
Addis Ababa University
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Declaration

I, the undersigned, declare that this research thesis is my original work and that all sources of materials used for the thesis have been correctly acknowledged.

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List of Abbreviations and Acronyms

AF- Aflatoxin

AFB1-Aflatoxin B1

AFB2- Aflatoxin B2

AFG1- Aflatoxin G1

AFG2- Aflatoxin G2

AFM1- Aflatoxin M1

AOAC- Association of Official Analytical Chemists

ARSO- East African Regional standard organization

EFSA -European Food Safety Authority

ELISA-Enzyme Linked Immunosorbent Assay

EU-European Union

FAO- Food and Agricultural Organization

FDA- US Food and drug Administration

Kg - Kilogram(s)

L - Liter(s)

Ml - Milliliter

Min -Minute

MRL – maximum residue level

FEHD- Food and Environmental Hygiene Department

HKSAR- Hong Kong special administrative region

HPLC- FLD-High performance Liquid chromatography coupled with florescence Detector

IARC-International Agency for Research on cancer

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Abstract

Milk is a key contributor to improve nutrition and food security particularly in developing countries; moreover these products are widely consumed by children. Milk has the greatest demonstrated potential for introducing AFM1 into the human diet and the possible presence of AFM1 in milk is turn out to be critical concern of the country. Aflatoxin M1 (AFM1) is a major carcinogenic compound that may be found in milk and dairy product resulting from ingestion of aflatoxin B1 by dairy animals. This study compare the intensity of Aflatoxin M1 contamination on milk from grazing and non- grazing cows collected from four potential milk source of the country, Debrbrihan, Sululta and Bishoftu and Addis Ababa which is a major market. A total of 21 samples from grazing and non grazing cows were evaluated for their Aflatoxin (AFM1). The main analytical technique implemented for aflatoxin analysis was Immunoaffinity column sample clean-up and Shimadzu High Performance Liquid Chromatography (HPLC) using fluorescent as a detector. Questionnaire also implemented to assess the knowledge, attitude and practice (KAP) aspect of the participants. Results revealed that, Aflatoxin level of the milk samples from grazing cows, in sululta Debereberhan and Bishoftu towns were in the range of (0.22-1.02) , (0-4.99) and (0-7.57) respectively. The Aflatoxin level from the non-grazing cows ranged from (14.2-28.96) Sululta, (3.28-42.87) Debereberhan and (3.21-43.47)in Bishoftu. The milk samples that were collected from Addis Ababa city had ranged from aflatoxin concentration of (2.34-76.07). One way anova results indicate that the mean value of cow's that were grazing is significantly different ($P<0.05$) from the milk samples of cow's that were non-grazing and milk collected from Addis Ababa.KAP results indicated negative relation between knowledge and aflatoxin concentration whereas practice had positive relation. These results suggest that mitigation should focus on type of feed and feed handling. Furthermore comprehensive and well-integrated approach needs to follow the value chain actors to manage aflatoxin risks and to pull aflatoxins out of human food chains.

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Key Words: milk, Aflatoxin, grazing, non grazing, Immunoaffinity column, HPLC, value chain

1. Introduction

1.1.Background

Milk is the most important source of calcium and phosphorus of human body and due to having essential amino acids, has an important status in supplying the body's protein needs. Studies have shown that there is a close relationship between consumption of milk and health status of people in terms of efficiency, Intelligence quotient (IQ), reducing the risk of infectious diseases, regulation of metabolic activities, decreasing blood pressure, increasing beneficial blood lipids (High-density lipoprotein), preventing from colon cancer and osteoporosis (Hjartaker., 2002). Milk is a key contributor to improve nutrition and food security particularly in developing countries and play a significant role in reducing poverty and malnutrition (Darsana Barnouin, 2013). However milk, as a liquid, is a highly variable product that rapidly loses its quality and spoils if not to be treated.

Mycotoxins are toxic secondary metabolites naturally produced by molds (*Aspergillus, Fusariumand Penicillium spp*.) (Asrjets, 2013) and are known to cause toxicities to humans and animals (Shetty, Trends Food SciTechnol,food principles, 2009). Aflatoxins are among the group of important mycotoxins which cause sub-acute and chronic effects such as liver cancer, chronic hepatitis, jaundice, hepatomegaly and cirrhosis in humans (Dorner, 2013). The toxin-producing mold was identified as Aspergillusflavus in 1960 and hence the toxin was given the name Aflatoxin by virtue of its origin (*A. flavus*→*Afla*). The moulds Aspergillusflavus and A. parasiticus produce exclusively aflatoxin B1, B2, G1 and G2, and all the other aflatoxins (ochratoxins, trichothecenes, zearalenone, fumonisins, tremorgenic toxins and ergot alkaloids) are derivates of these four (Dorner, 2013).

Milk has the greatest potential for introducing AFM1 into the human diet and the possible presence of AFM1 in milk and their products represents a worldwide concern, mainly because the major consumers are children, who are more susceptible to immunosuppressive, mutagenic, teratogenic, and carcinogenic effects (Sefidgar et al, 2011). The source of AFM1 in milk has

been reported to be Aflatoxin B1 (AFB1) present in feed of lactating animals, which gets transformed to 4-hydroxylated metabolite in liver and is excreted in milk.

The contamination of milk with AFM1 has been the interest of many researchers and there are many studies conducted in different countries. A recent study conducted in Ethiopia show that milk and dairy feeds in the Greater Addis Ababa milk shed are highly contaminated with aflatoxins in the range between 0.028 and 4.98 mg/L (Gizachew et al, 2016). However the method of detection in the study had its own limitations, as it used a relatively less sensitive method. Moreover the AFM1 level in milk from grass fed cows was not quantified. This study therefore aims to determine and compare the intensity of AFM1 contamination in milk samples from dairy farmers and dairy processors by using HPLC, which is a highly sensitive method of detection of aflatoxin. For the purpose of this study the cows of house hold dairy farmers are mostly grass fed, whereas cows of dairy processors depend on feed source such as mixed concentrate feed.

1.2.Problem Statement

Ethiopia is a country with a relatively favourable climate for breeding dairy cattle and that enables the country to have a substantial potential for dairy development. The milk production is dominated by smallholders' dairy farmers who contribute the major share of the gross marketed milk production. Natural pasture (grazing) and crop residues are the major feed resources used as feed for dairy production by these small dairy farmers. Non-conventional feed resources such as *atella* (beer left over) are also utilized by some dairy producers to supplement dairy animals. On the other hand, urban milk producers and dairy processors depend on agro-industrial byproducts such as concentrate cereal bran feeds and oil cakes (noug, cotton, linseed, safflower and sesame). In this respect, contamination of dairy cattle feed with AFB1 is of concern as it can be transferred into milk, where it appears as aflatoxin M1 (AFM1). A recent study that was done in aflatoxin contamination of milk and feeds in the greater Addis Ababa reveled that all feed and milk samples tested had aflatoxin concentration exceeding the limits of European Union. The study was conducted on agro-industrial byproducts used as feeds such as wheat bran, noug (*Guizotiaabyssinica*or Niger seed) cake, pea hulls, maize grain and brewer's dry yeast by using commercial enzyme-linked immunosorbent assay kits. The study had its limitations as it excluded milk samples from cows that were grazing. More over the use of ELISA immunoassay

can be justified by the fact that it is affordable, simple and easy to use and does not need expensive equipment such as liquid chromatography. However, several researchers mostly from developed countries combined Imunoaffinity column and Liquid Chromatography for specificity and confirmation of resultsInvalid source specified..

Considering the fact that milk consumption of the Ethiopian population is increasing and the Ethiopian dairy industry is growing, it becomes important to have an understanding about the extent of AFM1 levels in milk so as to prevent public health risks. Moreover no published data is available that assesses the knowledge, attitude and practice of house hold dairy farmers and dairy processors regarding aflatoxin. Therefore, this particular research will put effort to assess the knowledge, attitude and practice regarding aflatoxin in hold dairy farmers and dairy processors. A highly sensitive method of detection of AFM1 will be used to quantify and compare the intensity of Aflatoxin M_1 on milk samples from house hold dairy farmers and dairy processors.

1.3.Significance of the study

The study aim to indicate the multifaceted nature of aflatoxin contamination leads to holistic approach that will be mitigating the risk of human and animal exposure to this toxin. The resulting information will be valuable in planning mitigation strategies as part of an overall strategy to promote maternal and child health. It will also contribute to the reduction of healthcare costs, thus increasing the wellness and healthy life style of the consumers. Responsible bodies will use the information from the study to intervene and create awareness of aflatoxins and support risk mitigation practices along the entire dairy value chain. Policymakers and development organizations can use the outcome of the study to plan their policies and programs in the dairy sector.

1.4. Research Questions/hypotheses

1. Is there a difference in knowledge, attitude and practice regarding aflatoxin among house hold dairy farmers and dairy processors?
2. Is there a significant difference on the level of aflatoxin in milk depending on feed source and feeding approach?

1.5.Objective

1.5.1 General objective

- To assess the knowledge, attitude and practice regarding aflatoxin in milk in household dairy farmers and dairy processors
- To compare the intensity of Aflatoxin M_1contamination on milk samples from grazing and non- grazing cows in three milk producing areas and milk marketed in Addis Ababa.

1.5.2 Specific objectives

- To evaluate the intensity of aflatoxin M1 contamination on milk from grazing cows in Debrbrihan, Sululta and Bishoftu .
- To evaluate the intensity of aflatoxin M1 contamination on milk from non-grazing cows in Debrbrihan, Sululta and Bishoftu.
- To evaluate the intensity of aflatoxin M1 contamination on commercially marketed milk from Addis Ababa.
- To assess the knowledge and attitude and practices of milk farmers and processers to determine the factors for contamination of milk with Aflatoxin m1.

2. Literature review

2.1 Mycotoxins

In the 1960s more than 100,000 young turkeys on poultry farms in the United Kingdom died in a period of a few months from an unidentified disease, which was named "turkey disease". Ducklings and other poultry animals were also affected, and high mortalities were observed. A careful survey of the inputs and environment of the affected farms indicated that the disease was associated with feeds and specifically with peanut meal imported from Brazil. A disease with symptoms typical of turkey disease was reproduced when animals were fed the same peanut meal. Intensive investigations were then carried out on the suspected ingredient to identify the nature of the toxin, which was soon found to be of fungal origin. The toxin-producing fungus was identified as Aspergillusflavusand the toxin was accordingly called aflatoxin/Mycotoxin.

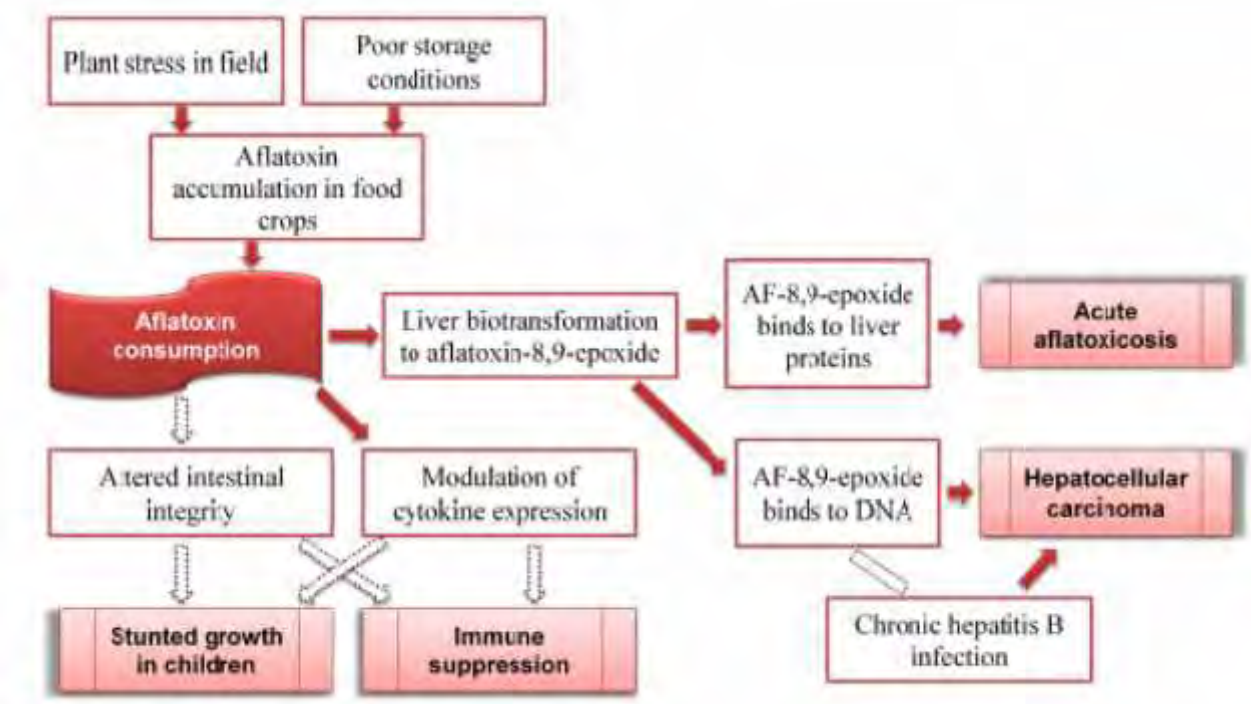
While all mycotoxins are of fungal origin, not all toxic compounds produced by fungi are called mycotoxins. The term Mycotoxin is derived from the Greek word 'mycos' meaning fungus(mould), and the Latin word 'toxicum', which means poison (Jouany et al, 2009). Mycotoxins are produced by fungi through their secondary metabolism. Mycotoxin concentration can therefore be independent of the growth of the fungi, which is associated with the primary metabolism. The diversity of the compounds formed and the specificity of the fungal strain for mycotoxin production result from the secondary metabolism, which is usually activated by signals from the environment (cold, heat, dryness, fungicide, etc.). Among the numerous mycotoxins, several groups have been identified, produced by the three major fungus genera Aspergillus, Penicillium and Fusarium .According to the same mycotoxin can be produced by several different fungi, and the same fungus can generate several mycotoxins (Jouany*et a*l., 2009).

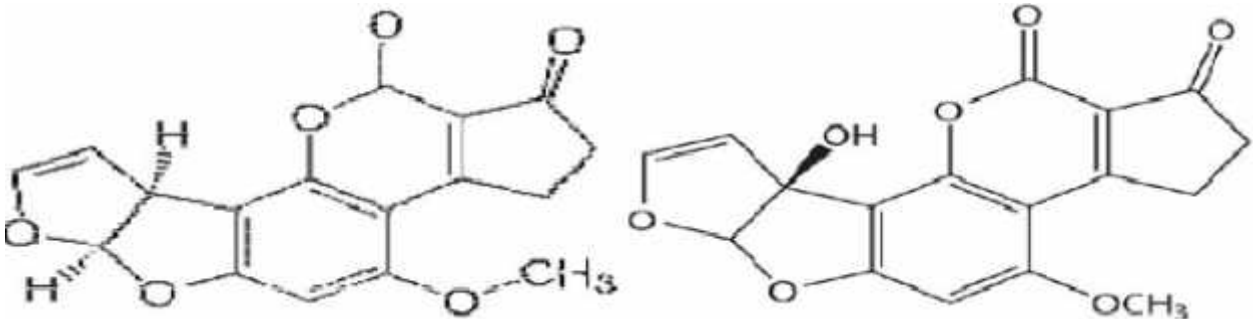
Mycotoxins present in food products and animal feeds are an important problem concerning food and feed safety and significant economic losses are associated with their impact on human and animal health (Shundo*et al*., 2009) In addition to being acutely toxic, some mycotoxins are now linked with the incidence of certain types of cancer and it is this aspect which has evoked global concern over feed and food safety, especially for milk and milk products.

2.2 Aflatoxins

Aflatoxins belong to the class of mycotoxins and it is a group of approximately 20 related fungal metabolites generally produced by Aspergillus species, namely A. flavus, A. parasiticus, A. ochraceoroseus, A. bombycis, A. nomius, A. fumigitus and A. pseudotamari (Soleimanyet al, 2007). These fungi belong to the class Hyphomycetes, subdivision Deuteromycotin and family Aspergillaceae (Mohammadi, 2011). Aspergillusflavus and A. parasiticus are economically important moulds that produce exclusively aflatoxin B1, B2, G1 and G2, and all the other aflatoxins are derivates of these four (Felicia et al.,2011,) . The four major naturally produced aflatoxins are known as B1, B2, G1, and G2. "B" and "G" refer to the blue and green fluorescent colors produced under UV light on thin layer chromatography plates, while the subscript numbers 1 and 2 indicate major and minor compounds, respectively (Felicia *et al*., 2011).

Aflatoxin B1, the most toxic of the aflatoxins, is the most potent naturally occurring chemical liver carcinogen known. Specific P450 enzymes in the liver metabolize aflatoxin into a reactive oxygen species (aflatoxin-8,9-epoxide), which may then bind to proteins and cause acute toxicity (aflatoxicosis) or to DNA and induce liver cancer (Khlangwiset, 2010) . Aspergillusflavus and A. parasiticus colonize a wide variety of food commodities including maize, oilseeds, spices, groundnuts, tree nuts, milk, and dried fruit (Bhat*et al*., 2006). Whether these fungi produce aflatoxin depends on drought stress and rainfall, suitability of crop genotype for its climate, insect damage, and agricultural practices (Khlangwiset, 2010). These fungi can also produce aflatoxin in "postharvest" conditions: storage, transportation, and food processing. Aflatoxin contamination is a particular problem in maize, oilseeds, spices, peanuts, tree nuts (almonds, pistachios, hazelnuts, pecans, Brazil nuts, and walnuts), milk (in the form of aflatoxin B1's metabolite aflatoxin M1), and dried fruit (Shephard, 2008). Maize and peanuts are the main sources of human exposure to aflatoxin because they are so highly consumed worldwide and unfortunately are also the most susceptible crops to aflatoxin contamination (Khlangwiset, 2010). Figure 1 depicts the pathway by which aflatoxin accumulates in food crops and contributes to various adverse human health effects.


Plant stress in field
Poor storage conditions
Aflatoxin accumulation in food crops
Aflatoxin consumption
Liver biotransformation to aflatoxin-8,9-epoxide
AF-8,9-epoxide binds to liver proteins
Acute aflatoxicosis
Altered intestinal integrity
Modulation of cytokine expression
AF-8,9-epoxide binds to DNA
Hepatocellular carcinoma
Stunted growth in children
Immune suppression
Chronic hepatitis B infection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OCH3

substance, AFM1 were found in in milk in concentrations of 1.5, 0.245, 13.7, 4.7, 12.4 and 20.2 mg/L respectively in high-yielding dairy cows producing up to 40 L of milk per day (Veldman*et al*., 1992). AFB1 and AFM1 had a strong correlation(r2 = 0.915), and he proposed the following equation to estimate the transfer of AFM1 in milk: AFM1 (ng/kg milk) = 10.95 + 0.787 × (µg AFB1 intake per day) This equation indicates that the animals must ingest less than 50 and 25 µg AFB1 per day to comply with the European regulatory levels of contamination in milk set at 0.05 and 0.025 µg/kg of milk for adults and infants, respectively. Thus cows must ingest less than 10 and 5 kg of feed contaminated at the maximum authorized level (5 µg AFB1/kg feed for dairy cattle) to maintain a safe level of AFM1 in milk.

2.4 Factors That Predispose Crops to Aflatoxin Contamination

Field and postharvest practices can predispose cropproduce to aflatoxin contamination. The risk of contamination is greater in developing countries where peasant farmers who constitute the majority face financial challenges and have little or no access to improved technology. The factors that influence mycotoxinproduction are either biological (biotic), environmental (abiotic) or nutritional (Diener and Davis, 1966; Okelloetal., 2010). Some of the biotic factors include cultivar susceptibility and growth stage, insect and bird damage and presence of other fungi or microbes and strain variation in the fungus while abiotic factors include mechanical damage, moisture, temperature, pH and other crop stresses such as drought, soil type, suitability of substrate, excessive rainfall, gaseous exchange andgaseous environment and preservatives and crowding of plants (CAST, 1989; CRA, 2011; Suttajit, 1989; Robens, 1990; William et al., 2004). Nitrogen stress is another biotic factor which can also predispose crops to aflatoxin contamination.

Most of the factors enumerated above are beyond the control of farmers in developing countries. For instance, unpredictable rainfall which is worsened by climate change makes crops grown in developing countries more prone to water stress and therefore a higher risk of aflatoxin contamination. Also, due to lack of access to improved technology, farmers in developing countries cannot test soils to determine their physicochemical characteristics before cropping.

2.5 Aflatoxin M1

AFM1 is a metabolite of Aflatoxin B1 (AFB1) and originally discovered in milk of humans and animals fed on moldy grains containing AFB1. With an intake of AFB1 for 2-60 mg / cow / day, AFM1 residues in milk could range between 1 and 50 ppb. There have been found differences between the amounts of AFM´s produced by different bovine species. The AFM1 distribution in some dairy foods made from contaminated milk is approximately: 40-60% in cheese, 10% in butterfat and <2% in buttermilk. AFM1 is highly soluble in water, so it is incomprehensible why this toxin is deposited in the cheese but not in the milk whey (Creppy, 2002). The carcinogenicity of AFM1 is about ten times less than that of AFB1, and for this reason has been included in the class 2B by International Agency for Research on Cancer.

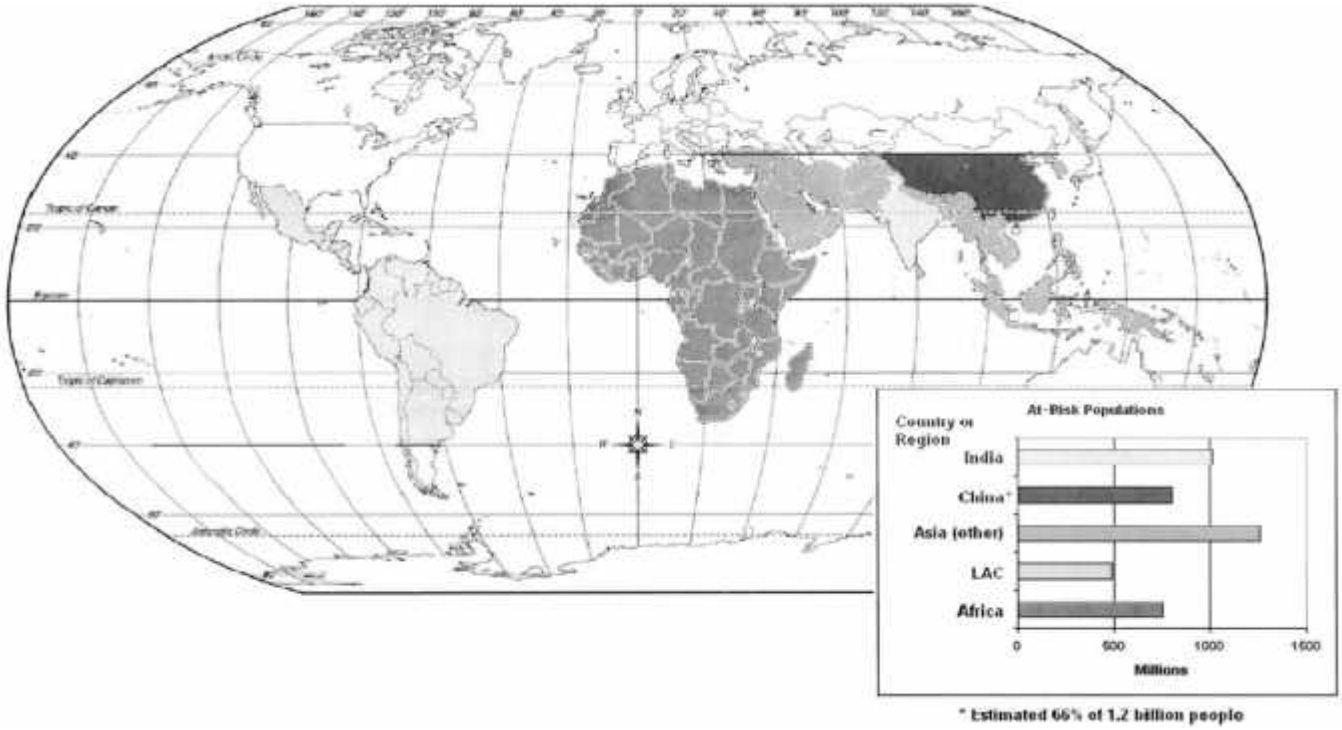
The quantity of AFM1 in the milk depends on the concentration of AFB1 in the contaminated feed. It has been reported that milk is one of the main risk factors of human exposure to AFM1. Infants are the foremost milk consumers, which make them more susceptible to the adverse effects of mycotoxins (Shundo, 2009). The lactating animal could be regarded as intermediate host also due to the biological transformation of AFB1 to AFM1 inside the animal body. Consequently, the farm animals may be considered as a reservoir for AFM1. The milk could be established as a major carrier of AFM1 which affects the human health. Generally, presence of Aflatoxins in animal or human bodies cause a disease named Aflatoxicosis, so the presence of AFM1 may be specified as Aflatoxicosis M1. The main target organ in mammals is the liver so Aflatoxicosis is primarily a hepatic disease. Aflatoxins also cause decreased milk production.

The occurrence of AFM1 in milk and milk products is a serious problem of food hygiene. Aflatoxin contamination in milk and products is produced in two ways. Either toxins pass to milk with ingestion of feeds contaminated with Aflatoxin, or it results as subsequent contamination of milk and milk products with fungi. AFM1 has been reported to cause certain hygiene difficulties in milk and milk products used for food. During the obtaining of cream, AFM1 disperses heterogeneously in milk. AFM1 is not destroyed during the pasteurization process or in yoghurt and cheese making.

2.6 Aflatoxins and Human Health

Exposure to Aflatoxin diet is the major way through which humans as well as animals are exposed to aflatoxins. Apart from this, exposure to aflatoxin can be through ingestion of contaminated milk containing Aflatoxin M1(metabolite of AFB1).Occupational exposure to aflatoxins in agricultural workers, people working in oil mills, and granaries have been reported (Sorenson et al 1984). Figure 3 depicts the countries which are exposed to aflatoxin contamination.

Aflatoxin carcinogenecity after ingestion, aflatoxin is metabolized by cytochrome p450 group of enzymes in the liver, where it is converted to many metabolic products like aflatoxicol, aflatoxin Q1, aflatoxin P1, and aflatoxin M1, depending on the genetic predisposition of the species. Along with the above another metabolite called aflatoxin 8, 9 epoxide is also formed. The amount of this metabolite decides the species susceptibility as this can induce mutations by intercalating in to DNA, by forming an adduct with guanine moiety in the DNA (Smela. *et al* 2001), which may lead to hepatic carcinoma. This was observed in most of the experimental models, and it is presumed that this is the reason for aflatoxin carcinogenecity (Katherine et al 1997 &Raile*yet al* 1997). Moreover species susceptibility to aflatoxin mainly depends on its liver detoxification systems, genetic makeup, age and other nutritional factors (Howard *et al* 1990.)


At-Risk Populations
County or Region
India
China*
Asia (other)
LAC
Africa
0
500
1000
1500
Millions
* Estimated 66% of 1.2 billion people

Aflatoxin and Hepatitis Many experiments conducted in different areas especially in China and in the African countries have shown high incidence of hepatitis B virus infection where dietary exposure to aflatoxins was prevalent. Subsequent research proved that both aflatoxins and hepatitis B virus act synergistically in the etiology of liver cancer (Montesano *et al* 1997 &Groopman*et al* 1996.)

Aflatoxin and Hepatitis Many experiments conducted in different areas especially in China and in the African countries have shown high incidence of hepatitis B virus infection where dietary exposure to aflatoxins was prevalent. Subsequent research proved that both aflatoxins and hepatitis B virus act synergistically in the etiology of liver cancer (Montesano *et al* 1997 &Groopman*et al* 1996.)

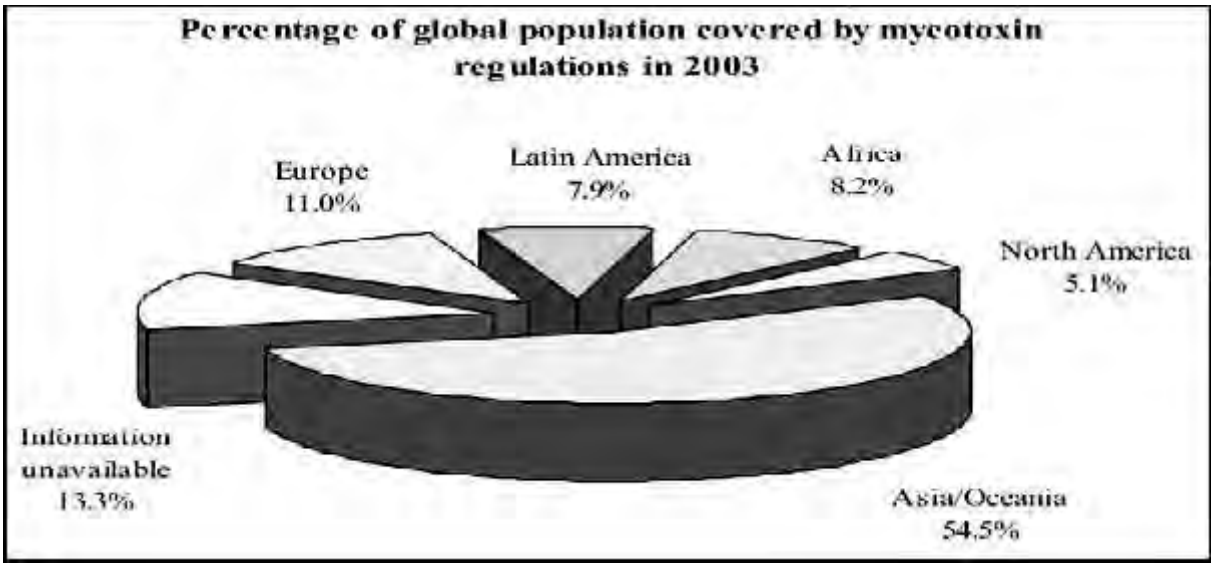
Aflatoxin and childrenFoetal and childhood environment, including the nutritional status of the pregnant mother and the infant, are considered critical for growth and risk of disease in earlier life. Mal-nourishment is one of the common problems in developing countries. Apart from these, they are also exposed to high levels of mycotoxins. Aflatoxins are the major among these. It has been proved that these aflatoxins are immunogenic, teratogenic, and they retard the growth among experimental animals. In the developing countries like India and China, the environmental conditions favor their production. High exposure of these aflatoxins occurs throughout these regions. A study in West Africa showed a significant correlation among the aflatoxin exposure and stunted growth in children who are exposed to aflatoxin right for neonatal stages (Gong *et al* 2002). Apart from that due to the capacity of aflatoxins to cross the placental barrier, can cause genetic defects at foetal stages itself (Maxwell *et al* 1998)

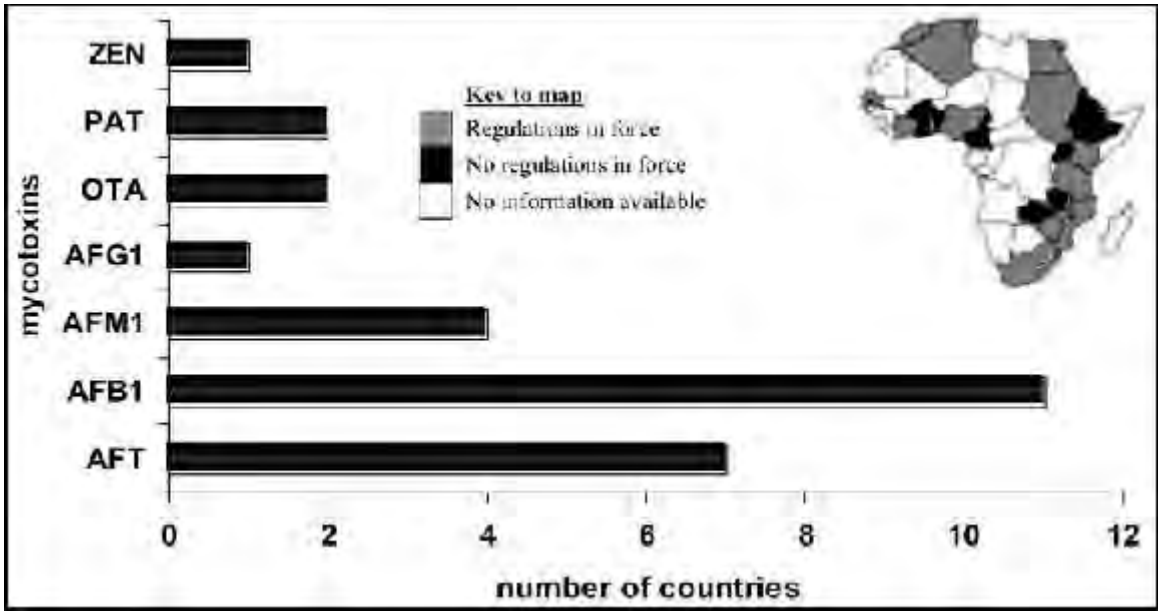
2.7 Economic losses due to Aflatoxins

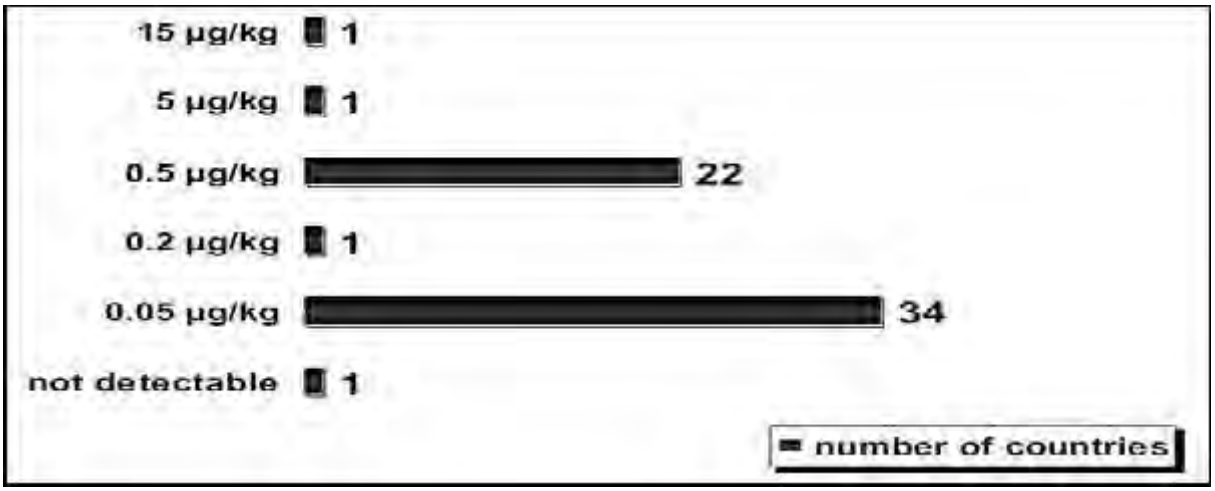
Developing countries suffer most from impact of enforcement of regulation by European and international agencies, particularly the former which is a major Importer of agricultural commodities from developing countries. The economic losses to developing countries are varied. The losses do not only arise from crop and livestock losses but also from costs associated with regulatory compliances (CRA, 2011). For instance, Bankole and Adebanjo (2003) reported that as a result of regulation, exports of agricultural products particularly groundnuts from developing

countries had dropped considerably resulting in major economic losses to producing countries. Losses from rejected shipments and lower prices for inferior quality can devastate developing country export markets (Bhat and Vasanthi, 2003). In 2011, Argentina, China, India and South Africa experienced 37, 60, 136 and 12 rejections, respectively (Codex Alimentarius Commission, 2014). The World Bank predicted that, policy change by the EU will reduce by 64% imports of cereals, dried fruits and nuts from African countries like Chad, Egypt, Gambia, Mali, Nigeria, Senegal, South Africa, Sudan and Zimbabbwe, and thus cost African countries about US$670 million in trade per year (Bankole and Adebanjo, 2003). An assessment reported that, the magnitude of the economic impacts of the health consequences associated with consumption of aflatoxin-contaminated food in developing countries is not known due to a lack of good data. According to them, the quantification of economic losses and estimation of the effects of aflatoxin on health will encourage Health Ministries to enforce standards and provide crucial advocacy to benefit the rural poor, such as improving their level of education about aflatoxin exposure. The toll of the effects on human health includes the cost of mortality, the cost of productive capacity lost when people die prematurely, the cost of morbidity, losses resulting from hospitalization and the cost of health care services, both public and private. There is intangible cost of pain, suffering, anxiety and reduction of the quality of life (Bhat and Vasanthi, 2003).

According to (Otsuki et al. 2001), compliance requirements on exporters impose costs on developing countries, such as the cost of upgrading production systems, processing and storage equipment, and quality control stations. The FAO has also highlighted a number of compliance problems which include lack of funds allocated to research on aflatoxins, scarcity of highly trained and experienced personnel, inadequate facilities for safe aflatoxin research, lack of maintenance of laboratory facilities and inadequate infrastructure (FAO/WHO, 1997). Contamination of maize, a staple in developing countries reduces its economic value which can result in large monetary losses and lead to the removal of large amounts from the market as a result of stringent regulatory limits (Riley and Norred, 1999).


Percentage of global population covered by mycotoxin regulations in 2003
Europe
11.0%
Latin America
7.9%
Africa
8.2%
North America
5.1%
Information
unavailable
13.3%
Asia/Oceania
54.5%


ZEN
PAT
OTA
AFG1
AFM1
AFB1
AFT
mycotoxins
Key to map
Regulations in force
No regulations in force
No information available
0
2
4
6
8
10
12
number of countries


15 µg/kg
1
5 µg/kg
1
0.5 µg/kg
22
0.2 µg/kg
1
0.05 µg/kg
34
not detectable
1
number of countries

Review of a decade survey of AFM1 in dairy milk

Due to AFs and AFM1 health hazard potential, it is therefore important to monitor and regulate the level of AFM1 in milk and milk products through control of animal feed quality for consumer's safety purposes (Whitlow, 2010) Aflatoxin M1 is a metabolite of AFB1 that can occur in milk and milk products from animals consuming feed contaminated with AFB1. Data from this review revealed that not much is done in regard to AFM1 surveys in many developing countries and particularly in African countries.. Numerous epidemiological studies have shown in some areas a correlation between high aflatoxin exposure and high incidence of hepatho carcinoma in several countries in Africa. Data in Table 1 show that only few African countries apart from South Africa (Dutton 2012). Egypt, Morocco, Nigeria (Mwanza, 2012). were involved in the survey for AFM1. However the following Middle East, Asian and Latin American countries are implementing controls of the toxin in both dairy and human milk Iran and Kuwait and Pakistan India, Argentina and Brazil (Sassahara, 2005). Data obtained clearly show that ELISA immunoassay technique has been the most used analytical method in the past decade to measure AFM1. This has also been confirmed in a survey done in Italy on AFM1 in dairy products by The use of ELISA immunoassay can be justified by the fact that it is affordable, simple and easy, to use and does not need expensive equipment such as liquid chromatography. However, several researchers mostly from developed countries combined Imunoaffinity column and Liquid Chromatography for specificity and confirmation of results [Sassahara, 2005 and Dutton, 2012). Survey in developing countries showed high levels of AFM1 contamination (> 0.05 µg/L) in many milk samples analysed as compared to data obtained from developed countries such as France ,Italy Portugal and Spain in which the results were mostly low or with very few samples above the European Commission regulation of 0.05 µg/L (Mwanza, 2012) . The explanation to this might be that, developed countries have imposed strict control on the quality of feed provided to animals which reduces chances for aflatoxin contaminations. Such regulations are not yet implemented or being implemented in developing countries. In addition, climatic conditions mostly tropical; hot and humid conditions favourable for aflatoxin producing fungi contamination in cereals recorded in most developing countries could be the pivotal reason for AFM1 contamination in milk.

2.10 Prevention and Management of Aflatoxin Contamination

According to Suttajit (1989), prevention of aflatoxin contamination can be primary, secondary or tertiary. The primary prevention is considered as the most important and most effective for reducing fungal growth and mycotoxin production. Some of the primary prevention practices include development of plant varieties resistant to fungi, lowering moisture content of seed after harvest and during storage, storing commodities at low temperature, application of fungicides and preservatives and control of insect infestation in stored bulk grains with approved insecticides. Secondary prevention includes redrying of products, removal of contaminated seeds, inactivation or detoxification. Tertiary prevention involves complete destruction of contaminated products and detoxification or destruction of mycotoxins to the minimum level. Some methods of preventing aflatoxin contamination include education and extension, rapid drying, physical separation, smoking, use of plant products, biological control, detoxification, seminars and workshops, adoption of good agronomic practices, early harvesting, sanitation, use of improved storage structures, synthetic chemicals, resistant varieties and fumigation. Studies proposed good agricultural practices such as rotating crops, irrigating to eliminate drought stress, controlling weeds, cultivating mould-resistant stocks and introducing biocontrols such as nonmycotoxigenic fungal strains. (Cassel et, al. (2012) recommended that aflatoxin contamination can also be prevented by keeping storage and feeding facilities clean. According to them, aflatoxin contaminated feed can be tolerated by some livestock particularly older animals but the risk becomes greater with increasing levels of contamination. They maintained that feed additives including organic acids like propionic, sorbic and benzoic acids and their salts such as calcium propionate, potassium sorbate and copper sulphate inhibit mould growth in feed.. The Codex Alimentarius Commission (2014) also recommended the implementation of good agricultural practices (GAP) and good manufacturing practices (GMP) by producers. Control of mycotoxin in Africa is a matter of importance not only for health implications, but also for improvement of the economy in the affected countries. According to (Darwish et al. 2014), a number of strategies for reduction and control of mycotoxins have been considered in different African countries. These include prevention of mould growth in crops and other feedstuffs, decontamination of mycotoxin-contaminated foods and continuous surveillance of mycotoxins in agricultural crops, animal feedstuffs and human food. Other control measures that have been tried in some African countries include segregation of contaminated peanuts in

Malawi, detoxification of peanut meal for export in Senegal, regulation of mycotoxins in animal feed according to the susceptibility of the animal species in Zimbabwe, selection of peanut varieties less susceptible to aflatoxin contamination in Bourkina Faso and improvement in produce handling practices during the 1960s in Nigeria and the 1990s in The Gambia (Bhat and Vasanthi, 2003).

According to Cassel et al. (2012), time of harvest is important in influencing the occurrence and levels of aflatoxin. For instance, harvesting maize above 20% moisture content followed by rapid drying to at least 14% within 24 to 48 h of harvest checks the growth of *Aspergillus*spp. and minimizes aflatoxin production. Chulze (2010) reported that it is possible to control aflaoxins in stored commodities by controlled atmospheres, preservatives or natural inhibitors; the use of antioxidants and essential oils is possible but the cost can be prohibitive on a large scale. In recent times, there have been initiatives aimed atMcontrollingaflatoxins in developing countries, especially Africa. One of such initiative is the Partnership for Aflatoxin Control in Africa (PACA), which is based on a Memorandum of Understanding that was signed between the African Union Commission and Mars Incorporated, aimed at sharing food safety resources and expertise to control aflatoxins in food crops which constitutes a significant threat and a major deterrent to use of key African raw materials in global supply chains (African Union Commission, 2015). Another initiative is the aflatoxin control in maize and peanuts project, which is aimed at developing and implementing holistic strategies to address aflatoxin contamination in maize and peanuts including developing and scaling up biological control technology interventions to improve the health and income of farmers and their families and generate wealth in the crop value chain (African Agricultural Technology Foundation, 2015). The project is funded by Bill and Melinda Gates Foundation and African Agricultural Technology Foundation (AATF) through the IITA and UK aid from the UK government, respectively.

2.11 Dairy production system in Ethiopia

The main source of milk production in Ethiopia is cow but small quantities of milk are also obtained from goat and camel in some region particularly in pastoralist areas. Four major systems of milk production can be distinguished in Ethiopia,

PastoralismEven though, information on both absolute numbers and distribution vary, it is estimated that about 30% of the livestock population are found in the pastoral areas. The pastoralist livestock production system which supports an estimated 10% of the human population covers 50-60% of the total area mostly lying at altitudes ranging from below 1500 m.a.s.l. pastoralism is the major system of milk production in the low land. However, because of the rainfall pattern and related reasons shortage of feed availability milk production is low and highly seasonally dependent.

The highland smallholder milk production The Ethiopian highlands possess a high potential for dairy development. These areas occupying the central part of Ethiopia, over about 40% of the country (approx. 490.000 km^2) and are the largest of their kind in sub-Saharan Africa (Tedla et al, 1989). In the highland areas agricultural production system is predominantly substance smallholder mixed farming, with crop and livestock husbandry typically practiced within the same management unit. In this farming system all the feed requirement is derived from native pasture and a balance comes from crop residues and stub grazing. The majority of milking cows are indigenous animals which have low production performance with the average age at first calving is 53 months and average calving intervals is 25 months. Cows had three to four calves before leaving the herd at 11-13 years of age, the average caw lactation yield is 524 liters for 239 days of which 238 liters is off take for human use while 286 liters is suckled by the calf. But also a very small number of crossbred animals are milked to provide the family with fresh milk butter and cheese. Surpluses are sold, usually by women, who use the regular cash income to buy household necessities or to save for festival occasions (Mugerewa). Both the pastoralist and smallholder farmers produce 98% of the country milk production (MOA, 1985 E.C).

Urban and peri-urban milk production this system developed in and around major cities and towns which have a high demand for milk. The main feeds sources are agro-industrial by products (Oil Seed Cakes, Bran, etc) and purchased roughage. The system comprises small and medium size dairy farms located mainly in the highlands of Ethiopia. Farmers use all or part of their land for home grown feeds. Generally, the primary of the production system is to sale milk as a means of additional cash income.

Intensive Dairy Farming This is a more specialized dairy farming practiced by state sector and very few individuals on commercial basis. Most of the intensive dairy farms are concentrated in and around Addis Ababa and are basically based on exotic pure bred stock. The urban, peri-urban and intensive dairy farmers are produce *2%* of the total milk production of the country.

Major constraint for dairy development system in Ethiopia

The livestock sub-sector in general and the dairy sub-sector in particular does not make a contribution to the national income considering with it size. The reasons for this are numerous and include both non-technical and technical constraints.

*Non-technical constraints*The non-technical constraints of dairy development generally include a variety of socio-economic and institutional considerations, which is most cases and are will common constraints to other agricultural sector in the country.

*Human population*The high rate of population increase (2.9-3 % per annual) is reckoned to affect livestock development. The demand for livestock products directly related with the annual population growth which the livestock production is lag behind with the rate of population growth. Moreover, high population growth has forced people to cultivate more and more land. The necessity to extend the cropping areas to support the increasing population in the highlands, the carrying capacity of the land is stretched beyond its limits, which resulted in law production performance of the livestock.

Livestock population One of the serious constraints to the livestock development in Ethiopia rest on the importance attached to the economic functions of the livestock found in various agro-ecological zones, overall, livestock in Ethiopia are used as input function, asset and security function.

Farming methods in Ethiopia have remained unchanged for centuries, cultivation is carried out using oxen drown traditional ploughs in the highland this demand high dependency on animal power (as an input function). High population growth has forced people to plough more land, which in turn demand more ploughing capacity. Therefore, to fulfil this demand more ploughing capacity requires for the presence of a higher cattle herd, which created pressure on grazing land and ultimately poor economies of peasant farm. The other economic benefit of livestock, as a source of additional income consideration as assets and security are also important, and due to low productive indigenous stock these functions requires to maintain large herd and demand additional area of grazing land. In the law lands the pastoral nomads maximum benefit from livestock through milk and meat (The output function): Furthermore, in order to overcome low productivity of their livestock and recurring draught large number of stock is maintained as security function as well (M. Tesfaye, 1991).*)*

Technical constraintsAnimal health Animal health and improved management is also one of the major constraint of dairy development in Ethiopia which cause poor performance across the productive system. Many of the problems result from the interaction among the technical and non-technical constraints themselves e.g. poorly fed animals develop low disease resistance, fertility problem, partly because the animal health care system relies heavily on veterinary measures, poor grazing management systems continue to cause high mortality and morbidity (e.g internal parasites), many of the disease constraints which affect supply are also a consequence of the non-technical constraints e.g. insufficient money to purchase drugs or vaccines.

2.12 Milk Aflatoxin in Ethiopia

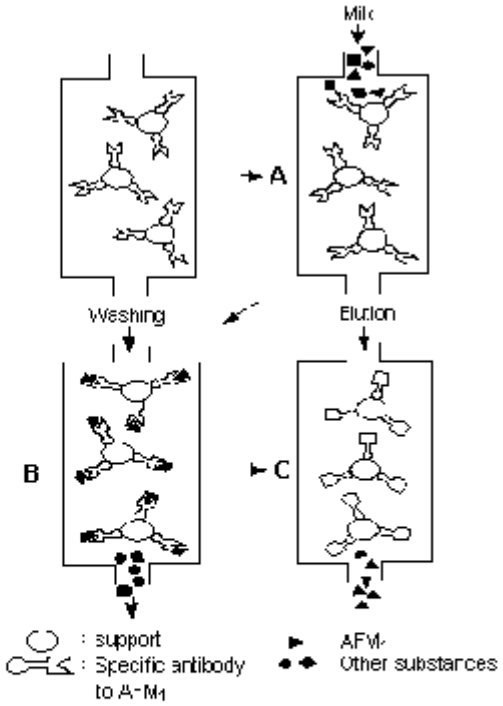
Studies have indicated aflatoxin contamination in Ethiopia in staple cereals (Ayalewet,al, 2006), red chili pepper and ground peas (Fufa&Urga, 1996) and furthermore, aflatoxin contamination in milk and dairy feeds has been reported for the first time in 2015. In this study, the researchers collected raw milk samples from dairy farmers and milk collectors in the Greater Addis Ababa milk shed and quantified the levels of AFM1 using ELISA. Also, animal feed samples were collected from dairy farmers and dairy feed processors and traders, and AFB1 levels were analyzed. Results showed the presence of AFM1 in all milk samples, and contamination level ranged between 0.028 and 4.98 mg/L. Overall, only nine (8.2%) out of a total of 110 milk

samples contained less than or equal to 0.05 mg/L of AFM1. Furthermore, 29 (26.3%) milk samples exceeded 0.5 mg/L. All the feed samples were contaminated with AFB1 ranging between seven and 419 mg/kg. Overall, out of a total of 156 feed samples collected, only 16 (10.2%) contained AFB1 at a level less than or equal to 10 mg/kg. At the same time, 41 (26.2%) of the feed samples contained AFB1 at a level exceeding 100 mg/kg. quantified. In this study Correlation between AFM1 in milk and AFB1 in feed. There was a moderate positive correlation between AFB1 in feed and AFM1 in milk collected from the corresponding dairy farm, with a correlation coefficient of 0.31. The average pass through of AFB1 in feed to AFM1 in milk was about 1%. Some dairy farms had discrepancies between the levels of aflatoxin contamination in their milk and feeds. Table 6 shows the results from eight dairy farms that had the highest level of AFM1 contamination (>1.0 mg/L) in their milk, and the corresponding AFB1 levels in the feed and the feed ingredients. Note that feed containing maize grain or beer-by products only had low levels of AFB1 (Gizachew et al, 2005)

2.13 Detection techniques of Aflatoxins

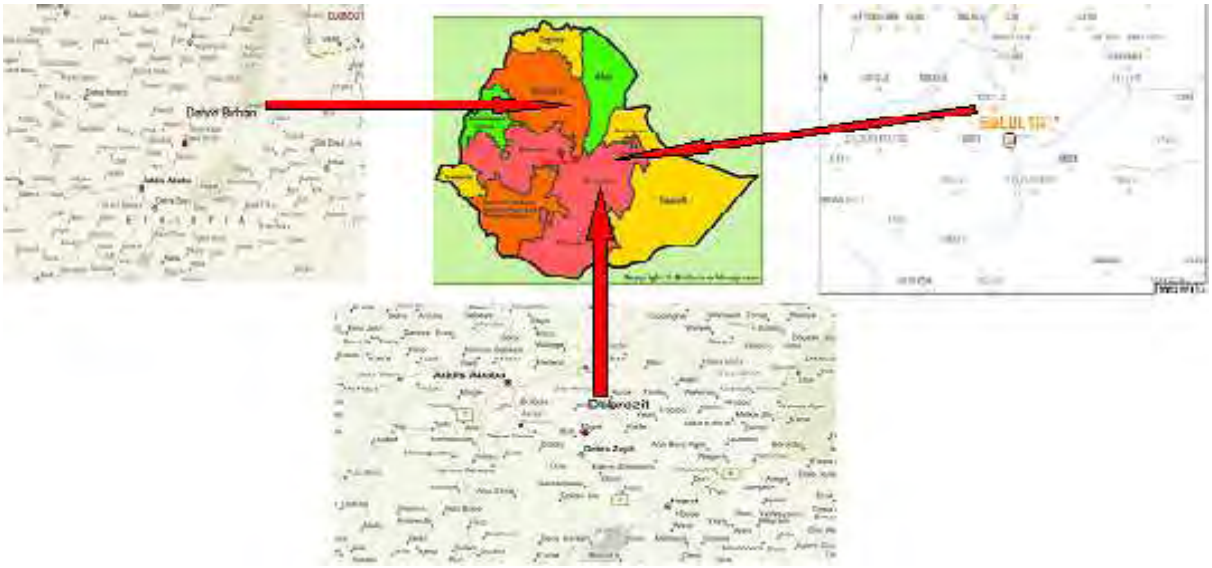
High performance liquid chromatography (HPLC)

Analytical laboratories moved away from TLC to HPLC determination with advances in HPLC methods in 1980s. High performance liquid chromatography is a very precise and highly automated quantification technique for aflatoxins analysis with high selectivity and sensitivity. Now-a-days, HPLC methods are widely used because of their superior performance and reliability as compared with TLC. HPLC methods have been developed for all major mycotoxins in cereals and other agricultural commodities. In the field of analysis of aflatoxins, HPLC is mainly used for final separation and detection of the analyte of the interest and extraction and clean-up techniques have to be applied prior to detection with HPLC. In HPLC, a liquid mobile phase or solvent is used to move the sample through the column. An immobilized liquid stationary phase is packed in the column. The analyte is then partitioned between the two phases as it passes through the column and thus leading to the separation of compounds due to the



Schematic diagram of immunoaffinity chromatography for concentration and purification of aflatoxin M_1

A, introduction of milk onto affinity gel column; B, removal of impurities; C, elution of aflatoxin M_1


Debre Berhan
Debre Zeit

All sampling bottles were washed with detergents and dried a day before milk collection. The bottles were rinsed with the sample itself on the day of sample collection.

Table1: Areas where milk samples were collected and coding

Area	**Grazing**		**Non- Grazing**	
	Farm Code	no of samples	Producer Code	no of samples
Deberbirhan	Farm1,Farm 2,Farm3	3	Farm1,Farm 2,Farm3	3
Bishoftu	Farm1,Farm 2,Farm3	3	Farm1,Farm 2,Farm3	3
Sululta	Farm1,Farm 2,Farm3	3	Farm1,Farm 2,Farm3	3
Addis Ababa	Brand, Brand 2, Brand 3			3

3.4 Chemicals and Reagents

Chemical and Reagents used in the sample preparation and analysis includes: Sigma Aldrich Aflatoxin Standards, HPLC grade Acetonitrile, Methanol, ultra-pure water, phosphate buffered saline solution (PBS: NaCl 8g/l, KCl 0.2 g /l, Na2HPO4 1.15 g /l, KH2PO4 0.2 g/l) and adjusted to pH to 7.4 using 0.1M HCl or 0.1M NaOHwere supplied by Ethiopian food medicine and healthcare administration and control authority (EFMHACA).

Mobile phase

The mobile phase used for aflatoxin analysis was a mixture of a de-ionized water-methanol-acetonitrile (65:25:15, v/v/v) at flow rate 1.2 ml/min. The mobile phase was vacuum filtered and sonicated for 30 minutes to degassed and Isocratic method was applied for analysis for better resolution. Distilled and ultrapure water were used throughout mobile phase preparation.

Standards:

Aflatoxin m1, standards purchased from Sigma Aldrich (St. Louis, MO, USA).

Immunoaffinity columns

AFLAPREP® (product code P07, P07/500, Batch number: CL 577, Glasgow UK) immune affinity column which contain antibodies against AFm1 with a capacity greater than 100 ng for aflatoxin B1 and recovery (85%-110%) was used for sample clean up. IAC was purchased from R-Biopharma Rhone Ltd, Scotland, UK.

Apparatus

Different laboratory apparatuses and instruments were used in the research. Here to list the major ones were : Laboratory Centrifuge, centrifuge tubes, vacuum pump, Immunoaffinity column, Whatman glass microfiber filter paper, lab stand with clump, nylon filter ,Volumetric and Graduated pipettes (1ml, 5ml, 10ml, 25ml and 50ml), micropipettes of (100, 500, & 1000) µl, volumetric flasks (10ml, 25ml, 50ml, 100ml, 500ml and 1000ml), Measuring cylinders (50ml and 100ml), Beakers (50ml, 100ml and 500ml), conical flasks (250, 500 and 1000ml), Ultrasonic bath (Sonicator), Wash bottle, sample collecting bottles, ice-box, Electronic balance, syringes (5ml and 10ml), Vials with screw cap. SHIMADZU HPLC system setup containing auto sampler, injector, oven, column, Link, Degasser, fluorescence detector and desktop computer with chromatography software were used.

Safety Measures

AFs are carcinogens and care should be exercised to avoid personal exposure and potential risk of contamination. All handling of pure compounds was done in the fume hood with protectivewear such as safety glasses, gloves, laboratory coat and a disposable face mask. The glass wares were washed with hypochlorite and dilute acid before re-using and the waste materials treated with hypochlorite before disposal.

3.5 Chromatographic condition

Chromatographic separation and detection was carried out using Shimadzu USA, HPLC instrument with LC software and fluorescence detector were used for analysis. separation was achieving with A Shim-pack FC-ODS reversed phase column (5µm, 250L x 4.6mm diameter). The operating condition were as follows: column temperature at 25°c temperature; flow rate

1.2ml min^{-1}; 25 minutes running time, 20µl injection volume; detection wavelength: excitation wavelength 365 nm/emission wavelength 440 nm; diluent methanol and Needle wash (Water: Methanol 90:10 v/v)

3.6. Chromatographic method validation

In order to obtain consistent, reliable and accurate data, method validation for the suitability of the analytical method for the determination of aflatoxins in the sample was performed on the following validation attributes. Toxin identification linearity and calibration curve, LODs and limits of quantification (LOQ), precision, recovery and working range

3.6.1 Identification of Aflatoxins Retention Time (RT)

Retention time was measured from the time at which the sample is injected to the point at which the display shows a maximum peak height for that compound. In this research identification of the RT for definite Aflatoxin m1 species was done to know the elution of the intended Aflatoxin species peak to be revealed there by to determine Aflatoxin m1 peak is detected the mobile phase itself injected in vial as a blank or control. Then of Aflatoxins m1 standards were injected.

3.6.2 Precision

In this research under the same experimental condition and in the same day repeatability test performed by choosing an interim standard in the working range that was 5 ppb of aflatoxinm1 standard by injecting ten times.

3.6.3 Linearity and Range

The linearity was studied by preparing aflatoxin m1 standard solutions from 6 different concentrations of the aflatoxin standards (0.5 ppb, 1 ppb, 3 ppb , 5ppb , 8 ppb and 10 ppb). The curve, for AFM1, was prepared by plotting regression line peak area (Y-axis) against [concentration (x-axis)]. The coefficient of correlation (R^2) was considered appropriate when > 0.99.

3.6.4 Limit of Detection (LOD), Limit of Quantification (LOQ)

Detection performance of the HPLC was determined by the limit of detection (LOD) which is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as

an exact value. LOD was determined by the amount of analyte that can be detected above baseline noise; typically, three times the noise level S/N > 3. On the other hand the limits of quantification (LOQ) as the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOQ was determined based on the amount of analyte which can be reproducibly quantitated above the baseline noise, that gives S/N >10.

3.6.5 Recovery

Recovery experiments were carried out by spiking two mixed aflatoxin standard concentration (0.25 ppb and 3 ppb) in to sample. Spiked and un-spiked sample were treated with the same extraction and clean up procedure for sample analysis. Recovery specifications with immunoaffinity clean-up are greater or equal to 85% and less than or equal to 110% for aflatoxins m1, (Certificate of analysis, R-Biopharm Rhone Ltd). Percent Recovery calculated according to the relation,

$$\% R = \frac{\text{(Spiked sample result - unspiked sample result)*100\%}}{\text{Known spike added concentration}}$$

3.7 Preparation of Standard Solutions

Aflatoxin standards were prepared in volumetric flasks by dissolving in HPLC grade mixture of Methanol and acetonitrile at different desired concentrations of (0.05 ppb, 0.1 ppb, 0.25ppb, 0.5 ppb, 1 ppb, 3 ppb , 5ppb , 8 ppb and 10 ppb). And the spiking, LOQ and LOD, testing standards depending on the type of analysis, which required careful micro-pipetting of the solution prepared. The standards prepared transferred to vials and stored, cooled at $4^{o}C$ and protected from light to avoid deterioration of the Aflatoxins in solution.

3.8 Procedures for Aflatoxin Analysis

3.8.1 Sample Extraction and Cleanup in liquid milk (similar to ISO 14501)

Milk samples was measured 100 ml into a glass beaker and heated to approximately 35-$37^{0}C$ centrifuged at (4,000 rpm for 10 minutes), and filtered through a Whatman glass microfiber filter

The elute Aflatoxins (m1) methanol solution were determined at parts per billion (µg/L) levels in milk by Immunoaffinity column cleanup and high performance liquid chromatography with fluorescence detection and calculated according to the following equation.

$$W = Wa \times VfVi \times 1/Vs$$

Where:-

W = amount of aflatoxin in the test sample in µg/L

Wa = amount of aflatoxin corresponding to area of aflatoxin peak of the test extract (ng)

Vf = the final volume of re-dissolved eluate (µL)

Vi = volume of injected eluate (µL)

Vs = volume of test portion (milk) passing through the column (mL)

3.9 Experimental Design

Completely randomized experimental designs were followed to see the level of aflatoxin in milk from grazing and non- grazing cows in three milk producing areas.

3.10 Statistical data analysis

For data analysis, Excel Microsoft office 2013 and IBM SPSS Statistics version 20 software were used. In the SPSS method, one-way analysis of variance (ANOVA) was performed to evaluate the levels of total aflatoxin means comparison between the study sites. A P-value of less than 0.05 ($P < 0.05$) was considered to show statistical significance. Assumptions of ANOVA were checked. As there was one dependent variable one way ANOVA used. Dependent variable, level of Aflatoxin in the study sites. As the independent variable the feeding type.

4. Results and Discussion

4.1 KAP Assessment on Aflatoxin among milk processors and Household farmers

Based on purposive sampling techniques assessments on KAP (Knowledge, attitude and practice) was done. A semi-structured questionnaire which may possibly figure out circumstance related to feeding approach product handling, testing and processing practice of house hold farmers and milk processors accordingly. On this survey, a total of 27 participants (9 milk house hold farmers, 18 milk processors) were participated and the response grouped in knowledge, practice and attitude.

4.1.1 Socio-demographic Characteristics of Household Farmers

As demonstrated below in table 2 from the summary, the majority of dairy farmers participated in the study were above the age of 27 years, mostly married and 75% of farmers (cattleman) were illiterate and . Their economic class was categorized in the under average and medium economic class where the majority 33 has a family income of ≤ 1000 birr per month on the average. On this survey, a total of 9 participants/farmers (cattleman) were participated and the response grouped in knowledge, practice and attitude.

Table 2: Socio demographic characteristics of dairy farmersin the study sites

Statement	Response		
	Frequency		Percentage
Age	20-30	5	55.5
	30-50	3	33.3
	>50	1	11.2
Sex	Male	6	66.6
	Female	3	33.3
Marital status	Single	2	22.2
	Married	7	77.8
Educational status	Illiterate	7	77.8
	Elementary school	2	22.2
Family income	<1000	3	33.3
	>2000	5	55.5
	>5000	1	11.2
Position in the house hold	Household head	7	77.7
	Spouse household head	1	11.1
	Relative of house hold head	1	11.1

4.1.2. Socio Demographic Characteristics of Professionals in dairy processing Plants

Regarding professionals work in milk processing, majority of them weren't married. Their experience on the dairy sector was an average of 3 years. The quality and food safety issues of the milk processing based on a purposive sampling. On this survey 18 professional employees in dairy processing particularly engaged in section of quality control and production are participated and the response summarized in two parts.

Table 3: Demographic Characteristics of professionals working in dairy processing

Statement	Response		
	Frequency		Percentage
Age	22-30	12	66.6
	30-50	6	33.4
Sex	Male	16	88.9
	Female	2	11.1
Marital status	Single	12	22.2
	Married	6	66.6
Educational status	Degree and above	14	77.8
	Diploma	4	22.2
income	<2000	6	33.3
	≥3000	10	55.5
	>5000	2	11.2
Occupation	Employee	18	100
	Share holders	0	0
Work experience on these area	< 2years	8	
	≥3years	8	
	>5years	2	

4.1.3 KAP of Household Dairy Farmers

As summarized in table 5 belowa total of 9participants were interviewed for their knowledge,attitude and practice (KAP) regarding aflatoxin contamination in farmers which mostly engaged on grazing. According to the result of (KAP) assessment, awareness of mould growth and formation of mycotoxin is very low among the dairy farmers.

Regarding on the awareness of mycotoxin or aflatoxin, 90% of respondents were not well aware of the toxin; rather they remind that there was an incidence that they were forced to dispose their milk due to occurrence of unknown disease on the raw milk. The entire respondent had no knowledge on the possible source of mycotoxins/ aflatoxins. The researchers were trying to figure something out why they were prefer feeding from field grazing than processed feed product if they were related with aflatoxin source , all of the respondents were respond that they just prefer grazing because of the accesses and cost rather they weren't related with aflatoxin

contamination. Related to these they prefer the feed product due to milk productivity than grazing.

From the respondent practices point of view around 80% of them weren't feed their cow exclusively on grazing sometimes there were a likelihood of feed source besides grazing. The respondent also reported that there were products which supplement their feed in addition to grazing around 70 % of them were feed traditional beer (tella) of waste (Atela). Around 80% of the respondent were reported that their cow was engaged on field gazing means the rest were feed them other sources dairy feed product like tradional beer waste (tela) and Other leftover food due mold growth and bran in addition to grazing. Regarding to their attitude of the respondent on the preference of grazing over other feed from productivity point of view above 50 % them were favorite dairy feed product rather grazing. The main reason that the respondents forced to use 66% of the report is feed products were not easy on the pocket and the rest 33 % was also due to accessibility of none of the respondent were to report from the quality and safety point of view.

Table 4: Summary of Aflatoxin KAP among dairy farmers

Statement			
Knowledge	**Response**	**Frequency**	**percentage**
Do you have any awareness on mycotoxins /aflatoxin /	Yes	1	11.2
	No	8	88.8
Knowledge on possible source of mycotoxins.	Yes	0	0
	No	9	100
Knowledge on advantages using field grazing feeding over other processed dairy feed product related to mycotoxin contamination	Yes	0	
	No	9	100
Knowledge on the effect of mould contaminated feed dairy products and health impact on consumer	Yes	0	
	No	9	100
Practice			
Can you guarantee as your cow feeding is exclusively on grazing.	Yes	7	77.8
	No	2	22.2
Type of feed mostly used beside grazing for milking cow	Traditional beer waste /Atela	7	90
	Other leftover food due mold growth and bran	2	10
At what level the cow is in use of on field grazing feeding	Most	7	77.8
	Least	2	22.2
Attitude			
Why you prefer grazing than feed	cost-effective	6	66.6
	Productivity	1	11.1
	Accesses	2	22.2
Which feeding mechanism is preferable fromthe two	Grazing	3	33.3
	Dairy Feed	6	66.6
how do you prefer grazing than dairy feed	Economical	6	66.6
	Access	3	33.3
	quality problem on dairy feed	0	

4.1.4. KAP of Professional in Dairy Processing Plants

Table 6 summarizes the responses of all 18 participants interviewed for their knowledge,attitude and practice (KAP) of mycotoxins / aflatoxin contamination in dairy processing from production and quality department in dairy processing.

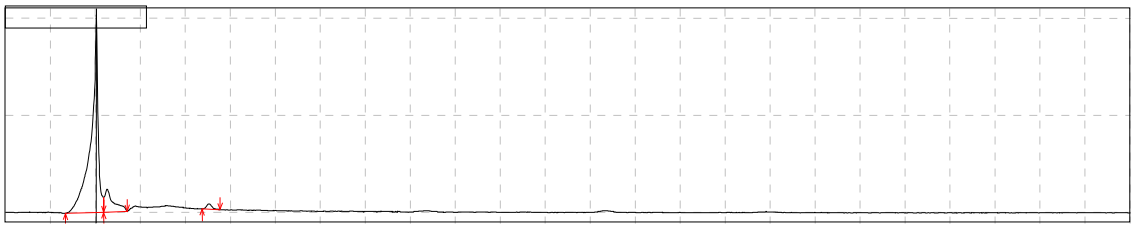
Concerning their knowledge on the preventive means of the respondent on monitoring/inspecting for the presence and absence of mycotoxin in the raw milk at reception section all of them were reported that they didn't have any awareness/and mechanisms to control before entering into the process line of the raw milk. Around 95 % of the respondents were familiarity on the root causes of mycotoxin presence in milk and conditions which favors it. Alongside prevalence of aflatoxins in milk they reported that 100% of them are expecting it. Regarding their practices related to aflatoxin concerns the researcher wants to know the main source of the raw milk from the option of their own dairy farm, individual farmers and cooperative raw milk suppliers 45% , 33% and 22% of them are from individuals, cooperatives and own farmers respectively. In these case respondents face limitation on poor data recording from the same plant the two technical personals responded in different way. From the previous information some of processing plant had their own dairy farming, so that regarding their feed handling practices around 90% of them were not follow good storage and handling practices. The very critical point which raised from the researcher their system in place that helps to trace and track to identify the source of the raw milk 100% of the respondents reported that there was no any system establish.

On the aspect of their attitude 90 % of them were not believe that pasteurization won't eliminate aflatoxin since once occur. About 78% of the respondents were assumes that there is a possibility of removing aflatoxin from the source with some intervention.

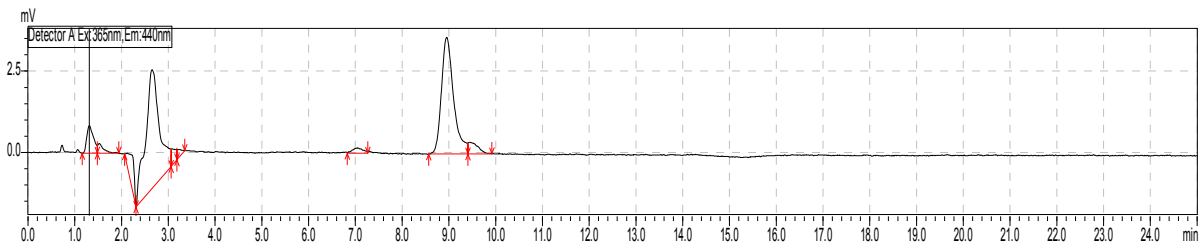
Table 5: Survey of Aflatoxin KAP among milk processors

Statement				
Knowledge		**Response**	**Frequency**	**Percentage**
1.	Awareness on monitoring and inspecting for the presence and absence of aflatoxin in the raw milk during delivery and before processing	Yes	0	
		No	18	100
2.	Knowledge about food toxin like mycotoxin	Yes	18	100
		No	0	0
3.	Knowledge on the root causes of mycotoxin presence in milk and conditions which favors it	Yes	17	94.6
		No	1	5.4
4	Knowledge expectance of mycotoxin present in milk and its significance	YES	18	100
		NO	0	0
Practices				
5	Storage conditions of feed if they have dairy farm alongside	Good	1	11.1
		Poor	3	88.9
6	Storage conditions of feed if they have dairy farm alongside	Good	1	11.1
		Poor	3	88.9
7	Do you have a strong system which helps to trace and track raw milk source	Yes	0	0
		No	18	100
8	Where is the source of raw milk	Their Own dairy farm	4	22.2
		Individual Farmers	8	44.5
		Cooperative Raw milk suppliers	6	33.3
Attitude				
9	Product testing like mycotoxin testing	Yes	0	0
		No	18	100
10	Assumption of mycotoxin eliminate by pasteurization	Yes	1	11.2
		No	17	88.8
11	Expectance of aflatoxins present in pasteurized milk and its significance	Yes	16	77.8
		No	2	22.2
12	Your assumption that possibility of removing aflatoxin from the source with some intervention	Possible	16	77.8
		Not possible	2	22.2

Detector A Ex:365nm,Em:440nmData
file Name:Afla m1 blanck 002.lcd



Datafile Name:Afla m1 008.lcd
Sample Name:Afla std m1 8ppb 008
Sample ID:Afla std m1 8ppb 008



4.2.2 Precision

As verified in Table 2 the precision was evaluated through the repeatability of the method by assaying ten replicate injections of aflatoxin standard at the same concentration (5 ppb), during the same day, under the same experimental conditions. It shows an acceptable %RSD which had a values of (<0.90and <2.1%) for the retention time and peak area respectively. A precision criterion the instrument precision (repeatability) and is normally expressed as the percent relative standard deviation for a statistically significant number of samples should be ≤ 5% RSD in FDA standard.(FDA, 2012)

Table 6: precision with repeated concentration

Precision (m1)				
series	**concentration (ng/g)**	**Area**	**Retention time**	
1	5 PPB	45434	8.956	
2	5 PPB	45287	8.556	
3	5 PPB	45637	8.518	
4	5 PPB	46342	8.476	
5	5 PPB	45174	8.443	
6	5 PPB	45712	8.417	
7	5 PPB	45872	8.382	
8	5 PPB	44898	8.35	
9	5 PPB	45023	8.373	
10	5 PPB	45658	8.301	
	mean	45507	8.4772	
	STD	410.1015	0.185312	
	RSD	0.901183	2.186008	

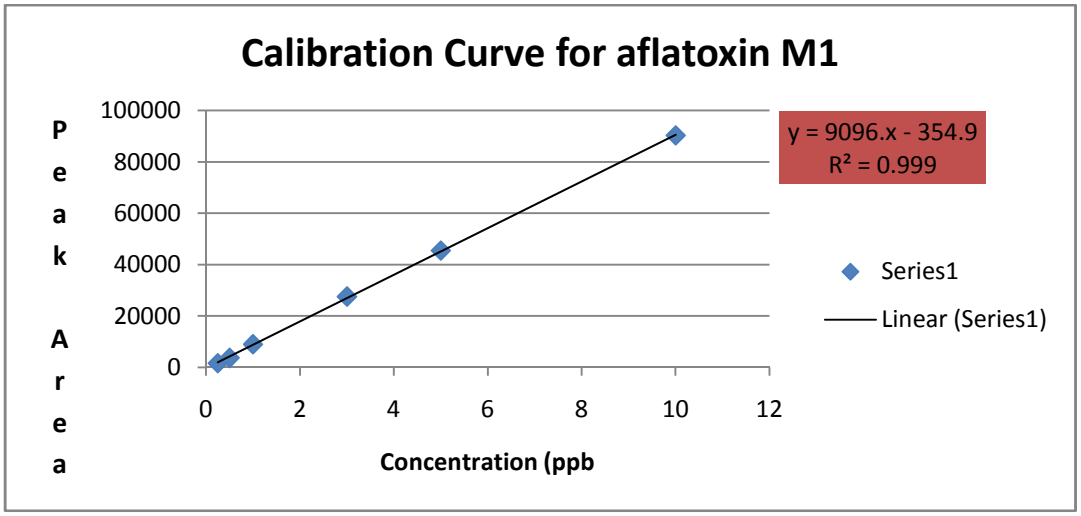

Calibration Curve for aflatoxin M1
Peak Area
100000
80000
60000
40000
20000
0
0
2
4
6
8
10
12
Concentration (ppb
y = 9096.x - 354.9
R² = 0.999
Series1
Linear (Series1)

Table 15: Limit of detection and quantification

LOD			LOQ	
Aflatoxin	Relative concentration of Aflatoxin m1 (ppb)	S/N > 3, Result	Relative Individual concentration of Aflatoxin (ppb)	S/N > 10, Result
AFL m1	0.25	3.62	0.5	15.40

4.2.5 Recovery Test

The recovery specification was conducted based on the company requirement R-Bipolar Rhone Ltd for the Immunoaffinity column. Greater than or equal to 85% and less than or equal to 110% of aflatoxin m1. After spiking the sample and data obtained for the corresponding spiking and un-spiking level, percent recovery calculation done.

4.3 Aflatoxin concentration in Milk Samples

The concentration of aflatoxin in milk samples from grazing and non-grazing cows from different regions covered under this study and milk from Addis Ababa is presented in Table 7 and Table 8.

Table 7: Aflatoxin concentration if milk samples from the study sites

Area	Farm	Aflatoxin Concentration (ppb)	
		Grazing (N=2)	Non-Grazing(N=2)
Sululta	Farm 1	0.692	14.86
	Farm 2	0.418	26.23
	Farm 3	0.519	25.08
Debrebirhan	Farm 1	4.214	22.34
	Farm 2	3.949	4.66
	Farm 3	0.79	38.17
Bishoftu	Farm 1	0.915	43.17
	Farm 2	5.583	21.09
	Farm 3	3.73	3.25

Table 8: Aflatoxin concentration if milk samples from Addis Ababa

Brand	Aflatoxin Concentration
Brand 1	25.48
Brand 2	61.78
Brand 3	2.53

The AFM1 contamination of milk samples from grazing cows is very low as compared with the milk from non-grazing cows in all study areas and pasteurized milk samples from Addis Ababa. A single grazing dependent dairy farm from both Debrebirhan and Bishoftu town had a relatively low AFM1 concentration as compared to its counterpart dairy farms (0.79 ppb and 0.915 ppb respectively). Likewise, the AFM1 concentration of milk from farm 2 in Debrebirhan was 4.66 ppb and from farm 3 in Bishoftu was 3.25 ppb, which indicates a wide variation among milk samples within the same area. Brand 3 milk from Addis Ababa town had also very low AFM1 level as compared to the other two milk samples (2.53 ppb).

The mean AFM1 concentration in milk samples from the study areas is given in Table 9. Accordingly, milk samples collected from grazing cows in sululta town had significantly lower AFM1 level (0.54 ± 0.32) than milk samples from Debrebirhan and Bishoftu (2.15± 33.3 and 3.26 ± 16.6 respectively). 50%, 33.3 % and 16.6 % of milk samples in Sululta, Debrebirhan and Bishoftu towns from grazing cows comply with the standards set by Ethiopia (<0.5 ppb).

Table 9: Mean aflatoxin concentration if milk samples from the study sites

Site of Milk sample	**Grazing**				**Non-Grazing**		
	Range (ppb) (n=no of samples)	Mean (ppb)	Mean % ≤ 0.5 (ppb)	Std deviation	Range (ppb) (n=no of samples)	Mean (ppb)	Std deviation
Sululta	0.22-1.02(*n=6*)	0.54	50	0.32	14.2-28.96 (*n=6*)	22.06	5.98
Debrebirhan	0-4.99(*n=6*)	2.15	33.3	2.19	3.28-42.87 (*n=6*)	21.72	15.31
Bishoftu	0-7.57(*n=6*)	3.26	16.6	2.68	3.21-43.47 (*n=6*)	22.50	17.89

Milk samples from non-grazing cows from Debereberhan had a mean lower aflatoxin value (21.72±15.31) when compared to mean aflatoxin levels of milk from cows from Sululta (22.06±5.98) and Bishoftu (22.5±17.89). All milk samples collected from non-grazing cows were highly contaminated with aflatoxin and none of them had levels below the Ethiopian standard. The milk samples that were collected from Addis Ababa town had the highest mean aflatoxin concentration (29.93±28.2) ppb (Table 10) and ranged from 2.34 ppb to 76.07 ppb.

Table 10: Mean aflatoxin concentration of milk samples from Addis Ababa

Site of Milk sample (n=no of samples)	**Range (ppb)**	**Mean (ppb)**	**Std deviation**
Addis Ababa (*n=6*)	2.34-76.07	29.93	28.2

4.4. Comparison of Aflatoxin Concentration in Milk from grazing and non-grazing cows

The mean aflatoxin concentration found in milk samples collected from grazing and non-grazing cows in three different study regions and in Addis Ababa is presented in Table 7. It was found out that cow's that were grazing had lowest mean aflatoxin concentration (1.98±2.21) ppb. Milk from cows that were non-grazing had higher mean aflatoxin concentrations (22.09±13.18) ppb and pasteurized milk that was marketed in Addis Ababa had the highest mean aflatoxin concentration (29.93±28.22) ppb.

One way anova results indicate that the mean value of milk from grazing cows is significantly different ($P<0.05$) from milk samples from non-grazing cows and milk marketed in Addis Ababa. The milk samples from non-grazing cows were not statistically different from the milk that was collected from Addis Ababa.

Table 11: Mean Aflatoxin concentration of milk from grazing and non-grazing cows

Feeding system (n=no of samples)	**Range (ppb)**	**Mean(ppb)**	**Std deviation**
Grazing (*n=18*)	0-7.57	1.98[a]	2.21
Non-Grazing (*n=18*)	3.2-43.47	22.09[b]	13.18
Addis Ababa (*n=6*)	2.3-76.1	29.93[b]	28.22

a-b any two means in the same column not followed by the same latter are significantly different

4.5Aflatoxin Concentration in milk from different Sampling Areas

The mean aflatoxin concentration in milk from the different cities where milk samples were collected irrespective of the type of feeding system is given in table 8. Accordingly milk from sululta had the lowest mean aflatoxin concentration (11.3±11.94) whereas the milk in Addis Ababa had the highest mean aflatoxin concentration (29.93±28.22). 25% of milk samples collected from sululta had aflatoxin concentrations lower than that of the Ethiopian standard whereas none of the milk samples from Addis Ababa meet the Ethiopia standard for aflatoxin in milk.

Table 12: Aflatoxin concentration in milk from the study sites

Site of Milk sample (n=no of samples)	Range (ppb)	Mean(ppb)	Mean % ≤ 0.5 (ppb)	Std deviation
Sululta(*n=12*)	0.22-28.96	11.30[a]	25	11.94
Debrebirhan (*n=12*)	0-42.9	11.94 [a]	16.7	14.60
Bishoftu(*n=12*)	0-43.5	12.88 [a]	8.3	15.80
Addis Ababa (*n=6*)	2.3-76.1	29.93[b]	0	28.22

a-b any two means in the same column not followed by the same latter are significantly different

A comparison of the results of the present study with those reported by other investigators indicated that the extent of contamination on Ethiopian milk from grazing cows by AFM1 is considerably higher. A survey conducted in Nigeria in 2014 reported that the incidence of AFM1 contamination in fresh cow milk from free grazing cows was (0.009-0.456) ppb, and 64% of the positive samples exceeded the limit set by the European Union (Oluwafemi et al, 2014). Another study conducted in small scale dairy farms Zimbabwe in 2016 indicated the level of AFM1 contamination in in the range 074-1.30 ppb and only 30% of the samples were positive. Makau*et al.* (2016) reported that all milk samples from rural dairy system had AFM1 contamination below the EU limits ranging between 0 and 0.041 ppb. Analysis of raw milk samples from rural Punjab by Iqbal et al (2013) indicated that 52% of tested samples (n=48) were contaminated with AFM1 with a mean of 0.04 ± 0.034 ppb. Elzupir et al (2009) determined AFM1 levels in 44 milk samples from different dairy farms and vendors in Sudan and found out that all milk samples had an average AFM1 concentration of 2.07 ppb, ranging between 0.22 and 6.90 ppb.

The source of contamination of milk from grazing cows can be attributed to the type of feeding system. It has been reported elsewhere that dairy cattle that feed on aflatoxin contaminated feeds produce contaminated milk (Johanna et, al 2008). Though the grazing cows under this study were meant to be exclusively grazing ones, this was not the case. The seasons of May and June (the period of sample collection) are characterized by their dryness and grazing feed will be scarce. Such being the case, dairy farmers depend on additional feeds and give their cows what is available without considering the contamination of the feed by molds.

The KAP assessment of household dairy farmers indicates that their cows were not exclusively grass fed and were given waste from traditional beer (78%) and other left over food (22%) which are favorable media for growth and proliferation of aflatoxin producing fungi. A recent study conducted in AFB1 contamination in dairy feeds in Addis Ababa revealed the presence of the toxin in left over beer in concentration 15±4 ppb (Gizachew et al, 2016). The storage practice of these feeds was witnessed to be poor and it contributes to the high amounts of AFM1 in milk samples. Bryden (2012) stated that high ambient temperatures and high relative humidity are very conducive for the development of certain fungus to produce mycotoxins like Aflatoxin B1 (AFB1) which will be carried onto the milk.

The findings of the current study regarding the AFM1 levels in milk from non-grazing cows are also high as compared with that of many African and Asian countries. 35% (n=85) of milk samples from large scale dairy farmers in Urban centers in Kenya were found to have levels of AFM1 exceeding the limit set by WHO/FAo (0.05 ppb) (Kang'ethe et al, 2009). About 42% of milk samples (n=48) from urban dairy farms in Pakistan exceeded the limits 0.05 ppb. Milk samples in this study contained AFM1 concentrations up to eight times more than that reported in recent study conducted in greater Addis Ababa. Getachew et al (2015) reported AFM1 levels in milk samples from greater Addis Ababa in the range between 0.028 and 4.98 ppb and only 26.3% of the samples (n=110) exceeded the limit set by US (0.5 ppb).

The higher levels of AFM1 in milk samples reported here could be due to several factors such as difference in method used for analysis, sample size and season of sample collection. HPLC system can determine the concentration of aflatoxin with more sensitivity, determining the small amounts of dietary aflatoxin (Pirestani et al, 2011). The variation in level of AFM1 in milk samples can also be due to the differences in the levels of AFB1 in feeds the cows were given. In the current study, KAP assessment has enabled the finding that dairy farmers used to store their feed in a way that encourages mold growth.

Milk samples from non-grazing cows from farm 2 in Debrebirhan and farm 3 in Bishofu had very low AFM1 levels among their respective categories. This could be due to the difference in the dose of AFB1 in feed given to the cows. The levels of AFM1 in the milk however, are significantly influenced by AFB1 dosage (Battacone*et al.* 2003). The difference in the breeds of the animals can also influence the level of AFM1 level in milk. The rate of absorption of

aflatoxin, and the excretion of aflatoxin M1 in milk, varies between individual animals as the extent of transfer fromfeed to milk is influenced by variousnutritional and physiological factors, including feedingregimens, rate of ingestion, rate of digestion,health of the animal, hepatic biotransformationcapacity, and actual milk production (Johanna Fink-Gremmels, 2008).

Pasteurized milk samples sold in the market of Addis Ababa had very high levels of AFM1 contamination as compared to other studies. A study conducted in Iran resulted in AFM1 contamination of pasteurized milk samples (n=47) in the range between 0.0008-0.058 ppb and only 2.1% of the milk samples exceeded 0.5ppb. Another study conducted on commercial pasteurized (n=79) and UHT (n=60) milk samples in Brazil show that 15.2% and 28.3% of milk samples were beyond the 0.05ppb (Garrido et al, 2003) Pasteurized cow milk samples (n=30) were contaminated with AFAM1 with mean value 0.0595ppb, range (0.146-0.217) ppb in Jordan (Omar, 2016). In Addis Ababa milk processors collect milk from different small holder dairy farmers in different areas and process it and this has contributed to the high levels of AFAM1.

Aflatoxicol was present in 13% of the ultra-pasteurized samples (n=580) at concentrations exceeding 0.05 ppb and in 8% of the samples exceeding 0.5 ppb in Mexico and levels were not influenced by pasteurization(Carvajal et al, 2003).

When it comes to levels of AFM1 in pasteurized milk, contradicting findings exist. There are findings which report higher levels of AFM1 in pasteurized milk due to concentration of the heat resistance of the toxin during evaporation (Carvajal et al, 2003, Fallah, 2010; Flores-Flores et al., 2015; Murphy et al., 2006; Hosein et al, 2009). However some authors have reported reduced levels of AFM1 by pasteurization (Langat et al, 2016). A review by Benkerroum (2016) cites the works of different investigators and states a reduction in levels of AFM1 in milk by heat treatment up to 12-40%.

5. Conclusion and Recommendation

5.1 Conclusion

The carry-over of AFB1 from contaminated feed into milkas its hydroxylated metabolite, aflatoxin M1 (AFM1) has been the interest of many countries. The current study indicates the presence of aflatoxin in 93% of the tested samples, 86% with greater values beyond the Ethiopian standards (0.5ppb).

Milk from non-grazing cows had significantly higher AFM1 contamination than milk from grazing cows and milk from Addis Ababa. The higher contamination level of AFM1 in the milk samples from non-grazing cows indicate that cows in the study area are exposed to AFB1-contaminated feeds. The elevated level of AFM1 in milk from Addis Ababa is due to collection of milk samples from dairy farms from neighboring areas not covered in this study. Moreover, the study areas are usually characterized by high temperature and humidity between the monthsofMay and June which is a period where milk samples were collected, which accounts for the high levels of AFM1 in milk. High ambient temperatures and high relative humidity are very conducive for the development of certain fungus to produce mycotoxins like Aflatoxin B1 (AFB1).

The current study indicated that feed of the cows is the factor that contributes significantly to the presence of AFM1 in milk. Though the current study did not determine the level of AFB1 in the feed of the cows, KAP assessment indicated that 88.9% of the feed for non-grazing cows were handled poorly, in a way that favors the growth of aflatoxin.

The sample size of pasteurized milk samples (n=3) analyzed here might not be enough to draw conclusion but the finding highlights the existing danger in pasteurized milk. Milk processors only check for the presence of water and do not check for aflatoxin when they buy milk from producers.

5.2 Recommendation

- Longitudinal studies that cover wide areas of the country, in different seasons of the country need to be conducted to draw conclusions regarding the severity of aflatoxin contamination in milk.
- Monitoring and supervision activities should be widespread and be part of the routine test in dairy industries and feed processors.
- Agencies that support the dairy sector of Ethiopia should focus on awareness creation among dairy farmers and feed processors to minimize aflatoxin contamination and to encourage the adoption of process-based guidelines good manufacturing practices.
- Since heat treatment cannot destroy aflatoxin in milk, dairy processors need to be enforced to implement quality assurance systems that ensure the absence of aflatoxin in pasteurized milk.
- Beside preventive strategies, several physical, biological,and chemical methods need to be investigated and implemented for their effectiveness to remove or inactivate molds in animal feed.
- Government and stake holders those are in the sector needs to sit together and develop short term and long term strategies/plans that helps to reduce and prevent the current aflatoxin level in the country
- Develop and supplement the disease surveillance, food monitoring, laboratory, and public health response capacity of affected areas and government agencies.
- Collaboration between the agricultural and public health communities, between the local, regional, national, and international governing bodies, and between different disciplines within public health and agricultural is necessary to reduce aflatoxin exposure.
- Sustainable good practices should be maintained for all feeds, feeding practices, milk contamination and animal health and records of practices and performance for all cases of aflatoxin contamination of milk

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Annex 1

QUESTIONNAIRE

PART 1.SOCIO-DEMOGRAPHIC CHARACTERISTICS

Socio Demographic Characteristics of dairy farmers (cattleman), in the study sites

Question Number	Question	Response	Instruction
1	Age (year)		
2	Sex	1. Male 2. Female	
3	Marital Status	1. Single 2. Married 3. Divorced 4. Widowed	
4	Educational status	1. Unable to Read and Write 2. Able to Read and write without formal education. 3. Primary school 4. Secondary school 5. Higher Institution 6. Other	
5	Monthly Family Income	…….Birr	
6	Position in the house hold	1. Household head 2. Spouse of household head 3. Child of house hold head 4. Relative of house hold head 5. Other (specify above)	

Socio Demographic Characteristics of dairy processing in the study sites

Question Number	Question	Response	Instruction
1	Age (year)		
2	Sex	1. Male 2. Female	
3	Marital Status	1. Single 2. Married 3. Divorced 4. Widowed	
4	Educational status	1. Degree and above 2. Diploma	
5	Monthly Income/ salary	…….Birr	
6	Occupation	1. Employee 2. Share holder	
7	Work experience on the area	1. < 2years 2. ≥3years 3. >5years	

PART II KAP QUESTIONNAIRE ON AMONG FARMERS/ DAIRY FARMERS /AND DAIRY PROCESSORS.

KAP QUESTIONNAIRE ON AMONG FARMERS

Question Number	Question	Response	Instruction
Knowledge (K)			
1	Do you have any awareness on mycotoxins /aflatoxins /	Yes No	
2	Knowledge on possible source of mycotoxins.	Yes No	
3	Knowledge on advantages using field grazing feeding over other processed dairy feed product related to mycotoxin contamination	Yes No	
4	Knowledge on the effect of mould contaminated feed dairy products and health impact on consumer	Yes No	
Practice			
1	Can you guarantee as your cow feeding is exclusively on grazing	Yes No	If No Q 2
2	Type of feed mostly used beside grazing for milking cow		
3	At what level the cow is in use of on field grazing feeding	Most Least	
Attitude			
1	Why you prefer grazing than commercial feed	cost-effective Productivity /yield Accesses	
2	Which feeding mechanism is preferable from **the two**	Grazing Dairy Feed	
3	how do you prefer grazing than dairy feed	Economical Access quality problem on dairy feed	

KAP QUESTIONNAIRE ON AMONGDAIRY PROCESSORS

Question Number	Question	Response	Instruction
Knowledge (K)			
1	Awareness on Monitoring/inspecting for the presence and absence of mycotoxin in the raw milk during delivery and before processing /	Yes No	
2	Knowledge about food toxin like mycotoxin.	Yes No	
3	Knowledge on the root causes of mycotoxin presence in milk and conditions which favors it	Yes No	
4	Knowledge expectance of mycotoxin present in milk and its significance	Yes No	
Practice (P)			
1	Storage conditions of feed if they have dairy farm alongside	Yes No	If No Q 2
2	Type of feed mostly used beside grazing for milking cow		
3	Where is the source of raw milk	• Individual Farmers • Cooperative Raw milk suppliers	
	Product testing like mycotoxin testing	• Yes • No	
Attitude (A)			
1	Assumption of mycotoxin eliminate by pasteurization	Yes No	
2	Expectance of aflatoxins present in pasteurized milk and its significance	Yes No	
3	Your assumption that possibility of removing aflatoxine from the source with some intervention	• Possible • Not possible	