness of the effects of aflatoxin in foods and feeds

Traders reported the following as effects of *Aspergillus* species in food and feed: change of colour: food traders (32 %) (17), feed traders (50 %) (25) and production of bad smell; food traders (26.4 %) (14), feed traders (64 %) (32) as the main effects. Although 56.3 % of the traders were aware of aflatoxin, only very small proportion of the traders reported aflatoxins as being harmful to human and animal health; food traders (3.7 %) (2), feed traders (8 %) (4). Other health hazard experienced by the traders associated with grains and animal feed storage included; allergies, coughing, sneezing, “homa” breathing problems, smell of flour, and chest complication (Table 4.3). Hypothesis was accepted; over 90 % of the traders were not aware of the harmful effects of flatoxin in foods and feeds in relation to human and animal health.
Table 4.3: Traders’ level of awareness on storage of foods and feeds, conditions that encourage aflatoxin contamination in foods and feeds and effects of aflatoxin on health

<table>
<thead>
<tr>
<th>Traders</th>
<th>Storage of foods and feeds</th>
<th>Conditions that encourage aflatoxin contamination</th>
<th>Effects of aflatoxin in foods, feeds and health</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Food traders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=53)</td>
<td>Cool dry place</td>
<td>High temperature</td>
<td>Change in colour</td>
</tr>
<tr>
<td></td>
<td>15 (28.3)</td>
<td>46 (86.7)</td>
<td>17 (32)</td>
</tr>
<tr>
<td></td>
<td>Well ventilated store</td>
<td>Moisture</td>
<td>Produced bad smell</td>
</tr>
<tr>
<td></td>
<td>18 (33.9)</td>
<td>26 (49.0)</td>
<td>Rotting/decaying</td>
</tr>
<tr>
<td></td>
<td>Packed in bags</td>
<td>Poor ventilation</td>
<td>Poisoning</td>
</tr>
<tr>
<td></td>
<td>28 (52.8)</td>
<td>3 (5.6)</td>
<td>2 (3.7)</td>
</tr>
<tr>
<td></td>
<td>Raised ground</td>
<td>Long term storage</td>
<td>Has no idea</td>
</tr>
<tr>
<td></td>
<td>25 (47.1)</td>
<td>3 (5.6)</td>
<td>36 (67.9)</td>
</tr>
<tr>
<td></td>
<td>Clean place</td>
<td>Insects and rats</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2 (3.7)</td>
<td>3 (5.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cover with polythene</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>overnight</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 (11.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Feed traders</strong></td>
<td>Cool dry place</td>
<td>High temperature</td>
<td>Change in colour</td>
</tr>
<tr>
<td>(N=50)</td>
<td>14 (28)</td>
<td>18 (36)</td>
<td>25 (50)</td>
</tr>
<tr>
<td></td>
<td>Well ventilated store</td>
<td>Moisture</td>
<td>Produced bad smell</td>
</tr>
<tr>
<td></td>
<td>9 (18)</td>
<td>45 (90)</td>
<td>32 (64)</td>
</tr>
<tr>
<td></td>
<td>Packed in bags</td>
<td>Poor ventilation</td>
<td>Compact/ caking</td>
</tr>
<tr>
<td></td>
<td>42 (84)</td>
<td>3 (6)</td>
<td>24 (48)</td>
</tr>
<tr>
<td></td>
<td>Raised ground</td>
<td>Long term storage</td>
<td>Poisoning</td>
</tr>
<tr>
<td></td>
<td>35 (70)</td>
<td>2 (4)</td>
<td>4 (8)</td>
</tr>
<tr>
<td></td>
<td>Well arranged</td>
<td></td>
<td>Texture change</td>
</tr>
<tr>
<td></td>
<td>9 (18)</td>
<td></td>
<td>6 (12)</td>
</tr>
</tbody>
</table>
<pre><code>                                                             | Has no idea                                     |
                                                             | 10 (18.8)                                       |
</code></pre>
4.4 Sources of traders’ merchandise and factors considered during purchase

Traders mainly get the foods and feeds from the feed stores, farmers and the suppliers (middle men). A large proportion of traders 45.60 % (47) obtained their goods from the suppliers (middle men) while 22.30 % (23) got their goods from the farmers (Table 4.4).

<table>
<thead>
<tr>
<th>Source</th>
<th>Frequency (N = 103)</th>
<th>Percentage N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suppliers</td>
<td>47.00</td>
<td>45.60</td>
</tr>
<tr>
<td>The farmers</td>
<td>23.00</td>
<td>22.30</td>
</tr>
<tr>
<td>Store outlets</td>
<td>33.00</td>
<td>32.00</td>
</tr>
</tbody>
</table>

In purchasing the foods and feeds, quality, price and demand were traders’ major driving force during purchase, a large proportion of the traders 64.10 % (66) considered quality, 17.50 % (18) considered price of the commodity while 9.70 % (10) consider demand as first determining factor. Others factors given first priority during purchase are nutrition value 2.90 % (3) and type of breed 2.90 % (3) (Table 4.5).
Table 4.5: Factors considered by the traders when purchasing foods and feeds

<table>
<thead>
<tr>
<th>Factor</th>
<th>1st priority</th>
<th>2nd priority</th>
<th>3rd priority</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency</td>
<td>%</td>
<td>Frequency</td>
</tr>
<tr>
<td>Quality</td>
<td>66.00</td>
<td>64.10</td>
<td>19.00</td>
</tr>
<tr>
<td>Price</td>
<td>18.00</td>
<td>17.10</td>
<td>30.00</td>
</tr>
<tr>
<td>Demand</td>
<td>10.00</td>
<td>9.70</td>
<td>14.00</td>
</tr>
<tr>
<td>Nutrition value</td>
<td>3.00</td>
<td>2.90</td>
<td>3.00</td>
</tr>
<tr>
<td>Type of breed</td>
<td>3.00</td>
<td>2.90</td>
<td>5.00</td>
</tr>
<tr>
<td>Availability</td>
<td>-</td>
<td>-</td>
<td>12.00</td>
</tr>
</tbody>
</table>
4.5 Levels of aflatoxins in human foods and cattle feed

4.5.1 Aflatoxins in human foods

Three types of human foods, (Maize, rice and groundnuts) both processed and non-processed were sampled. Most of these foods recorded high aflatoxin mean levels above the recommended maximum residue limit (20ppb) by WHO/FDA/KEBS standards. Mean aflatoxin level in non-processed maize (49.70±14.20), processed maize (101.20±21.30), non-processed rice (38.20±10.50), processed rice (63.90±14.50), non-processed groundnuts (54.60±14.80), and processed groundnuts (120.90±27.20). Levels of Aflatoxin detected from maize, rice and groundnuts were compared. The findings indicated that, higher levels of aflatoxins were detected in groundnuts than in maize and rice (F = 1.98, df = 2, P = 0.144). (Appendix VII; Table 4.6). Hypothesis rejected, foods and feeds in Nairobi County had traces of aflatoxins.

4.5.2 Comparison of aflatoxin levels between processed and non-processed human foods

Levels of aflatoxin in processed maize (101.20 ± 21.30 ppb) were significantly higher than that in the non processed maize (49.70 ± 14.70 ppb). Using paired sample T – test to compare aflatoxin level in processed maize to that of non processed maize, the result showed that, there was no significant differences in aflatoxin levels ( T = 2.02, P = 0.054). Aflatoxin levels in processed rice (63.90 ± 14.50 ppb) were slightly higher than that in the non processed rice (38.20 ± 10.50 ppb), but with no significant difference (T = 1.43, P = 0.163). Level of aflatoxin
in processed groundnuts (120.90 ± 27.20 ppb) was higher than that in the non processed groundnuts (54.60 ± 14.80 ppb). In groundnuts, the result showed that there was a significant difference in aflatoxin levels (T = 2.14, \( P = 0.043 \)). Generally, when the levels of aflatoxin detected in all processed foods were compared to the levels in all non processed foods using a paired T-test, the results showed that, there was a significant difference (T = 3.36, \( P = 0.002 \)). Higher levels of aflatoxin were detected in processed foods (95.30 ± 12.70ppb) than in non processed foods (47.50 ± 7.60 ppb) (Appendix VII; Table 4.6).
Table 4.6: Aflatoxin levels (ppb) in processed and non processed human foods and cattle feed in Nairobi County, Kenya

<table>
<thead>
<tr>
<th></th>
<th>Maize</th>
<th>Rice</th>
<th>Groundnuts</th>
<th>Cattle dairy meals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non processed</td>
<td>Processed</td>
<td>Non processed</td>
<td>Processed</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>49.70</td>
<td>101.20</td>
<td>38.20</td>
<td>63.90</td>
</tr>
<tr>
<td><strong>Std Error</strong></td>
<td>14.20</td>
<td>21.30</td>
<td>10.50</td>
<td>14.50</td>
</tr>
<tr>
<td><strong>T – Value</strong></td>
<td>2.02</td>
<td>1.43</td>
<td>1.43</td>
<td>1.24</td>
</tr>
<tr>
<td><strong>P - Value</strong></td>
<td>0.054</td>
<td>0.163</td>
<td>0.043*</td>
<td>0.223</td>
</tr>
</tbody>
</table>

NB * Indicates mean values are significantly different at $P \leq 0.05$
4.5.3 Comparison of aflatoxin levels between cattle dairy meals (brand A and B) and non-processed maize

In both feeds and maize the mean level of aflatoxin was higher than the WHO/FDA recommended residue limits of 20 ppb. Aflatoxin levels in brand A (77.60 ± 16.00 ppb) however, was slightly higher than that in brand B dairy meal (48.60 ± 12.00 ppb). Aflatoxin levels in brand A and brand B cattle feeds were compared using two-sample T-test. The result showed that, there was no significant difference in aflatoxin levels (T = 1.45, P = 0.153) Table 4.6. Using a two sample t-test to compare the levels of aflatoxin in non-processed maize to dairy meal brand A and to brand B, results indicated that there was no significant differences (t = 1.24, P = 0.223) and (t = 0.06, P = 0.954) respectively (Appendix VII). A comparison between levels of aflatoxin between human foods and cattle feeds showed that the levels of aflatoxin in Human food (71.40 ± 7.80ppb) were slightly higher than the levels of aflatoxin in cattle feeds (63.60 ± 10.00 ppb). A two sample t-test showed that there was no significant difference (T = 0.61, P = 0.546 4.2) (Appendix VII).

4.5.4 Comparison of the aflatoxin levels in human foods from selected open air markets (Toi, Kawangware, Uhuru, Kangemi, and Nyamakima)

4.5.4.1 Processed and non-processed maize

The levels of aflatoxin nono-processed maize in Toi market (113.64 ppb) were slightly higher than in the all the other markets. The highest level of aflatoxin in processed maize was recorded in Uhuru market (145.23 ppb). Levels of aflatoxin
in non processed maize was not significantly different in the markets (F = 1.55, df = 4, P = 0.256). The levels of aflatoxin in processed maize in the various markets was not significantly different (F = 0.59, df = 4, P = 0.677) (Table 4.7).

4.5.4.2 Processed and non-processed rice

The highest level of aflatoxin in non-processed rice was recorded in Uhuru market (84.50 ppb). The levels of aflatoxin in non-processed rice in the various markets was not significantly different (F = 2.32, df = 4, P = 0.121). The highest level of aflatoxin in processed rice was recorded in Kangemi market (96.71 ppb). The levels of aflatoxin in processed rice in the various markets was not significantly different (F = 1.37, df = 4, P = 0.307) (Table 4.7).

4.5.4.3 Processed and non-processed groundnuts

Toi market levels of aflatoxin in non-processed groundnuts (104.17 ppb) was slightly higher than in the all the other markets. Levels of the aflatoxin in non-processed groundnuts was not significantly different in the markets (F = 1.00, df = 4, P = 0.446). The highest level of aflatoxin in processed groundnut was recorded in Toi market (234.00 ppb). Levels of aflatoxin in processed groundnuts in the various markets was significantly different (F = 3.30, df = 4, P = 0.052) Table 4.7. Hypothesis rejected; High levels of aflatoxins were detected in processed foods and feeds than in non processed foods and feeds.
4.7: Levels of aflatoxin in non-processed and processed human foods from selected open air markets in Nairobi County, Kenya

<table>
<thead>
<tr>
<th>Market</th>
<th>Maize Non-processed</th>
<th>Maize Processed</th>
<th>Rice Non-processed</th>
<th>Rice Processed</th>
<th>Groundnuts Non-processed</th>
<th>Groundnuts Processed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>S.E</td>
<td>Mean</td>
<td>S.E</td>
<td>Mean</td>
<td>S.E</td>
</tr>
<tr>
<td>Uhuru</td>
<td>15.61*</td>
<td>5.77</td>
<td>145.2</td>
<td>31.20</td>
<td>84.50</td>
<td>36.00</td>
</tr>
<tr>
<td>Kangemi</td>
<td>35.70</td>
<td>26.50</td>
<td>53.60</td>
<td>40.70</td>
<td>18.25*</td>
<td>4.57</td>
</tr>
<tr>
<td>Kawangware</td>
<td>33.60</td>
<td>18.90</td>
<td>100.00</td>
<td>48.20</td>
<td>16.21*</td>
<td>1.95</td>
</tr>
<tr>
<td>Nyamakima</td>
<td>54.40</td>
<td>31.20</td>
<td>70.70</td>
<td>56.50</td>
<td>54.30</td>
<td>23.20</td>
</tr>
<tr>
<td>Toi</td>
<td>113.64</td>
<td>48.80</td>
<td>136.70</td>
<td>66.50</td>
<td>10.10</td>
<td>2.03</td>
</tr>
</tbody>
</table>

* Safe aflatoxin level (below 20 ppb)
4.6 Prevalence of aflatoxins in foods and feeds in Nairobi County, Kenya

The prevalence of aflatoxins in human foods sold in the open markets and feeds in AGROVET stores were calculated based on the number of samples having aflatoxin levels above the maximum residue limit of 20 ppb as recommended by KEBS and FDA. For each of the food stuff and feed category, the prevalence is as shown in table 4.8.

4.7: Prevalence of aflatoxins in the foods and feeds in Nairobi County, Kenya.

<table>
<thead>
<tr>
<th>Foods</th>
<th>Maize</th>
<th>Rice</th>
<th>Groundnuts</th>
<th>Brand A</th>
<th>Brand B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N-P</td>
<td>N-P</td>
<td>N-P</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>50%</td>
<td>56.25%</td>
<td>43.75%</td>
<td>62.5%</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75%</td>
<td>51.85%</td>
<td>60%</td>
<td></td>
</tr>
</tbody>
</table>

N-P: Non processed  P: Processed
CHAPTER 5: DISCUSSION

5.1 Conditions in which foods and feeds were stored in Nairobi County

Storage of foods in open air markets was unsatisfactory. For instance packing and storing grains on raised platform covered with polythene paper under temporary shade overnight, is a method that was practiced by food traders that encourages moisture accumulation. Unlike food traders, majority of feed traders stored their feeds in cool dry place (store), in bags and on raised wooden pellets. This is consistent with the findings by Strosnider et al. (2006) who reported that it is advisable to keep the grains from contact with the earth, raising them on wooden pallets or on concrete floor and ensuring adequate ventilation in a storage facility helps to prevent an increase in moisture content, insect and rodent infestation during storage, and is a critical measure against aflatoxin contamination. Displaying of unpacked grains foods in the open during the day was common practice among food traders that exposes the food to fungi colonization. This is consistent with findings by Lunyasunya et al. (2005) who reported that majority of the farmers do not protect their maize stovers against scorching sunshine, termites and pests damage and more importantly mould colonization.

High temperature and moisture content were the main factors contributing to aflatoxin contamination. Although some traders employed the correct practices of eliminating these elements during storage, others did little to prevent moisture acquisition during storage. The findings by Hawkins et al. (2005) confirms that
high levels of humidity, temperature and poor aeration during storage are important factors that may contribute to aflatoxin contamination. In another study, Smith et al. (1985) further emphasizes that aflatoxin contamination can occur when food commodities are stored under high moisture and temperature conditions.

Nylon sacks were widely used for storage of grains and feeds as compared to sisal sacks. Storage of foods in nylon bags in relation to aflatoxin contamination in the previous studies was found to be unsatisfactory. Mwihia et al. 2008 in his study on aflatoxins level on locally grown maize from Makueni County noted that nylon sacks were widely used as grain storage containers (89%). He reported that these sacks maintain moisture, are impervious to free air circulation within the grain store and may promote aflatoxin contamination. He also noted that their wide spread use could be attributed to their cheap cost and easy availability than the sisal sacks which are known to keep minimal moisture content hence reduce aflatoxin contamination which were used by only 10%.

5.2 Effects of aflatoxin in foods and feeds and their impact on human and animal health

Change of colour on food and feeds could be attributed to the presence of *A. flavus* and *A. parasiticus* whose colonies on substrate appear yellow–green and yellow respectively. This is consistent with past studies by Maren (2002) who reported that *A. flavus* spread yellow-green colonies and *A. parasiticus* dark yellow green
canidial areas. She further noted that bad smell and decaying could be due to activities of *Aspergillus* fungi which normally lead to food and feed decay, and that members of *Aspergillus* genus are more heat tolerant and xerophilic than other fungal genera, therefore are very common food and feed spoilage organisms.

The low awareness of traders on the harmful effects of aflatoxins to human and animal health may be an indication of poor public awareness. This is supported by the results obtained in seminar and workshop attendance; whereby few traders (24%) who had attended some training related to handling and storage of foods and feeds were more aware of aflatoxin and applied knowledge on prevention of aflatoxin contamination. Currently no literature has been published on awareness level of aflatoxins contamination among food and feed handler; however, much has been reported on its toxicity effects on human and animal health by many researchers. For instance, Pier (1992) reported that aflatoxin poisoning in dairy cattle produce a wide range of harmful effect such as decrease in feed utilization, reduced appetite, liver and kidney damage, gastrointestinal dysfunction, reduced milk production anemia and death.

Both food and feed traders in open markets and in AGROVET stores respectively reported of suffering from other health hazards when handling food and animal feeds such as allergies, coughing, sneezing, breathing problems and other chest
complications. This is consistent with findings by Lunyasunya et al. (2005) who reported that handling of mould infested feeds, hay stovers, silage and other feeds may be harmful due to the presence of mycotoxins and actinomycetes which are responsible not only for poisoning but also for allergy diseases affecting man known as ‘farmers lung’ disease. This is particularly serious in asthmatics and patients suffering from cystic fibrosis.

5.3 Aflatoxin levels in foods in selected open air markets and feeds in AGROVET stores

The studied 96 food samples processed and non-processed contain aflatoxin levels above 20ppb the maximum residue limit. This finding confirms similar findings by Muture et al. (2005) in his study of aflatoxin levels in maize and maize products in Makueni County of Kenya. He found out that over half of the samples did not comply; they had aflatoxins levels greater than the Kenya regulatory limit (20ppb) and therefore would be regarded as unfit for human consumption. Similar findings were established by Lewis et al. (2005) where 55 % of maize products sampled during 2004 aflatoxicosis outbreak had aflatoxins levels greater than Kenya regulatory limit of 20 ppb. Lunyasunya et al. (2005), reporting on a case of an out break of jaundice with high Case fatality Rate (CFR) in Makueni and Kitui County, out of the 342 maize samples collected, a total of 182 (53.2%) had aflatoxin level above the recommended maximum limit of 20ppb.
Groundnuts contained high levels of aflatoxin than maize and rice. This is in consistent with findings of Bankole et al. (2004) in Nigeria who reported that groundnuts indeed harbour high concentrations of aflatoxins. He analyzed 106 dry roasted groundnuts of 500g each for moisture content and aflatoxins. The result indicated that most samples were contaminated with aflatoxins, Aflatoxin B₁ was found in 64.2% of the samples and B₂, G₁ and G₂ occurred in 24.6%, 11.3% and 2.8% respectively.

The high mean level of aflatoxin in foods and the poor storage practices among food traders in open air markets may be attributed to low level of awareness of aflatoxin contamination among food traders whereby only one third of the traders were aware of aflatoxin contamination. The storage contrast among food and feed traders can be attributed to their level of awareness of aflatoxin contamination, whereby aflatoxin awareness was found to be significantly high among cattle feed traders compared to food traders. Therefore, low level of awareness and poor storage practices may have been the major contributing factors to aflatoxin contamination.

Food traders in open markets obtained their grains from suppliers (middle men). If these suppliers were also not conversant with aflatoxin contamination, then they may serve as a source of aflatoxin contamination from one region of Kenya to another. This confirms findings by Lewis et al. (2005) who noted that, there is a
possibility that contaminated grains from affected areas of Makueni County may enter the distribution system through migrant traders and then to the markets resulting in widespread aflatoxin contamination of market grains. He further reported that contaminated grains sold in the market act as a source of continuous exposure of the consumer to aflatoxin contamination.

All the 54 feed samples obtained from AGROVET stores had traces of aflatoxins, whose levels exceeded FDA maximum residue limit for aflatoxins in feeds for dairy cattle (20ppb). This confirms findings by Kang’ethe et al. (2007) who reported that out of 70 feeds obtained from farmers, 98.6% had traces of aflatoxins B₁ while 83% (58) had aflatoxin levels equal or above 0.01mg Kg⁻¹ the Kenya Bureau of Standards. In another study, Jacques (1988) reported that most cases of animal poisoning by aflatoxin can be traced to the growth of fungi in poorly handled feeds.

Kenya is a large milk producer in East Africa with projected milk production capacity of 3885 million liters per year and a consumption of 76.7% capital per year (Speedy, 2003). Milk is highly consumed by a large population of residents of Nairobi and the surrounding environments. Nairobi County is the source of the feeds used in the surrounding regions. In the suburbs of the city such as Ongata Rongai, Ngong, Ruai, and surrounding counties, there are individuals who keep cattle for milk production for domestic use and for commercial purposes. These
individuals depend on AGROVET stores within Nairobi for their cattle feed supply. If these feeds are contaminated then this may pose risks of aflatoxin poisoning to the animals and eventually man.

5.4 Levels of aflatoxins in processed and non-processed foods and cattle feed

Foods and feeds samples collected from open air markets and AGROVET stores respectively, showed levels of aflatoxins higher than recommended in processed foods and feeds. Contamination may have occurred before or after processing since Aspergillus spores may be present in the air in a contaminated environment (Lunyasunya et al., 2005). Aflatoxin levels were higher in processed than non-processed foods and feeds which would be attributed to the fact that processed food and feeds tend to form suitable substrate with easily absorbable nutrients. This is consistent with findings by Fox et al. (2008) who noted that mycotoxins greatly resist decomposition and being broken down by digestion. They remain in the food products, even temperature treatment such as cooking and freezing do not destroy mycotoxins.

This is further confirmed by Yousef et al. (1989) reported that aflatoxin M₁ is relatively stable in raw milk and is unaffected by pasteurization or processing into cheese or yoghurt. This means that contaminated milk will give contaminated milk products. In another study, Dada et al. (1983) confirmed that processing aflatoxin contaminated grain yields contaminated product in a case where she
used *A. flavus*- contaminated sorghum, for making *ogi* porridge (fermented sorghum made into porridge) 30% of aflatoxin B₁ was found in *ogi* porridge. In contrast Turner *et al.* (2002) reported that cooking or boiling which is the commonest method of preparing maize-based foods, a staple diet with high aflatoxin exposure can reduce aflatoxin to a limited extent. However, Bankole *et al.* (2004) warns that breakdown of aflatoxins by various means does not guarantee safety of the contaminated substance. At times, this breakdown is reversible and may lead to another form of aflatoxin. Beside, reaction products have not been subjected to detailed examination.

The purpose of assessing aflatoxin contamination in cattle feeds sold in AGROVETS and foods sold in open markets within Nairobi County was to highlight the existing danger of aflatoxin contamination of these foods and feeds which possibly leads to animal and human poisoning in the urban population. Therefore, creating awareness of aflatoxins in human food and cattle feed traders will go along way in improving public health through improvement of quality and hygiene.
CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

i. Fifty six percent of food and feeds traders were aware of aflatoxin. However, awareness of aflatoxin contamination was high among cattle feed traders (83.3 %) than food traders (34 %).

ii. The results showed that within open air markets in Nairobi County, foods were stored in unsatisfactory conditions while feeds were stored in fairly good conditions.

iii. Foods: Maize (49.7±14.2), rice (38.2±10.5) and groundnuts (56.6±14.8); and feeds (63.6±10) were found to have significantly high mean level of aflatoxin above the recommended level by FDA and KEBS (20ppb). However, groundnuts had highest aflatoxin levels (54.6±14.8) followed by maize and rice in that order.

iv. Higher mean levels of aflatoxins were associated with processed foods (95.3±12.7ppb) than with non processed foods (47.5±7.6ppb).

v. Although good proportion of traders were aware of aflatoxin contamination, a significant proportion of food traders (96.3 %) and feeds
traders (92%) were not aware of the harmful effects of aflatoxins in foods and feeds respectively.

vi. Majority (42.7%) of the traders were secondary leavers. A large proportion (76.7%) of the traders had not attended any form of training in form of workshops or seminar on handling and storage of feeds and foods. Training creates awareness of aflatoxin contamination.

vii. Prevalence of aflatoxins in foods within open air markets and feeds in AGROVETS stores was; Non processed maize (50%), rice (43.75%), groundnuts (50%) and processed maize (56.25%), rice (62.50%), groundnuts (75%) and feeds (55.93%).

6.2 Recommendations

According to the findings of this study the following recommendations were drawn:

i. There is need to campaign for seminars and workshops by stockholders such as public health and ministry of Agriculture to create awareness among food and feed handlers against poor practices that contribute to aflatoxin contamination.
ii. There is need for better food storage facilities within the open air markets. The county government should also improve the existing building structures within the markets and give proper guidelines in regard to storage.

iii. There is need to look for mechanisms of assessing foods that which enter in the markets in regard to contamination so as to help curb spread of aflatoxin from one region to another.

iv. Long term solutions to food and feeds contamination should include strengthening nationwide surveillance, increase food inspection in market area and feeds in AGROVET stores to ensure their safety.

v. The study recommends that more research to be done on other foods sold in open air market like beans, sorghum, finger millet, milk and spices etc.

vi. It is important for a research to be done to verify whether there is a relationship between source of food and aflatoxin contamination.

vii. Studies should be done to verify whether there is a relationship between current rising cases of liver cancer and aflatoxin contamination.
REFERENCES


**Smith, J. E. and Moss M. O. (1985).** Mycotoxins formation, analysis and significance, John Wiley and Son Ltd, Chichester, Great Britain p.148


factor for primary liver cancer: a three year study of corn harvested in Haiman China by H. P. C. C. and ELISA. *Food Chemical Toxicology*, **35**: 1143-1150.


APPENDICES

Appendix I: Questionnaire used to assess traders’ awareness of aflatoxin contamination in foods and feeds

1. What is your level of education?
   - Primary education
   - Secondary education
   - College level
   - Other (specify)__________________________

2. What types of foods and feeds do you trade in?
   - Foods
   - Feeds

3. Where do you purchase the foods and feeds (farmers /suppliers/store outlets)?
   - Foods
   - Feeds
4. What factors do you take into account in purchasing the foods and feeds?

Arrange in order of significance.

i) 

ii) 

iii) 

iv) 

5. How much do you purchase in:

   Day (kg) 

   Week (kg) 

   Month (kg) 

   Year (kg) 

6. i) Do you know what aflatoxin is?

   a. Yes 
   
   b. No 

   ii) If yes, what are the effects of aflatoxins in foods and feeds?
7. i) Have you attended any training related to handling and storage of foods and feeds?

Yes

No

(ii) If yes, specify the type of training (workshop or seminar) ______________

(iii) How do you store your foods and feeds so as to prevent aflatoxin contamination during storage?

(iv) What encourages the contamination of aflatoxins in foods and feeds?

8. List any health hazard associate with grains and animal feeds storage.

i)

ii)

iii)

iv)
Appendix II: Table used in recording sample details

The following categories of foods were sampled: non-processed (maize grains, unpolished rice and groundnuts). A total of 96 human food samples were collected from the open markets.

<table>
<thead>
<tr>
<th>Store outlet/ Open markets</th>
<th>Sample no.</th>
<th>Type of sample</th>
<th>Quantity of sample</th>
<th>Level of aflatoxin concentration</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>2</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kawangware</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kangemi</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Nyamakima</td>
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<tr>
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</tr>
<tr>
<td></td>
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<tr>
<td></td>
<td>3</td>
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</table>
### Appendix III: Table of random numbers

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<td>131</td>
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Select a row from 1 to 40 and a column from 1 to 12. Use the number at the intersection of the selected row and column.

Source: Soil Conservation Surveys Guidebook (Forest Practices code of British Columbia Act, 2001)
Appendix IV: Foods displayed in open air market in Nairobi County, Kenya 2012
Appendix V: Foods stored under polythene paper overnight in a food open air market in Nairobi County, Kenya 2012
Appendix VI: Storage of feeds in an AGROVET store in Nairobi County, Kenya 2012
Appendix VII: Statistical and Data analysis

(a) One-way ANOVA: Aflatoxin levels in various Foods

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<th>MS</th>
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Individual 95% CIs For Mean Based on Pooled StDev

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<tr>
<th>Level</th>
<th>N</th>
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<td>Groundnuts</td>
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<td>87.75</td>
<td>92.49</td>
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<td>75.80</td>
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Pooled StDev = 75.19

(b) Paired T-Test and CI: Non processed, Processed

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<td>Non -processed</td>
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<td>48</td>
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95% CI for mean difference: (-76.5, -19.2)
T-Test of mean difference = 0 (vs not = 0): T-Value = -3.36 P-Value = 0.002
(c) Two-Sample T-Test and CI: food, feeds

Two-sample T for food vs feeds

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Difference = mu food - mu feeds
Estimate for difference: 7.8
95% CI for difference: (-17.7, 33.2)
T-Test of difference = 0 (vs not =) : T-Value = 0.61 P-Value = 0.546 DF = 107

(d) Two-Sample T-Test and CI: Non -P. Maize, brand B

Two-sample T for non p. maize vs brand B

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Difference = mu Non- P. Maize - mu brand B
Estimate for difference: 1.1
95% CI for difference: (-36.7, 38.8)
T-Test of difference = 0 (vs not =): T-Value = 0.06 P-Value = 0.954 DF = 33

(e) Two-Sample T-Test and CI: Non P. maize, brand A

Two-sample T for non p. maize vs brand A

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Difference = mu Non- P. Maize - mu brand A
Estimate for difference: -27.0
95% CI for difference: (-71.2, 17.1)
T-Test of difference = 0 (vs not =): T-Value = -1.24 P-Value = 0.223 DF = 39
(f) Two-Sample T-Test and CI: Non-processed maize, processed maize

Two-sample T for Non processed vs Processed

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Difference = mu Non-Processed maize - mu Processed maize
Estimate for difference: -51.5
95% CI for difference: (-104.1, 1.0)
T-Test of difference = 0 (vs not =): T-Value = -2.02 P-Value = 0.054 DF = 26

(g) Two-Sample T-Test and CI: Non processed rice Processed rice

Two-sample T for Non processed vs Processed

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Difference = mu Non-Processed rice - mu Processed rice
Estimate for difference: -25.7
95% CI for difference: (-62.5, 11.1)
T-Test of difference = 0 (vs not =): T-Value = -1.43 P-Value = 0.163 DF = 27
(h) Two-Sample T-Test and CI: Non-processed groundnut, processed groundnuts

Two-sample T for Non-processed vs processed

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Difference = mu Non-Processed groundnut - mu Processed groundnut
Estimate for difference:  -66.4
95% CI for difference: (-130.4, -2.3)
T-Test of difference = 0 (vs not =): T-Value = -2.14 P-Value = 0.043 DF = 23