ADDIS ABABA UNIVERSITY
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AFLATOXINS, HEAVY METALS, AND SAFETY ISSUES IN DAIRY FEEDS, MILK AND WATER IN SOME SELECTED AREAS OF ETHIOPIA

BY

REHRAHIE MESFIN GEBREHIOWT

A THESIS PRESENTED TO THE SCHOOL OF GRADUATE STUDIES OF THE ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE PHD DEGREE IN BIOLOGY (APPLIED MICROBIOLOGY)

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DEDICATION

The thesis is dedicated to the almighty God who owe the infinite knowledge and strength and St. Merry for her eternal support in my life.

DECLARATION

I declare that the thesis, hereby submitted by me for the PhD degree in Applied Microbiology to the School of Graduate Studies of Addis Ababa University is my own work and has not been submitted by me or anybody else where. Materials obtained from other sources are duly acknowledged in the thesis.

Signed on February 2018, the School of Graduate Studies, Faculty of Life Sciences, Addis Ababa University.

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LIST OF ABBREVIATIONS

ACCSQ	Asian Consultative Committee on standards and quality
ACCSQ	Asian and Pacific Standard and Asian Consultative Committee on standards and quality
AFB1	AflatoxinB1 found in feeds
AFM1	AflatoxinM1 found milk
AOAC	Association of Official Analytical Chemists
APHRD	Animal and plant health regulatory directorate
ARSO	Regional Organization for Standardization
As	Arsenic
BSI	Britain standards
CAC	Codex Alimentations Commission
Cd	Cadmium
CEN	European Committee for Standardization
CP	Crude Protein
Cr	Chromium
CSA	Central Statistics Agency
^{o}C	Degree Centigrade
ECETS	European Committee for Electro technical Standardization
ECAE	Ethiopian Conformity Assessment Enterprise
ECOPA	Ethiopian Consumers Protection Associations
EAAPP	East African Agricultural Productivity Project
EAFA	Ethiopian Animal Feed Industry Association
ECETS	European Committee for Electro technical Standardization
ELISA	Enzyme-linked Immunosorbent Assay
EMDTI	Ethiopian Dairy and Meat Technology Institute
EPA	Environmental Protection Authority
E U	European Union
ETSI	European Telecommunications Standards Institute
ES	Ethiopian standards
ESA	Ethiopian Standard Agency

ETB	Ethiopian Birr
ETSI	European Telecommunications Standards Institute
FAO	Food and Agriculture Organization
EPA	Environmental Protection agency
^{o}F	Degree Fahrenheit
FMHACA	Food Medicine and Health Administration and Care Authority
GAP	Good Agricultural Practices
GDP	Gross Domestic Product
GFAAS	Graphite Furnace Atomic Absorption Spectrophotometer
GHP	Good Hygienic Practices
GIS	Geographic information System
GLM	General Linear Model
GLP	Good Labeling Practices
GMP	Good Manufacturing Practices
GPP	Good Packaging Practices
HACCP	HACCP Hazard Analysis and Critical Control Points
HCL_4	Perchloric acid
HNO_3	Nitric acid
H_2O_2	Hydrogen peroxide
IEC	International Electro-technical Commission
IGAD	Inter-Governmental Authority on Development
IS	Indian standards
ITU	International Telecommunication Union
Kcal	Kilo calorie
MoA	Ministry of Agriculture
MOFED	Ministry of Finance and Economic Development
ME	Metabolizable Energy
NCC	National Codex Committee
NRC	National Research Council
ISO	International Organization for Standardization
Pb	Lead

QSAE	Quality and standards authority of Ethiopia
SAS	Statistical Analysis System
SAQI	South African Quality Institute
SD	Standard deviation
SE	Standard error
SNNP	Southern Nations, Nationalities and Peoples Region
WHO	World Health Organization
µg/kg	Microgram per kilogram
µg/L	Microgram per liter
OD	Optical density
OEML	International Organization of Legal Metrology
pg/ml	Picogram per milliliter
TFAF	Task Force on Animal Feeding
TCFA	Texas Cattle Feeders Association
UNIDO	United Nations Industrial Development Organization
USDA	United States Department of Agriculture
U.S.A.	United States of America
WTO	World trade Organization

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Evaluation of Aflatoxins, Heavy Metals and Safety Issues in Dairy Feeds, Milk and Water in Selected Sites of Central Ethiopia

Rehrahie Mesfin, Fassil Assefa and Getnet Assefa

Abstract

The production of wholesome milk is controlled by the quality and safety of feed supply. Aflatoxins and heavy metals are some of the major factors that affect the quality of feeds and water sources that are transferred and eventually get bio-accumulated in livestock species and humans via meat, milk and milk products. Monitoring dairy production inputs using technical tools and gathering appropriate information on perception, experience and indigenous knowledge of stake holders along the feed and milk chains are relevant in assessing how processing, storage and distribution of feeds and water sources to ensure safety of milk and milk products. The objective of this study was to determine aflatoxin B1 (AFB1) in feeds and aflatoxin M1 (AFM1) in milk and heavy metals cadmium (Cd), lead (Pb), arsenic (As) and chromium (Cr) in feeds, water, and milk samples from West Shoa, East Shoa and Hawassa, Ethiopia. A total of 205 samples consisting of 115 concentrate feeds, 45 roughage feeds and 45milk samples were collected for the detection and quantification of aflatoxin using Enzyme-linked Immunosorbent Assay (ELISA). A total of 90 samples (30 feeds, 30 water and 30 milk) were collected for determination of heavy metals using Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS). Stakeholders' perception and experience of handling feeds and water sources were evaluated by interviewing peri-urban farmers, feed processors, feed retailers and urban dairy producers using semi-structured questionnaires and field observations. The results showed half of the feed samples (81) were free from aflatoxin, and the remaining (79 samples) were within the EU standard of 5µg/kg and the USA standard of 20µg/kg. The pattern of afltoxin contamination showed that concentrate feeds were more contaminated (7.67 ± 0.80 µg/kg) than roughage feeds (0.41 ± 0.14 µg/kg); hay (0.72 ± 0.25 µg/kg) was more contaminated than straw (0.05 ± 0.05 µg/kg) and oilseed cake based concentrate feeds were more contaminated (13.09 ± 1.12 µg/kg) than concentrate feeds without oilseed cake (2.78 ± 0.66 µg/kg). The average AFB1 of feeds in Bishoftu (9.76µg/kg) was significantly higher ($p<0.05$) than the sampling sites in Holetta (6.33µg/kg) and Hawassa (1.19µg/kg). The AFB1 of feeds handled by dairy producers was significantly higher ($p<0.05$) (9.35 ± 1.04µg/kg) than feed retailers (6.91 ± 1.09 µg/kg) and

feed manufacturers (7.50 ± 1.43 µg/kg). The AFM1 of milk was in a range and average of 0–0.146 µg/L and 0.054 µg/L respectively of which 29% of the milk samples did not contain aflatoxin, and 58% of them had AFM1 level within the EU permitted limit of 0.05µg/L and 42% of the samples were less than the U.S.A. recommended limit of 0.5 µg/L. The AFB1and AFM1 levels of milk samples collected from the study locations were in the order of Hawassa < Holetta < Bishoftu. With regards to heavy metals, the data showed that concentrations of heavy metals in teff straw in Holetta and Bishoftu were 1543.54 ± 318.70 µg/kg and 1486.92 ± 279.73 µg/kg, respectively. The overall concentration of heavy metals in teff straw was in the order of Cr > As > Pb > Cd. The water samples taken from Mojo areas (Eastern Shoa) showed the highest of heavy metals (43.64 µg/L - 86.89 µg/L) with very high concentration of Cr (300.56 µg/L). In general, the average concentration of heavy metals in livestock water in Eastern shoa (Akaki to Mojo) (28.08 ± 7.02 µg/L) was significantly higher ($p<0.05$) than the levels of heavy metals in water collected from Western Shoa (Holetta/Welmera) (1.96 ± 0.28 µg/L) and the levels of the heavy metals was in the order of Cr > As > Pb > Cd. With the exception of pH of water from Mojo Lake (10.37) and Gelan dye factory (8.9), the rest of the water samples collected from Bishoftu and Holetta areas were within the legal pH limit of 6.5-8.5 for livestock drinking. The overall concentration of heavy metals in cow milk samples was in the order of Cr > Cd > Pb > As. The concentrations of Cd and As in milk were within the permissible limits. However, 60% and 73% of the milk samples from Holetta and Bishoftu respectively for Pb and, all the milk samples in both study locations for Cr were above the permissible limits indicating poor quality of milk due to environmental pollution. The data from the interview of stakeholders showed that 91% of the farmers sometimes encountered mold formation in roughage feeds due to lack of good harvesting and stacking practices. Most of the farmers admitted to feeding light moldy feeds to their livestock by diluting with uncontaminated ones. Most of the respondents (67%) used extreme moldy feeds for firewood; and 33% of the interviewees damped the extreme moldy feeds into landfills. Farmers recognized two causes of water contaminants associated with health and production problems in livestock. Accordingly farmers from Eastern Shoa (100%) were aware of the effect of industrial effluent as the most important hazard for dairy production; whereas 66% of the farmers from Eastern Shoa and 34% of the respondents from Western shoa identified leech problems in water bodies in dry season. Farmers also had indigenous knowledge to tackle the leech problem in that 69% of the farmers used bucket for selectively scooping water

from the water body to exclude the leech from being consumed by animals; whereas 50% of the respondents treated animals with chopped tobacco and onion. The majority of the feed processors (64%), feed retailers (82%) and dairy producers (56%) reported that they did not use palate for placing their concentrate feeds implying that there is probability of mold contamination in times of prolonged storage. Among the respondents, 88% of feed processors, all feed retailers and most (96%) of the dairy producers recognized that wheat bran was the most mold susceptible feed ingredient. Majority of the feed processors (67%), feed retailers (73%) and dairy producers (58%) stored their concentrate feeds for a short period of about 1 month. Majority of the feed processors (74%), feed retailers (87%) and most dairy producers (91%) did not encounter mold formation in their concentrate feed because of the small amount of concentrate feed they hold and shorter storage time. To overcome mold formation in concentrate feeds, 64% of the feed processors gave enough space between stored feed and the wall. Further research needs to be undertaken along the feed and milk production and distribution chains using other techniques such as HPLC, GC and multi-mycotoxin assay using LC-MS-MS taking into account different storage conditions such as use of palate, ventilation, and duration of feed storage on aflatoxin. The effect of mold growth in feeds on nutrient composition needs to be investigated. There is also a need for further investigation on heavy metals from soils and fodder feed samples grown in similar study locations.

Key words: *Arsenic, cadmium, dairy producers, concentrate feed, hay, storage time, mycotoxins*

Chapter 1. Introduction

1.1. Background of the Study

Globally, agriculture is one of the major sectors that help to ensure food security and economic growth. The livestock subsector contributes 40-85% of the world agricultural GDP and 75% of the rural and 25% of urban population depends on livestock for their livelihood (Nabarro and Wannous 2014). In most African countries livestock is the mainstay of 60%-80% of rural households for their incomes, nutrition, food security, manure, in the smallholder mixed crop/livestock and pastoral systems (Smith, 2015).

Livestock production is an integral part of agriculture occupying 30% of the terrestrial world (Thornto, 2010). About 1.3 billion of the world population is employed in the livestock market chain and livestock production supports the livelihood of 600 million poor smallholder farmers in the developing world (Thornton, 2010). The share of livestock production to the agricultural GDP was 33% in the developing world and 53% in the developed world. Livestock products also support 17 to the kilocalorie and 33% to the protein consumption (Thornto, 2010) globally.

Ethiopia is believed to have the largest livestock population in Africa with 56.71 million cattle, 29.33 million sheep, 29.11 million goats (CSA, 2015), and livestock resources are the sole source of livelihoods for about 10 million pastoral communities (Little *et al*., 2001). The total economic benefits from the sector from export of livestock and livestock products in the year 2008-2009 was estimated to be 113 billion ETB and 37.5 million US dollar, respectively. According to the estimate of 1999-2009 GDP of Ethiopia, livestock production contributed 13.15% to the GDP of the country (IGAD, 2017).

Ethiopia has good potential for dairy production and with the growth of urbanization, tourism industry and rising of income, there is an increasing demand for good quality milk and milk products (Francesconi, 2006). However, the productivity of livestock/dairying is constrained by different factors such as poor genetic potential, diseases problems, scarcity and poor quality of feeds (Smith, 2015) and poor quality of water (Matt and Sonja, 2012) which are the most important inputs for enhanced productivity, safe and good quality meat, milk and milk products.

Mycotoxin contamination of feeds through fungal growth is the major constraint for supply of quality grains and feeds. Aflatoxins are one of the different types of mycotoxins produced by

fungal growth which flourishes under warmer temperature and appropriate moisture conditions (Ramesh and Siruguri, 2013). Apart from low quality feeds, water sources contaminated with heavy metals from industrial effluents affect the quality of dairy products (Swarup and Dwevedi, 1998; Kaplan *et al.,* 2010). Fodder feeds are the other reservoir of heavy metals (Rozso *et al*., 2003) which enters the food chain through livestock and channels to human being through livestock products (Raj *et al.,* 2006; Kaplan *et al*., 2010).

1.2. Statement of the Problem

Ethiopia has different agro-ecologies suitable for rearing of different livestock species. The country has also a range of rainfall pattern (1000-1900 mm/year) and temperature (0-30^{o}C) conditions that are suitable for production of high-yielding dairy cows and cultivation of different forage species (Ahmed, 2002) which is considered as an opportunity for increased production the dairy industry.

However, there are constraints that challenges the dairy sector such as poor genetic potential of indigenous animals, animal diseases, feed shortages (Keskin, 2006), poor quality feeds (Smith, 2015) and poor quality water (Matt and Sonja, 2012).

To overcome theses constraints livestock research in Ethiopia has been focusing on generating technologies that help to increase the quantity of feeds and productivity of livestock and less attention was given to the quality of feeds and water.

However, with the increasing rate of human population, urbanization and growing of public income, there is an increasing demand for good quality milk and milk products (Francesconi 2006). The ever increasing trend of tourism industry in Ethiopia and the issue of globalization is also good impulse for maintaining the quality of milk and milk products.

The production of good quality milk is determined by the supply of good quality feeds and good quality water (Upadhaya *et al*., 2010). However, the quality of feeds and water is challenged by various contaminants. Mycotoxins (Ramesh *et al*. 2013) & heavy metals (Enb, *et al*., 2009) are among the major contaminants that deteriorate feeds, milk and water.

Aflatoxins are one of the different types of mycotoxins produced by fungus species in warmer temperature and in appropriate moisture conditions (Ramesh and Siruguri, 2013). Among the 14 known types of aflatoxins (Chandra *et al*., 2013), B1, B2, G1, G2 were distinguished by their fluorescent light under ultraviolet medium as dominant and common aflatoxins groups (Reddy and Waliyar 2000). Among these groups of aflatoxins, aflatoxin B1 is the most prevalent, potent and class 1 carcinogenic (Feddern, *et al*., 2013).

When aflatoxin B1 in feeds is ingested by lactating animals, it is hydroxylated and metablized in the liver in to aflatoxin M1 and released in to milk (Chandra *et al*. 2013).

Aflatoxins have received considerable attention as they pose risks related to reduction of yield and quality of grains and feeds. They also affect the health and productivity of livestock species. In human being, exposure to aflatoxin causes liver cancer (in adults), stunted growth & increased susceptibility to diseases (in children). Aflatoxin also affects domestic and international trade and the economy in general (Ramesh *et al*. 2013; Chandra *et al*., 2013).

Apart from aflatoxins, water and feeds can be contaminated by heavy metals (Lars, 2003). Heavy metals emanate from weathering of rocks, industrialization, domestic sewage & agrochemicals (Nicholson *et al*., 1998). Soils, water and air are the main reservoirs of heavy metals (Alloway, 1995) and consequently affect the safety of feeds and foods as well as human health along the food chain (Birghilla, *et al*., 2008; Reilly, 1991). Among the various entrance routes of toxic heavy metals to the food chain, the major ones are contaminated feeds and waters (Derakhshesh and Rahimi, 2012).

Heavy metals are capable of causing health problems by accumulating in vital organs such as kidney and liver and disrupt their normal function (Rajaganapathy *et al*., 2011). Heavy metals also interfere with the normal process of milk synthesis and affect milk yields in dairy cattle (Khan *et al*., 2012). They also interfere with the metabolism of other essential elements by displacing them from where they do vital biochemical and enzymatic reactions in the body (Dawd, 2010). Cadmium, lead, arsenic and chromium are among the major toxic heavy metals that pose pollution on soils, water and air and transfer to crops, vegetables and feeds; then through the food chain they pass in to livestock species and human being. Heavy metals are known for their toxicity even when taken in very small concentration (Akan *et al.*, 2009).

It was reported that problems related to management of industrial effluent are becoming a concern in Ethiopia, since industries do not implement pollution control activities and they discharge their wastes into nearby water bodies, open land with little or no treatment (Fisseha *et al*., 2012). In addition, the information on contamination status of livestock feeds and milk with aflatoxins (Dawit *et al*., 2016) and heavy metals (Dawd 2010; Tasew *et al*. 2014 and Muluken, 2014) are very scanty.

Against backdrop of all these constraints, there is an interest to study on contaminants and pollutants in comprehensive way in relation to livestock feeds, milk and water with particular emphasis on aflatoxins and heavy metals.

1.3. Objective of the Study

General objective

The overall objective of this study was to determine the level of aflatoxins and heavy metals in dairy feeds, milk and water, assess safety issues of agricultural products and risks of contaminations on livestock species in the central highlands of Ethiopia.

Specific objectives

1. To determine the contamination status of dairy feeds and milk with aflatoxins under laboratory condition
2. To determine the contamination status of dairy feeds, milk and water for heavy metals
3. To assess the feed storage practices employed by feed manufactures, feed retailers and dairy producers and identify risks of contamination on livestock.

Chapter 2. Literature Review

2.1. Livestock feed resources in Ethiopia

Though, Ethiopia has great potential for increasing livestock production, however, productivity is constrained by feed shortages and poor quality of the available feeds. According to Zewdie (2010), the issue of the quantity and quality of available feed resources for livestock requirement has not been yet well addressed in most livestock production areas of the country. According to Betre (2000), the feed requirement of indigenous livestock species in the Ethiopian highlands was estimated to be 55 million tones of dry matter (DM) compared to the estimated currently available 40.1 millions tones. This indicates that the annual DM production could satisfy only two-third of the total DM requirements of the livestock. Due to this, during the dry season animals lose weight which is an indicator of feed shortage (Funte *et al*., 2010).

Livestock feeding in Ethiopia is based on grazing or browsing mainly on natural pastures and stubble grazing following crop harvest (Table 2.1). Crop residues are the second most important roughage feed source, but they are low in protein and digestibility (Tesfaye *et al*., 2009). There is seasonal variation in feed availability and quality and feed supply is adequate especially in September, October and November, and satisfactory in December, November, January and February, but it is scarce in March, April and May (Endale *et al*, 2016).

During dry season, livestock feed is in short supply and is also of poor quality (based on crop residues). The remaining feed resources to livestock species included improved pasture and forage crops, agro-industrial by-products and other non-conventional feeds. In recognition of the increased price of agro-industrial by-products, the development of improved forage crop or improving the existing natural pasture through over sowing is alternative means of feed supply (Alemayehu *et al*., 2016).

Table 2.1. Available livestock feed resources in Ethiopia

Feed resource	Percent of the total feed
Grazing	56.2
Crop residue	30.1
Improved forage feed	0.3
Hay	7.4
Agro-industrial by-products	1.2
Conventional feeds	4.8

Source: CSA, 2014/15

2.1.1. Natural pasture

Natural pasture encompasses naturally occurring grasses, legumes, herbs, shrubs and tree foliage that are used as animal feed through grazing or browsing. Natural pasture is the main feed resource and is particularly important during wet season (Endale *et al*. 2016) and it constitutes the main highland pastures which are low in production of dry matter and the availability of herbage for the grazing animal which is restricted to four or five months of the wet season over most of the natural grasslands (Ulfina *et al*., 2013). In Ethiopia, the large numbers of indigenous grass species and the great variation within the species make the country to be rich in diversified pasture grass species. To date, there are a total of 736 grass species from 181 genera documented in Ethiopia, of which 164 species from 68 genera were reported to be important (medium to high level) for pasture and forage purpose-any (Alemayehu, 2005). The productivity of natural pastures in the Ethiopian highlands ranges from 1-2 ton DM/ha on freely drained and infertile soils and it could vary from 6-10 ton/ha on seasonally water logged fertile area (Adugna, 2008).

Livestock grazing is the predominant form of land use in pastoral areas which receive less than 600-700 mm annual rain fall. However in the highlands, better soils are used for cropping and the slop of hills and the seasonally water logged areas are allocated for grazing (Adugna, 2008). However, with the increasing human population and use of land for crop cultivation, the land available for grazing is steadily shrinking as it is being converted to arable lands, and is restricted to areas that have little value such as hill tops, swampy areas, roadsides and other marginal land.

2.1.1.a Herbaceous Legumes: The highlands are also rich in different herbaceous leguminous species and they tend to increase with increasing altitude. There is a wide diversity of annual and perennial *Trifolium* species, annual *Medicago, Vigna*, *Lablab*, *Neonotonia*, and others in the highlands, particularly above 2,000 meters. A total of 358 herbaceous forage legume species

from 42 genera have been documented in Ethiopia and reports indicated that about 58 species from 31 genera are potentially important for pasture and forage-any (Alemayehu, 2005).

2.1.2. **Hay from natural pasture**

Fodder conservation in the form of hay and silage is not a common practice in many parts of Ethiopia with the exception of the central highlands around Addis Ababa (Adugna, 2008). With increasing human population and utilization of land for crop cultivation, land allocated for production of hay is getting diminishing from time to time. Hay production areas are limited to swampy areas of the central highlands in North and West shoa zones particularly in Selalie and Ada Berga areas where there is a long practice of commercial hay production from natural pastures or medaw grass. Hay production is also limited to some protected areas like churches and other institutions like colleagues, universities and police stations. As a result the supply of hay to commercial dairy and beef producers in the country is limited resulting to extreme higher input cost of dairy production.

The locally produced native hay serves as useful source of roughage/ basal diet for dairy cattle and the nutritive value could be very variable depending on the botanical composition or the species of the forage crop, the stage of maturity at harvesting time and the practice employed during harvesting, drying and storage conditions (Adugna, 2008).

2.1.3. Cultivated forage and pasture species

Production of forage and pasture depend on availability of species that are adapted to the climate, edaphic and biotic factors prevailing in the environment. Suitability of forage species to a given area is judged based on dry matter yield, persistence, adequate feed quality, compatibility with other species and ease of propagation and establishment (Adugna, 2008).

Cultivated forage and pasture are mainly important as cut-and-carry source of feed and as a supplement to crop residue and natural pasture. The most commonly cultivated forage crops in the highlands include grasses like elephant grass, Rhodes grass, guinea grass and oats. Among the herbaceous legumes, the most common ones include desmodium, vetch, Lucerne, lablab and cowpea. The most common fodder tree legumes include Tagasaste, sesbania leucaena, calliandra calthyrsus, gliricidia, sepium and pigeon pea. Production of improved forages should focus on those species that have high biomass yield potential such as Napier grass, Rhodes grass, guinea

grass and buffel grass (Adugna, 2008). Leguminous forages are important sources of nitrogen, fermentable organic matter and minerals in crop residue and poor quality natural pasture diets.

2.1.3.a Napier grass: is one of the most promising and high yielding fodder giving dry matter yield that surpasses most tropical grasses and is adapted to many areas of Ethiopia. It grows well in a range of altitudes starting from low to higher altitude of 2000 m.a.s.l. It performs good in high rainfall humid and sub-humid areas but its deep root system allows it to survive in the dry seasons and the optimum temperature for its growth is 25-40 ^{0}C (Adugna, et al., 2008). Napier grass can be utilized as animal feed in different forms such as cut-and-carry green feeding, grazing and ensiling and it can also make good hay if cut at a young stage but becomes too coarse if harvested late. It is very compatible with legumes such as silver leaf and green leaf desmodium and leucaena. The annual dry matter yield of Napier grass varies between 10-40 ton DM/ha with an average of 16 ton DM/ha (Adugna, 2008).

2.1.3.b Rhodes grass: Rhodes grass is one of the cultivated grasses that is suitable for hay making. It grows under a wide range of soil condition except very heavy and very acid soils. It is also known for being drought tolerant and it performs best where the amount of annual rainfall ranges between 750 and 1500 mm. the CP content of Rhodes grass varies from 3-7% depending on stage of maturity and handling conditions (Adugna, 2008).

2.1.3.c Oat: oat is a tall annual cereal, widely grown as a fodder in temperate and sub tropical countries and performs very well in the highlands of Ethiopia. It may be grown in pure stand or in mixture with legumes such as vetch and oat hay has crude protein content of 6% (Adugna, 2008).

In suitable areas yield of oat-vetch mixtures (8-12 tons/ha) improved pasture and forage grasses (6-8 tons/ha) and tree legumes (10-12 tons/ha) was recorded and have been incorporated widely into the crop-livestock system. However, due to increased human population and utilization of land for crop production, land available for cultivation of forage crops is diminishing resulted to limited utilization of improved pasture and forages species (Alemayehu, 2002). Forage legumes tend to increase with increasing altitude and they are particularly abundant above 2,200 meters. There is a wide range of annual and perennial *Trifolium* species, and annual *Medicago* species. At lower altitudes native legumes are less abundant but browse trees and shrubs are the dominant plants (Alemayehu, 2002).

2.1.4. Leguminous fodder trees and shrubs

Browse trees or shrubs are important animal feeds in Ethiopia especially in the arid, semi-arid and mountain zones, where large numbers of the country's livestock are found. They can also applicable by integrating them with food crops. Leguminous fodder trees and shrubs provide protein, vitamins and mineral that is lacking in grasslands pastures and help to enhance livestock productivity (Alemayehu *et al*., 2016).

In Ethiopia there are 179 browse species from 51 genera, which is not exhaustive, of which 51 species from 31 genera are recorded as promising browse species. Currently 185 accessions from 41 species and 18 genera are systematically collected and conserved by ILRI (Alemayehu, 2005).Use of leguminous fodder trees and shrubs also assist in increasing soil fertility, controlling soil erosion and providing firewood and timber. These legumes are well adapted to the current edaphic and grazing condition, they can be readily/easily integrated into farming systems, and they retain their feeding value into the dry season and show great success in the higher potential areas of the country (Alemayehu, 2005).

There is also considerable opportunity for the use of fodder tree-legumes in agro forestry. Woody legumes can be grown as fodder hedge planted around the backyard. Beyond serving as animal feed, they can be utilized for firewood, for construction, use of farm equipment, wind breaks and for stabilizing bunds and gullies. Thus promotion of fodder trees-legumes in the agro-forestry system is a good opportunity for extension of a forage development program within farming systems which can contribute to environmental protection and natural resource management (Alemayehu, 2005). Thus, forage production strategies can be developed as agro-forestry systems including contour forage banks and or under sowing in a forestry or horticultural system (Alemayehu *et al*. 2016).

Table 2.2. Recommended species for contour forage strips in the agro-forestry system

Altitude (m)	Browse legumes	Forage legumes	Grasses
<2000	Leucaena	Siratro	Panicum
	Sesbania	Axillaries	Setaria
	Pigeon pea	Silver leaf	Vetiveria
		Greenleaf	
		Vetch	
		Verano stylo	
2000-2400	Treelucerene	Green leaf	Phalaris
	Sesbania	Axillaries	Setaria
	Pigeon pea	White clover	
		Native clover	
		Vetch	
		Alfalfa	
>2400	Treelucerne	White clover	Phalaris
		Native clover	
		Vetch	
		Maku lotus	
		Alfalfa	

Source: Alemayehu, 2002

One of the best opportunities for highland farmers to utilize the existing forage species is through integration of pasture and forages in the farming system. Integration of pasture and forage species can be done using different methods such as backyard, under-sowing and over-sowing. These strategies allow farmers proper use of their land for cultivation of crop/pasture and forage/trees, from where their products can be used for food, feed and firewood respectively. This practice was demonstrated in the highlands where wheat and barley have been under-sown with Lucerne, annual clovers, tall fescue, perennial rye grass, *Setaria* and *Phalaris.* All under-sown forages established successfully except Lucerne and there was no significant reduction of cereal yield. The establishment of forages was much better under wheat than under barley (Alemayehu, 2005). Cowpea, pigeon pea and *Phaseolus acutifolius* integrated in the cropping system can be used for food and feed especially during the dry season (table 2.3). At research sites in the mid-altitude areas, maize was under-sown with Desmodium, phasey bean, *Chloris* (Rhodes grass), *Panicum* and *Cenchrus* after the first weeding. Almost all forages established, and there was no maize yield reduction (Alemayehu, 2005). Since they contain good nutritional

composition, such as protein, vitamins and minerals, improved forage crops are used as supplemental feed.

Some perennial grasses can be planted vegetative, for example, *Festucaarundinacea*, *Phalaris arundinacea*, and *Setaria sphacelata* are well adapted to waterlogged conditions and easily established by root splits (Alemayehu, 2005).

Table 2.3. Forage species recommended for under sowing and intercropping in Ethiopia

Altitude (m)	Browse legumes	Forage legumes	Grasses
<2000	Not appropriate	Cow pea	Not appropriate
		Verano stylo	
		Greenleaf	
		Wynn cassia	
2000-2400	Not appropriate	Siratro	Not appropriate
		Greenleaf	
>2400	Not appropriate	Vetch	Not appropriate
		White clover	
		Native clover	

Source: Alemayehu, 2002

The other opportunity for development of improved forage species is through the application of backyard forage establishment. In Ethiopia there are different forage species recommended to be cultivated in different agro ecologies of the country (table 3).

Table 2.4. Recommended back yard forage species in Ethiopia

Altitude (m)	Browse legumes	Forage legumes	Grasses
<2000	Leucanea	Green leaf	Rhodes grass
	Sesbania	Silver leaf	Elephant grass
	Pigeon pea	Alfalfa	Panicum grass
2000-2400	Sesbania	Alfalfa	Phalaris grass
	Pigeon pea	Vetch	Elephant grass
	Treelucerne	Verano stylo	
>2400	Treelucerene	Alfalfa	Phalaris grass
		Vetch	Oats

Source: Alemayehu, 2002

2.1.5. **Crop residues**

Crop residues are the second most important feed resource for livestock after grazing on natural pasture and cereal straws are the dominant crop residues in the highlands (Getnet and Ledin 1999). Crop residues include straws, stovers and haulms. Cereal straws include teff straw, wheat straw, barley straw and cereal stovers consists of maize stover and sorghum stover. Haulms of legume crop include haricot bean, field pea, chick pea, lentil, bean pea and cowpea (Dereje, et al., 2014; Rehrahie unpublished data). With the increasing human population, increasing expansion of land for cultivation of food crops, the decline of communal grazing lands, private pastures and forest areas as feed resources enhance the increase in the use of crop residues and purchased feeds (Bennin *et al*., 2003).

The nutritional value of crop residues is variable depending on the species and variety of the crop, time of harvest, handling and storage conditions. Cereal straws and stovers have lower nutritive value than the haulm from legume grain and/or vines from root crop such as sweet potato. The haulms of pulse crops (grain legumes) represent good quality roughage with a crude protein content of 5-12% (Adugna, 2008). In spite of the fact that these feeds resources represent a vast resource which is still not fully exploited, they fail to meet the productive functions of livestock species, as they contain low protein content and digestibility (Yitay *et al*., 2000).

Therefore, livestock nutrition need to be supplemented with improved feeds that are rich in nutrients since in particular dairy cattle cannot obtain their nutrient requirements from grazing and crop residues therefore, they need to be supplemented with feeds that are rich in energy and protein content like concentrates feeds (Kudo *et al*., 1994). Recently, market oriented dairy producers are changing their production systems from entirely depending on hay as sole roughage feed to a mixture of hay and different concentrates (agro-industrial by-products) as supplemental feeds for crossbred dairy cattle.

2.1.6 Agro-industrial by-products

Agro-industrial by-products are the by-products of primary processing of crops obtained from small scale and large scale flour milling, oil extracting, sugar cane and beer producing factories which can be called concentrate feeds and they are characterized by high energy and/or protein contents (Adugna, 2008).

2.1.6.1 Oilseed cakes

They are the residues or cakes that are produced as by-products during extraction of oil from oilseeds which include noug seed cake, cottonseed cake, linseed cake, peanut seed cake, sesame seed cake, sunflower seed cake and cakes obtained from other oilseeds. Oilseed cakes are rich in protein and serves as protein supplementation in concentrate mixture and they contain 20-50% protein and 0-12% fat depending on the type of the oilseed and method of extraction, but low in essential amino acids cystine and methionine and have variable and low lysine content. Oilseed cakes can also supply considerable amount of energy ranging from 2.03 - 3.7 Mcal ME/kg (Adugna, 2008)

2.1.6.1.a. Noug seed cake

Noug seed cake is one of the oilseed cakes commonly used as protein supplement for livestock in Ethiopia. About 84802.34 tones of noug seed is processed every year in the country from which about 50% noug seed cake is produced (Adugna, 2008). The protein content of noug seed cake varies from 28-38% with most values lying between 30-35%. The fat content varies from 2.1-12.6% with an average of 8.4% and an energy value of 2.37 Mcal ME/kg DM. It has high fiber (34.4% NDF and 8.4% lignin) content and low digestibility of 61.7% compared to most other oilseed cakes (Adugna, 2008). Noug seed cake can be highly lignified if the seed is not dehulled before extraction. When added to energy source feeds, noug cake can improve feed intake, digestibility and animal performance.

2.1.6.1b. Cottonseed cake

It is a by-product obtained after extraction of oil from cottonseed using either of the two extraction methods (mechanical or solvent) oil extraction methods. It has high protein content usually more than 36% and the protein content of most cottonseed cakes is usually around 40% (Adugna, 2008). It is an excellent protein supplement for ruminants, but it is deficient in vitamin D, carotene and calcium, but rich in phosphorous and it also contains a toxic substance known as gossypol which could be harmful to very young animals that does not yet have functional rumen.

However, gossypol toxicity is not harmful to adult ruminants because of their ability to detoxify free gossypol in their rumen (Adugna, 2008).

Cottonseed has a thick husk which is rich in fiber and low digestibility and the presence of the husk lowers the nutritive value of the cottonseed cake. Thus un-decorticated cottonseed cake is suitable only for feeding of adult animals (Adugna, 2008). The husk can be completely or partially removed by the process of decortications, a process that involves cracking and riddling. Removal of the husk lowers the fiber content of the cake thereby improving the digestibility of the other nutrients. In general the crude protein content of cottonseed cake may vary from 21-55% depending on the oil extraction method and whether the seeds were decorticated or not before extraction (Adugna, 2008).

2.1.6.1. c. Linseed cake

Linseed cake has a crude protein content of about 30% and it is one of the most popular supplements because of its high protein content and palatability. Linseed cake has a slight laxative effect which helps to keep the animal healthy. It is unique among the oilseed cakes because it contains about 3-10% mucilage. It appears to have a conditioning effect on animals, thus it is useful for fattening animals as it can produce rapid gain and attractive appearance on fattened animals (Adugna, 2008)

2.1.6.1. d. Groundnut or peanut cake

Peanut cake is produced when oil is extracted from peanut, which is composed principally of the kernel with some portion of the hull. It has higher protein content of around 50% than the other oilseed cakes. It is palatable, high quality protein supplement; although the protein is lower in methionine content compared to the other oilseed cakes. Peanut cake contains 2-7% oil and low fiber content (Adugna, 2008). Peanut is predominately produced in Hararghe, Eastern part of the country and the main producer of peanut cake is Hamaresa edible oil factory near the town of Harar (Adugna, 2008).

2.1.6.2. Flour milling by-products

Wheat bran, wheat short, wheat middlings and rice bran are produced during milling of grains to produce flour for human consumption. The different milling byproducts are mostly utilized by market oriented livestock production.

2.1.6.2. a. Wheat bran

Wheat bran is the most common milling by-product used for livestock feeding in Ethiopia (Rehrahie unpublished data). Wheat screening is broken or shriveled kernel plus some foreign materials such as weeds. Wheat grain consists of about 85% endosperm, 15% bran and 3% germ (Adugna, 1980). It is physically fibrous and flakey produce and it is the outer kernel plus some flour with a protein content of 14-18% and metabolizable energy content (ME) of 12MJ/kg and has high phosphorous (1%) but low Ca (0.1%) content. Wheat bran is palatable and known for its laxative characteristics because of its swelling and water holding capacity which is due to its high fiber and non-starch carbohydrate content (Adugna, 2008). It is one of the energy source concentrates containing easily digestible carbohydrate in the rumen supplying higher energy to the animal. Wheat bran contains high protein content of about 17% and it is a good source of most of the water soluble vitamins except niacin. The crude protein in wheat bran has a relatively high digestibility of about 75% (Adugna, 2008). The fiber and energy content of wheat bran depend on the quality of wheat being milled and the processing methods used. When added to protein source feeds, wheat bran can improve feed intake digestibility and growth performance of animals.

2.1.6.2. b. Wheat middling

It is a by-product of wheat milling industry containing a higher proportion of germ and flour than wheat bran and it includes screenings, bran, germ and flour remnants and has. About 92% of the energy value of maize grain and contains more protein than maize. It contains about 40% NDF, which is highly digested in the rumen. Wheat middlings are highly palatable and can be included in the ration at a level of about 15-25% of the total ration dry matter (Adugna, 2008).

2.1.6.2. c. Rice bran

Rice production is a recent introduction to Ethiopia. About 86% of the rice produced is in Amhara Regional State and the remaining 14% is produced in Oromiya, Benshangul-Gumuz and Gambela regions. In areas where rice is cultivated and processed, rice bran rice hulls and broken rice grains are produced as by-products. Rice bran consists of the fibrous outer layer of the grain, some hulls and chipped grain. It may also contain calcium carbonate, which is added during the milling process. Rice by-products are characterized by high fiber and low energy content (Adugna, 2008). The bran is the most nutritious part of the rice crop with a protein content of about 9% and fat content of 8%. Rice bran is an excellent source of vitamin B and E. The high oil content in rice bran increases its energy value but it may cause rancidity under warm and humid climatic condition thereby reducing palatability and increasing toxicity (Adugna, 2008).

2.1.6.2. d. Occasional surplus grain or grain damaged during processing

These grain by-products are produced during processing and seed cleaning. Such by-products of cereal grains have higher digestibility of 80-85% and have a protein content of about 8-12%, but are low in calcium and can be supplemented with limestone to correct the calcium deficiency. Excess consumption or rapid introduction of grains can cause sickness such as bloat and acidosis and death of animals (Adugna, 2008).

2.1.6.2. e. Molasses

These are by-products produced from sugar cane processing and they contain high level of sugar and are easily digestible in the rumen furthermore, they are good sources of drought feed supply. Molasses is also good sources of minerals such as calcium, potassium, sulfur and trace minerals, they are deficient in nitrogen and phosphorous. Molasses are concentrated sources of feed that can be stored for a very long period of time. Since the protein content of molasses is negligible, they are usually fed with high quality protein such as urea. Molasses is often used as a carrier for urea because they are palatable and provide some minerals (Adugna, 2008).

2.1.6.2. f. Brewery by-products

The different breweries existing in different regions of the country are supplying brewery residues which can be utilized as supplemental feed to livestock species (Rehrahie, unpublished data). Apart from commercial beer factories small scale home breweries and distilleries that can produce alcoholic beverages supply by-product of Tella atela and Arekie Atella which can be used as supplemental feed to livestock species. The crude protein (CP) and ME content of

brewery residue, Tella Atela and Arekie Atela have been reported to be 24.4% and 8.4 MJ/kg, 20.2% and 9.7 MJ/kg and 17.8% respectively (Adugna, 2008).

Despite the inadequate supply of agro-industrial by-products, farmers have the understanding of the benefit of concentrate supplementation for dairy cattle. However, the ever increasing prices limit producers from using it (Benin *et al*., 2003).

2.2. Fungi and mycotoxins

2.2.1. Fungi

Fungi are single cell or muticellular heterotrophic organisms with no chlorophyll which live by absorbing nutrients from organic matter. Fungi include mildews, molds, mushrooms, rusts, smuts, and yeasts (Sarah *et al*., 2008). Fungi are heterotrophic, and the molds form filamentous hyphae that produce spores and they can produce metabolites that are toxic to animals and humans (USDA, 1999). Reproduction in fungi occurs through the production of sexual and asexual spores and fungal infection begins from spores at any of the various stages of crop production and storage processes (Sarah *et al*., 2008).

2.2.2. Mycotoxins

Mycotoxins are secondary metabolites produced by several fungal species which severely affect the metabolic activities of plants, animals, and humans. So far, over 300 mycotoxins have been identified but the dominant types include aflatoxins, those produced by *Aspergillus* spp, Ochratoxin which are produced by *Penicillium* spp, Fumonisins produced from *Fusarium* spp and Ergot which are released by *Claviceps* spp (Derek, 2010) and these mycotoxins have been implicated in acute mycotoxicosis in both humans and farm animals (Ramesh and Siruguri, 2003).

Mycotoxins have been described for many decades, but they have only been chemically characterized with the discovery of aflatoxins in 1960s (Feddern, *et al*., 2013). Mycotoxins can develop during production, harvesting, or storage of grains, nuts, and other crops (Ramesh and Siruguri, 2003). Prolonged exposure for mycotoxins through diet has been linked to kidney and liver cancer as well as weakening of the immune-system (Rios *et al*., 2013).

Mycotoxins occur more frequently under tropical climate where high temperature and humidity are prevailing. In addition, diets in many tropical countries are more heavily concentrated on crops corn and nuts that are susceptible to mycotoxins; consequently, chronic health risks are particularly prevalent in such countries (Ramesh and Siruguri, 2003). Some mycotoxins are produced before harvest (DON, ergot); some occur following harvest (fumonisin, ochratoxin); and a few predominantly occur during storage (aflatoxin) (Ramesh and Siruguri, 2003).

Generally, tropical climatic conditions such as high temperatures and moisture, monsoons, unseasonal rains during harvest, and flash floods lead to fungal proliferation and mycotoxins

production. Poor harvesting practices, improper storage and poor transport and marketing conditions also contribute to fungal growth and proliferation of mycotoxins.

Among the mycotoxins, aflatoxins (Aflatoxin B1, B2, G1 and G2) raise the most concern and among the different forms of aflatoxin, aflatoxin B1 is found widely and in higher concentrations throughout the world in foods such as maize, peanuts and peanut cottonseed products. Outbreaks of aflatoxic hepatitis in humans have been reported in India, Kenya, and Malaysia (Ramesh and Siruguri, 2003).

Epidemiological studies carried out in several parts of Africa and Asia showed a correlation between exposure to aflatoxins and liver cancer. The risks associated with exposure to aflatoxins are enhanced by simultaneous exposure to hepatitis B and possibly hepatitis C viruses. Studies carried out in some West African countries including Benin, Gambia, and Togo indicated chronic exposure of population groups and fetuses to dietary aflatoxins (Ramesh and Siruguri, 2003).

Children exposed to aflatoxin become stunted, underweight, and more susceptible to infectious diseases in childhood age and in a later life. Many acute disease outbreaks from exposure to DON have also been reported in China and India. Consumption of ergot in pearl millet and other grasses has resulted in acute nausea, vomiting, and dizziness in India and East African countries, and gangrene, a classic ergot poisoning symptom in Ethiopia (Ramesh and Siruguri, 2003). Consumption of moldy sorghum or maize contaminated with fumonisin has been associated with occurrences of esophageal and liver cancers and with the development of neural tube defects in womb (Ramesh and Siruguri, 2003).

Table 2.5. Types of mycotoxins and corresponding toxigenic fungi

Mycotoxin	**Fungus species**
Deoxynivalenol (DON, vomitoxin), Fusarium graminearium	*Fusarium graminearium*
	Fusarium culmorum
T2 toxin & HT2 toxin	*Fusarium poae*
	Fusarium langsethiae
Fumonisins	*Fusarium verticillioides*
	Fusarium proliferatum
Aflatoxins B_1, B_2, G_1 & G_2	*Aspergillus flavus*
	Aspergillus parasiticus
Ochratoxin A	*Aspergillus ochraceus*
	Penicillium verrucosum
Ergot (ergotamine, ergometrine)	*Claviceps purpurea*

Source: Derek (2010)

Mycotoxic molds attack the kernels of grains thereby reducing the nutrients such as fat, protein, and vitamin content of the grain. Mycotoxin ingestion, inhalation or dermal absorption can cause different diseases and even death (USDA 1999). It was estimated that around 25% of the cereals produced in the world in 2009 was infected by fungi that had negative impact and food safety risks on growers, cereal food business operators and animal feed manufacturers (Food Business Africa, 2017). According to Derek (2010) it is not possible to completely eliminate fungal infection and mycotoxin contamination, but it is plausible to minimize to a lower level.

2.2.3. Aflatoxins

The word aflatoxin is the combination of three words "a" for *Aspergillus* genus, "fla" for the species *flavus* and toxin, meaning poison (Feddern, *et al*., 2013). Aflatoxins are characterized by colorless to pale-yellow crystals, intensely fluorescent to ultraviolet light, emitting blue (aflatoxins B1 and B2) or green (aflatoxin G1) and green–blue (aflatoxin G2) fluorescence, from which the designations B and G were derived and aflatoxin is classified in to four common groups as B1, B2, G1, and G2. Contaminated grains and byproducts of grain are the most common sources of aflatoxin.

Aflatoxin B1 is recognized by the International Agency for Research on Cancer as one of the most naturally occurring toxic and carcinogenic substances found in nature (Feddern, *et al*., 2013). Aflatoxin producing fungi *spp* and the moulds naturally originate in the soil and decayed vegetation in which risks of contamination begins with planting and can be worsened later during post harvest practices through inappropriate harvesting, handling, storage, processing, and transport practices (Ephrem, 2015).

The degree of contamination during crop production and after harvest also depends on environmental conditions that are optimal for the growth of fungi. Aflatoxins are very slightly soluble in water (10–30 μg/mL), insoluble in non-polar solvents, freely soluble in moderately polar organic solvents such as chloroform and methanol especially in dimethyl sulfoxide (Feddern *et al.*, 2013). They are unstable to ultraviolet light in the presence of oxygen, pH extremes (<3, > 10) and to oxidizing agents. The lactones ring of aflatoxins is susceptible to alkaline hydrolysis and degraded by ammonia or sodium hypochlorite (Feddern, *et al*., 2013).

Table 2.6. Physical and chemical properties of aflatoxins

Aflatoxin	Molecular formula	Molecular weight	Melting point
B1	$C_{17}H_{12}O_6$	312	268-269
B2	$C^{17}H_{14}O_6$	314	286-289
G1	$C^{17}H_{12}O_7$	328	244-246
G2	$C^{17}H_{14}O_7$	330	237-240

Source: Feddern *et al*. (2013).

Aflatoxins have the ability to contaminate agricultural commodities and can cause sickness or death in humans and animals. The risk of aflatoxin contamination of food and feed in Africa has increased due to environmental, agronomic and socio-economic factors (Ephrem, 2015). Tropical countries that are located between 40ºN and 40ºS latitude offer suitable growing conditions for mycotoxigenic fungi (Food Business Africa, 2017).

2.2.4. Factors affecting aflatoxin production

High temperatures and high humidity favor infection of crops by *Aspergillus* species (Cassel *et al.* 2011). Temperatures between 80^0F and 100^0F and relative humidity of 85% (corresponding to 18% moisture in grain) are optimum for growth of *Aspergillus* spp. Growth of the fungus is poor below 55^0F, but if the grain is moist enough, toxins can still be produced. Mature grains especially corn that remains in the field or corn that is stored without adequate drying can be subjected to *Aspergillus* growth and aflatoxin production.

Damage by pests (birds, mammals, and insects), prolonged stresses due to drought and nutrient stresses during pollination can increase the chance of *Aspergillus* spores infection to crops (Derek, 2010). Heavy rain can also cause spores to splash onto fruits and grains. Aflatoxin M1 and M2 which are the hydroxylated metabolites of aflatoxin B1 and aflatoxin B2, respectively are found in milk from cows that ingest aflatoxin contaminated feed. Once aflatoxin is produced, it is stable to heat, cold and light and is difficult to detect because aflatoxins are colorless, odorless and tasteless and they exist in uneven distribution in grain bins (Cassel *et al*., 2011).

2.2.5. Agricultural products susceptible to aflatoxin contamination

Aflatoxin producing fungi can contaminate several food commodities, including many of Africa's important staple cereal crops. Corn, nuts (peanuts), pistachio, Brazil nuts, oilseeds (cotton seed), copra, dried meat of coconut are some of the commodities with greater risk of

aflatoxin contamination (Cornea *et al*., 2011). This is because peanuts, cottonseed and copra are rich in edible oils which are very good substrates for growth of fungi (Idris *et al*., 2010).

Commodities which are moderately susceptible to aflatoxin contamination in the field include wheat, oats, millet, barley, rice, cassava, soybean, beans, pulses and sorghum. Other commodities such as cocoa beans, linseeds, melon seeds and sunflower seeds have been infrequently contaminated with mycotoxins with lower important rate compared to other commodities (Bankole *et al*., 2010). Corn silage is also a source of aflatoxins, because the ensiling process does not destroy toxins already present in silage (Cassel *et al*. 2011).

2.2.6. The challenge of aflatoxin in Africa

Aflatoxins tend to affect African countries quite drastically because the entire continent falls within the 40^0 N and 40^0 S of the equator. Aflatoxins are of great concern in tropical and subtropical regions (Michael, 2003). The prevailing climatic conditions in the tropics such as high temperature and drought, poor agricultural practices, bad postharvest handling systems such as improper processing, transportation and storage conditions all aggravate afalatoxin problem which is further exacerbated by the dietary reliance on staple foods such as maize and groundnuts which are highly susceptible to aflatoxin (Darwish *et al*., 2017).

Though aflatoxins cannot be seen by the naked eye, it is wise to suspect aflatoxin contamination if grains are damaged and rotten by birds, insects and other rodents. The presence of aflatoxins in foods has adversely affected not only the grain milling sector of the economy but also the health of the population and the ability of the continent to trade with the rest of the World (Bhat and Vasanthi, 2003). Consumption of large amount of toxins in a short period of time causes acute toxicity leading to death. The largest outbreak reported in the world was in 2004 when 317 cases were reported with 125 deaths which were due to aflatoxin problems of maize products in Kenya (Lewis *et al*., 2005). Consumption of small dosses over long time results to chronic effects to consumer (Darwish *et al*., 2017). Aflatoxins bind to DNA and disrupt genetic coddings, thus promoting carcinogenesis. In Africa, among other mycotoxins, aflatoxins have been implicated in human disease including liver cancer (Darwish *et al*. 2017).

2.2.7. Aflatoxin contamination of agricultural products in different countries

A long-term study (2003-2009) showed that 84%, 8%, 2% and 4% of nuts and nut products, fruits and vegetable, cereal products and herbs and spices were respectively contaminated with aflatoxins (Table 2.7 and Table 2.8).

Table 2.7. Level of aflatoxin contamination in cereal crops

Country	Commodity	MaximumAFB1 (µg/kg)
India	Ground corn meal	410
Ghana	Dried roasted corn	336
India	Mixed snakes	184.07
UK withdraw material from Ghana	Kenkey (Maize based by-products)	134
Ghana	Fermented Banku flour	57
Ghana	Maize flour	56
Thailand	Black rice	52.2
India	Corn meal in retail packs	46.2- 47
Hong Kong	Egg cake	45
Malaysia	Glutinous rice bales with peanut butter	35
Canada	Roasted red rice flour	32
Pakistan	Broken rice	11.5 – 28
Pakistan	Brown Basmati rice	12 – 27
Pakistan	Long grain white rice	18.9
Poland	Long grain white rice	16.7
Bangladesh	Rice flakes	12.7

Source: RASFF (2011)

Table 2.8. Level of aflatoxin contamination in herbs and spices

Country	**Commodity**	**Maximum AFB1µg/kg**
India	Ground turmeric and whole nutmerg	950
Indonesia	Whole nutmerg	419.9
India	Ground and broken nutmerg	238.5
Peru	Dried paprika	218.5
Ghana	Suya pepper	192.5
India	Chili powder	48
India	Dried red chili	24
China	Red pepper powder	24
India	Dry whole chilies	20.5
India	Ginger	18.6

Source: RASFF, 2011

2.2.8. Production losses and health risks of aflatoxins

Aflatoxin contamination can affect the entire supply chain for susceptible crops. The health risks on animals and humans depend on the level of mycotoxin contamination and on the quantity of contaminated food consumed. The sum total of action can impact food security, food availability, food access (by affecting incomes), and utilization of food (http://abtassociates.com/Aflatoxin-Stakeholders-Conference-Related-Matrie.aspx 2017).

In general, contamination of agricultural products by aflatoxin negatively affects agriculture, trade, and health. Consumption of high levels of aflatoxin contaminated foods by human being leads to acute poisoning (aflatoxicosis) and even death. Chronic exposure to low levels of contamination in crops directly or through livestock products increases liver cancer risks in humans and animals and can suppress the immune system. Although all ages are affected, young children are most susceptible to aflatoxin exposure which makes children stunted in their growth (Dhanasekaren *et al.,* 2011). Aflatoxin contaminated foods are also rejected from specific export shipments and leads to increased inspection and production losses (http://abtassociates.com/Aflatoxin- Stakeholders-Conference-Related-Matrie.aspx 2017).

It has been estimated that at least 25% of the grain produced each year worldwide is contaminated with mycotoxins (Upadhaya *et al*., 2010). Consumption of aflatoxin contaminated maize (aflatoxicosis) which resulted in 125 human deaths has been reported in Kenya (Derek, 2010).

2.2.9. Aflatoxicosis and livestock production

Mycotoxins can occur in livestock feed and if the toxins are consumed by animals they can channel into milk or meat and become a food safety hazard for human being (Ramesh and Siruguri, 2003). The problem of aflatoxin has resulted in reduction of livestock and poultry productivity (Bennett and Klich, 2003) and it has also led to higher susceptibility to infectious diseases in livestock and kidney and liver cancers in human beings (Rios *et al*., 2013).

Clinical signs of aflatoxicosis in animals include gastrointestinal dysfunction, reduced reproduction performance, and reduced feed utilization efficiency, anaemia, and jaundice. Young and nursing animals may be affected as a result of the conversion of aflatoxin B1 to the metabolite aflatoxin M1 excreted in milk of dairy cattle. Aflatoxins cause liver damage, decreased milk and egg production in livestock and poultry respectively. Aflatoxin also causes

infection due to immune suppression and embryo toxicity in animals consuming low dietary concentrations (Dhanasekaren *et al*., 2011). Aflatoxin B1 is considered by the International Agency for Research on Cancer (IARC) as having produced sufficient evidence of carcinogenicity in experimental animals to be classified as a carcinogen (IARC, 1993).

The problems associated with aflatoxins also have negative implications on domestic and export trades. The overall losses due to food and feed spoilage, reduction in livestock productivity, morbidity, mortality and reduced selling prices of contaminated meat, milk, and egg devastate the domestic and export market and pose an economic loss (Bhat and Vasanthi 2003). In Indonesia, Philippines and Thailand, 5% of the maize and peanuts produced were discarded due to contamination of aflatoxins (Bhat and Vasanthi 2003). The annual cost of aflatoxin contamination and other molds in these countries in terms of product spoilage, human health effects, and losses in the poultry and pork sectors was calculated to be 477 million dollars (Bhat and Vasanthi 2003). In the United States estimated value of maize lost due to aflatoxin contamination was $225 million per year (Betran and Isakeit, 2003). In the 1980s' in Malawi, the share of groundnut exports collapsed from 64% to 0.2% due to aflatoxin contamination (Rios *et al*., 2013).

2.2.10. Diagnosis and treatment of aflatoxicosis

Aflatoxicosis is a herd rather than an individual cow problem. If aflatoxicosis is suspected, the ration should be analyzed immediately. If it is present, the source should be eliminated immediately. Diagnosis of aflatoxicosis is often difficult because of the variation in clinical signs, gross pathological conditions and the presence of infectious diseases due to the suppression of the immune system (Caseal *et al*., 2011). On the farm, more than one mold or toxin may be present in the contaminated feed, which often makes definitive diagnosis of aflatoxicosis difficult.

Treatment should be directed at the severely affected animals in the herd and further poisoning prevented. Levels of protein in the ration and vitamins A, D, E, K and B should be increased as the toxin binds vitamins and affects protein synthesis. Good management practices to alleviate stress are essential to reduce the risk of secondary infections that must receive immediate attention and treatment (Caseal *et al*., 2011). Aflatoxicosis can only be prevented by feeding rations free of aflatoxin.

The tolerance of animals for aflatoxin infection depends on the age and sex of the animal, its health status, and overall management level of the farm (Caseal *et al*., 2011). To avoid contamination of milk, lactating dairy cattle should not consume greater than 20µ/kg in the total ration to prevent from passing the limits of 0.5µ/kg in milk set by Food and Drug Administration of the United Nations (Caseal *et al*., 2011). Consequently, calves should not receive milk from cows that are fed with feeds containing > 20 µ/kg.

Table 2.9. FDA maximum level of aflatoxin in animal feeds and food

Species	Commodity	Maximum level (ppb)
Dairy animals	All feeds and feed ingredients	20
Human	Milk	0.5
Human	Any food except milk	20
Poultry & dairy animals	Corn and other grains	20
All species	Animal feed other than corn or cottonseed meal	20
Breeding beef cattle, breeding swine or mature poultry	Corn and other grains	100
Finishing swine of ≥100 lbs	Corn and other grain	200
Finishing beef cattle	Corn and other grain	300
Beef cattle, swine, poultry	Cotton seed meal	300

Source: Carlson and Ensley (2003)

This does not suggest that feeding at these levels or below will reduce or eliminate the potential for aflatoxicosis for there are no clear cut safe feeding levels as it varies with individual animals (Caseal *et al*., 2011). Ingestion of aflatoxins at levels even lower than those listed in the guidelines may cause some undesirable side effects and depends on such factors as age, sex, and general health of the animals (Caseal *et al*., 2011). Monitoring animal health closely and discontinuing the use of contaminated feed immediately if undesirable effects are noticed are important practices to tackle the problems of aflatoxicosis (Caseal *et al*., 2011).

2.2.11. Control measures of aflatoxin

The control of management of aflatoxins in requires an integrated approach. A combination of technology solutions, effective regulations and standards, and enabling environment could bring about mitigation and control of aflatoxin on the continent. A number of strategies for reduction and control of mycotoxins have been considered in different African countries such as prevention of mould growth in crops and other feed stuffs, decontamination of mycotoxin-

contaminated foods, continous surveillance of mycotoxins in agricultural crops, animal feed stuffs and human food (Darwish *et al.*, 2014).

It is important for producers to realize that good agricultural practices (GAP) represent the primary line of defence against contamination of cereals with mycotoxins, followed by the implementation of good manufacturing practices (GMP). Better post-harvest storage practices to ensure that cereals are cooled quickly and evenly to prevent wet spots and should be maintained at a moisture content that does not promote mould growth (Finnegan, 2010). Control measures across the entire grain supply chain can increase the accessibility of aflatoxin-free foods to households for both domestic consumption and international market. During post harvest, screening and decontamination of grain can be employed using color or density sorting in order to remove contaminated grains from a grain lot before they are used for food processing (Ephrem, 2015).

The use of plant essential oils and atoxigenic strains of *Aspergillus flavus* have been reported to reduce aflatoxin levels (Derek, 2010). Application of ash was found to be effective ways to reduce aflatoxin. Kirui (2016), from Kenya reported that, maize cob ash was effective in reducing the aflatoxin level of maize and maize products.

Currently a bio-control method of aflatoxin mitigation is being tired in Africa. The bio-control product for aflatoxin management currently under testing in Kenya is Aflasafe KE01. Aflasafe KE01 is a mixture of spores of the bio-control atoxigenic strains (non-toxigenic indigenous *Aspergillus flavus*) that are coated onto sterile grain (sorghum), which serves as carrier and food for the fungi.

The atoxigenic strains grow and multiply on, and disperse from, the carrier to initiate displacement of aflatoxin-producers in the field. The product is applied 2–4 weeks prior to crop flowering. It is broadcasted on the crop and soil at an application rate of 10 kg/ha. In Kenya, field testing for an even lower rate of 5kg/ha is underway, translating to a direct cost benefit to the farmer through reduced pricing as a farm input (Bandyopadhyay, *et al*., 2016).

This method has several advantages including modifications to fungal communities caused by application of bio-control strains carry over through the value chain, discouraging contamination in storage and transport, even when conditions are very favourable to fungal growth. Positive influences of atoxigenic strain applications carry over between crops and provide multi-year benefits, with a single application of atoxigenic strains benefiting not only the treated crop but

also rotation crops and second season crops that miss a treatment and that because fungi can spread, as the safety of fungal communities within treated fields improves, so does the safety of fungal communities in areas neighbouring treated fields. The net effect is that the crop is protected throughout the supply chain (Bandyopadhyay, *et al*., 2016). It is not possible to completely eliminate fungal infection and mycotoxin contamination (Derek, 2010). Aflatoxins in cereals are fairly resistant to further food processing and the most effective way to reduce the contamination is to prevent it before happening in the process of crop production.

To minimize the negative effects of mycotoxin toxicity on animals that consume contaminated feeds, utilization of aflatoxin binders (adsorbents) was also reported to be advisable. These compounds are often added to animal feeds and inhibit intestinal absorption of mycotoxins and thereby prevent their deleterious effects in animal production. The most common binders include hydrated sodium calcium aluminosilicate (HSCAS), sodium or calcium bentonites and zeolites. These binders have great impact on improving animal production and health by providing quality products to consumers of animal products (Feddern *et al*., 2013).

The rapid progress in genomic research of both host plants and fungal pathogens could lead to a better understanding of the mechanisms of aflatoxin formation, pathogencity of the fungus, and crop-fungus interactions (Guo, 2009). Efforts are being made to understand fungus biology and the mechanism of aflatoxin biosynthesis using genomic tools. Thus, developing novel strategies to control aflatoxin contamination is the ultimate goal for scientist in the future. This will contribute greatly to achieve the goal of devising novel strategies to eliminate pre-harvest aflatoxin contamination resulting in a safer food and feed supply (Guo, 2009).

2.2.12. Legislations to control aflatoxin contamination

Currently, there are different international organizations including the U.S.A., E.U., CODEX alimentariuos, ISO, FAO/WHO, FDA, IDF that can establish international standards and limits of mycotoxin levels to ensure safety and quality of foods and feeds. For example EU general food safety legislation 178/2002 (EU 178/2002) imposes legal obligations for food business operators (including growers and food and feed manufacturers) to ensure that the food and feed they sell should comply the limits set by the standards setting organizations. It also requires them to identify, review and control critical points in their processes for food safety.

According to EU Regulations 1881/2006 (EU 1881/2006), the maximum contents of aflatoxin B1 and total aflatoxins (B1, B2, G1 and G2) in cereals and cereal products allowed are at 2µg/kg

and 4µg/kg, respectively. The maximum limits of aflatoxin B1 and total aflatoxin in maize grain subjected to sorting before human consumption are 5µg/kg and 10µg/kg respectively. In order to minimize human exposure to these toxins, EU regulations on its directive of 2002/32/EC has set maximum limits for aflatoxin B1 in animal feed, which are in the range of 5-30 µg/kg (Derek, 2010). Total aflatoxin implies the sum total of B1, B2, G1 and G2, in which the product under investigation is detected only for its status of aflatoxin positive or negative which means the detection does not go for further detecting the different forms of aflatoxins B1, B2, G1 and G2.

There are also national standards established by different states and countries which are set for enforcement in their own state and can be implemented in courtiers that have trade relations with them. For example the Regulations of England (regulation 2009, No. 1223) guides and controls the contaminants status in foods in England and food business operators are not allowed to mix or dilute cereal lots containing mycotoxins above the standard already established for their own nation.

2.3. Heavy metals and their properties

Heavy metals are those elements having a density of > 5 g/cm3, atomic weight 63.55 - 200.59 (Lars, 2003) and a specific gravity > 4.0 (Cruz *et al.,* 2009). Cadmium (Cd), Mercury (Hg), Lead (Pb) and Arsenic (As), are among the major toxic heavy metals posing problem to the environment (Akan *et al.*, 2009).

Heavy metals are deposited in soils in the form of solid and in solution as free ions or adsorbed to soil colloidal particles. Soils naturally having greater surface area like clay soils and other soils that are enriched with humus have higher opportunity for metal sinks via sorption up on converting to metal oxides (Alloway, 1995). Heavy metals have been used by human being for industrial production of different commodities such as synthetic fertilizers, fungicides and other agricultural chemicals. They have the ability to persist in the environment for years, posing long term risks to life (Reilly, 1991).

Long-term application of different agrochemicals such as herbicides, insecticides, fungicides, rodenticides, nematocides, different disinfectants, open dumping and improper disposal of industrial wastes on land leads to accumulation of heavy metals on soil and water (Ogundiran, *et al*., 2012; Rajaganapathy *et al*., 2011).

The transfer of heavy metals in to food chain takes place near smelting or mining areas, agricultural land, pastures grown over soils irrigated with waste water, drainage channels contaminated with industrial waste water, domestic contaminated water and eating foods that are grown over contaminated soils which can pose health risks in humans and farm animals (Reilly, 1991).

Heavy metals that are found in water bodies especially around industrial areas can enter the soils and groundwater and consequently bio-accumulate in the food webs which can adversely affect the biota. Then they are transferred from water and forage to livestock species and humans via meat and milk (Mor, 2005; Ogundiran, *et al*., 2012; Rajaganapathy *et al*., 2011).

2.3.1. Health risks due to exposure of heavy metals

Food and feed are the main carriers of heavy metals to human beings and animals (Ahmad, *et al.,* 2013). Long term dietary intake of food contaminated with heavy metals results in the accumulation of heavy metals in the body which contribute to the development of various disorders on growth, biological functions and hormonal system (Orisakwe *et al*., 2012). The

main threats of heavy metals to human being are associated with their accumulation in important body organs such as liver and kidney (Rajaganapathy *et al*., 2011).

Although the health effects of heavy metals have been known for a long time, exposure to heavy metals is even increasing in some parts of the world (Järup, 2016). Heavy metals have the ability of interfering with the normal process of milk synthesis and affect milk yields in dairy cattle (Khan *et al*., 2012). They are also capable of displacing other essential minerals from where they do vital biochemical and enzymatic reactions in the metabolism process (Dawd, 2010). The effects of heavy metals on the environment and human health are briefly mentioned below.

2.3.2. Cadmium

Cadmium is a relatively rare element, released to the air, soil, and water mainly by human activities. It is found as a mineral combined with other elements such as oxygen (cadmium oxide), chlorine (cadmium chloride), or Sulphur (cadmium sulphate, cadmium sulphide). The major sources of cadmium pollution are smoke from cigarettes, airborne industrial contaminants, batteries, fungicides, incineration of tires /rubber/ plastic, e.t.c. Phosphate fertilizers and urban sewage sludge also contains significant amounts of cadmium (NRC 1980).

Waste from zinc smelting and zinc plating operations have been sources of contamination that have resulted in cadmium intoxication. Consequently, high concentrations of cadmium (up to 10000 µg /kg) have been found in forages grown in fields near industrial zinc plating sites and where urban sludge has been used as a fertilizer (NRC 2001).

The increased level of cadmium at lower pH level in soil results in an increased uptake of cadmium by plants. This then is transferred to livestock via cadmium contaminated forage feeds. Cadmium has special property in that it can be detected in small amounts in milk because it is not increased by high dietary concentrations due to the inhibited transfer rate of cadmium from blood to milk as a result of metallothionein binding of cadmium in the blood cells.

The maximal tolerable cadmium level in the diet of cattle was set at 500µg/kg in an effort to avoid adding cadmium to the diet of humans consuming products primarily derived from dairy cattle (NRC, 1980).

Diets containing from 5000-30000 µg/kg cadmium generally decrease animal performance by interfering with copper and zinc absorption, resulting in symptoms usually associated with copper and zinc deficiency. Diets containing more than 5000 µg/kg cadmium can cause copper concentrations in liver to decline (NRC 2001). Ruminant diets containing more than 30000

µg/kg of cadmium cause anorexia, reduced growth, decreased milk production, and abortion (NRC 2001).

2.3.3. Lead

Lead is a non-essential element, potential pollutant and a very toxic metal that readily accumulates in soils and sediments. It is the most common industrial metal that has become widespread in air, water, soil and food. It is slightly soluble in water and is transported mainly through the atmosphere (Sharma & Dubey, 2005).

Lead halides and lead bromo-chloride, once added to gasoline as engine valve lubricants are emitted from automobile exhaust during combustion and continue to contaminate the environment. Lead is also abundant in ash, auto exhaust, battery manufacturing, bone meal, canned fruit and juices, car batteries, cigarette smoke, coal combustion, and other industrial products (Thürmer *et al.*, 2002).

Lead has the ability of been easily absorbed and accumulated in different plant parts (Ahmad *et al*., 2013) and it is a cumulative tissue poison that gets stored in different parts of the body especially in bones, liver, kidney and brain. Besides, direct ingestion of lead leading to increased blood lead levels, accumulated lead in the body also acts as a significant source of blood lead burden and nervous system disorders (Mukesh, *et al*., 2008).

The problem of lead poisoning in animals which has been widely recognized needs a special attention for the environmentalist and health personnel and it is the most common cause of toxicosis in livestock species (NRC 2001). In acute cases, lead toxicity in cattle results in depressed milk yield and death within 24 hours (Khan *et al*., 2016).

It is established that 3-10% of the ingested lead is absorbed by adult ruminants (NRC 2001). Elevated dietary calcium, phosphorus, iron, zinc, fat, and protein decrease the absorption and retention of lead (NRC 2001). Lead accumulates in bone and readily passes into milk so that increased dietary concentrations of lead results in increased lead concentration in milk (NRC 2001).

Clinical toxicosis with lead interferes with the normal function of metal-dependent enzymes. Lead interferes with protein synthesis and causes microcytic hypochromic anemia (NRC, 2001). Chronic exposure to low levels of lead is not associated with clinical symptoms in cattle because bones sequester lead and release it gradually to the blood for excretion. The concentration of lead

in the kidney cortex was often >50000 µg/kg and in the liver is >20000 µg/kg /kg (fresh tissue) which leads to death from lead poisoning (NRC, 2001).

Therefore, the maximum tolerable dietary lead is set at 30000µg/kg (NRC, 1980). Young animals tend to be more susceptible to lead toxicity than adults because they have a higher rate of absorption of lead (90 % *vs.* 10%) and are more likely to exhibit pica (NRC, 2001). In humans, low exposure to lead is associated with a loss of cognitive powers. Acute intoxication with lead causes impaired neurologic function resulting in blindness and irritability (Radostits *et al.,* 1994). Lead toxicity also causes intestinal pain, abortion and accumulates in the kidney cortex leading to impaired renal function (NRC, 2001).

Children are particularly susceptible to lead exposure due to high gastrointestinal uptake and the permeable blood–brain barrier and neurotoxic effects lead at lower levels of exposure than anticipated (Järup, 2016).

2.3.4. Arsenic

Arsenic is the twentieth most abundant element on earth and its inorganic forms such as arsenite and arsenate compounds are lethal to the environment and living creatures (Mazumder, 2008). Arsenic is a metalloid element found in the earth's crust and can be easily available by natural sources such as volcanic activity and weathering of minerals, and by anthropogenic activity causing emissions in the environment, such as ore smelting, burning of coal and specific uses, such as arsenic-based wood preservatives, insecticides and herbicides and wood preservatives (Järup, 2016). Thus, it is mainly found from groundwater in connection with mineralized zones and sedimentary aquifers (water bearing rock). Arsenic occurs in organic and inorganic forms. The most toxic forms of arsenic are the inorganic arsenic (III) and (V) compounds and the inorganic arsenic trioxide is well known as a rat poison,

Humans may encounter arsenic by natural means, industrial source, or from unintended sources. Drinking water may get contaminated by use of arsenical pesticides, natural mineral deposits or inappropriate disposal of arsenical chemicals. Deliberate consumption of arsenic in case of suicidal attempts or accidental consumption by children may also result in cases of acute poisoning (Mazumder, 2008).

Cattle fed with poultry manure containing 18 mg/kg organic arsenic/kg of diet did not show signs of toxicity (NRC 2001). The maximal tolerable dietary arsenic was set at 50 mg/kg and 100 mg/kg for inorganic and organic forms, respectively (NRC, 1980). Complications linked to long-

term arsenic consumption include: cancer liver disease diabetes nervous system complications, such as loss of sensation in the limbs and hearing problems digestive difficulties (Paddock, 2017).

Arsenic also inhibits the growth of rumen bacteria, weight loss, reduction of milk yield, liver and kidney damage in ruminant animals (Mukesh, *et al*., 2008). Arsenic becomes toxic when it is provided in large amounts to dairy cattle (NRC 2001). A deficiency of arsenic has been reported in goats and pigs fed diets containing >50000µg arsenic/kg of diet (NRC 2001). The maximal tolerable dietary arsenic was set at 50000 µg /kg and 100000 µg /kg for inorganic and organic forms respectively per kg of diet (NRC 1980).

Deficiency signs consisted of impaired reproductive performance and lower weight gains in second generation animals. Organic arsenicals have been used in swine and poultry for their antibiotic and anti-coccidial properties (NRC 2001). Cattle fed poultry manure containing 18000 µg /kg organic arsenic in a diet exhibited no signs of toxicity (NRC 2001).

2.3.5. Chromium

Chromium is a naturally occurring element found in rocks, soil, plants, animals, and in volcanic dust and gases. In the environment chromium exists primarily in the trivalent and to some extent in hexavalent forms. The trivalent chromium (III) is an essential nutrient in the diet, but it is required in a very small amount. Chromium plays a role in glucose and lipid metabolism and serves as a co-factor of chromodulin, which is known as insulin-resistance factor and plays a significant role in some physiological functions of animals and human beings (Zodape *et al*., 2012). Trivalent chromium is also responsible for stimulating the activities of insulin in the body and controls level of blood cholesterol (Ogabiela *et al*., 2011).

Several studies in cattle have demonstrated a favorable response to chromium supplementation, especially if the animals are under some physiologic stress. The findings included improved cell mediated immune response, improved energy status (reduced liver triglyceride accumulation), improved dry matter intake and milk production in cows (NRC 2001). In these studies the basal rations and the supplemental diet contained a chromium content of <1.6 mg/kg and 0.5-10 mg/kg diet respectively. Inclusion of chromium in the diet of stressed feedlot calves has also resulted in reduced morbidity (NRC 2001).

Chromium toxicity is primarily associated with exposure to hexavalent (Cr^{+6}) which was observed to be at least five times more toxic than the trivalent one. This is because hexavalent chromium passes into the interior of cells much more readily than trivalent chromium (NRC 2001). If significant amounts of chromium reach the cell nucleus, there can be a variety of pathologic changes and damage in the DNA (Wise, *et al*., 2008).

In general, chromium compounds have been found to be mutagenic and carcinogenic including kidney problems. The maximum tolerable concentration of chromium in the diet for livestock was set at 3000 mg/kg for the oxide form and 1000 mg/kg for the chloride form of the trivalent forms of chromium (NRC, 2001).

2.4. Issues of safety, quality and standards of agricultural products

2.4.1. Definitions

Safety: Safety refers to the absence of unacceptable levels or undesirable substance, chemical element and of disease causing microbes in a product which can cause human health problems **(**FAO/WHO, 2003)

Hazard: Hazard is the status of being dangerous or toxic and the consequences could be an adverse health effect or even death (Schweihofer, 2013). Animal hazard is any biological, chemical, or physical substance in feeds or water that is likely to cause illness in animal in the absence of its control. Human hazard is any biological, chemical, or physical substance in foods including livestock products that is likely to cause illness in humans in the absence of its control (Schweihofer, J. 2013).

Standard: A standard is a document that provides requirements, specifications, guidelines or procedures that can be used consistently to ensure that materials, products, processes and services are fit for their purpose. A standard is a guideline established by consensus and approved by a recognized body and aiming for the promotion of optimum community benefits. Standards are varied in character, subject and medium. They cover disciplines dealing with all technical, economic and social aspects of human activities.

Standards are developed by technical committee's which are coordinated by specialized body, and ensure that barriers between different areas of activities and different brands are mitigated. Standards reflect the results of joint work involving all competent parties concerned and validated by consensus to represent all relevant interests, producers, users, laboratories, public authorities, consumers and e. t. c. Standards are developed based on experiences and lead to material results in practice such as on products, goods and service, test methods e t c. Standards are reviewed periodically as dictated by circumstances to ensure their currency, and therefore evolve together with technological and social progress. Standards are documents which are recognized as valid nationally, regionally or internationally, as appropriate. As a general rule, all standards are not mandatory there are some standards established for voluntary application. But in fields or commodities that require serious safety and electrical installations, the application could be obligatory and compulsory (http://www.ethiomarket.com/qsae/ 2016).

2.4.2. Types of standards

National standards: The national standard is programmed and studied under the authority of the national standard body. The standardization process is conducted by standards committees which can obtain assistance from groups of experts. These committees or working groups are made up of qualified representatives of industrial circles, research institutes, public authorities, and consumer or professional bodies. Every country possesses its own national standardization system and the central, more representative national standards body participates within the regional or international bodies.

Regional standards: Regional standards are studied under the authority of the regional standard body with head office in Nairobi, Kenya. An example of regional standards is African Regional Organization for Standardization (ARSO) which was founded in 1977, comprised of 24 African standard bodies and to date, it has published about 400 African Regional Standards. The objectives of ARSO is to promote social, industrial and economic development and provide consumer protection and human safety by advocating and establishing activities concerning standardization in Africa and to promote the harmonization of the views of its members and their contribution and participation at the international level in the field of standardization. The technical work is related to the operation of activities in the following main fields: Preparation and issuance of African Regional Standards, Quality Assurance activities, African Regional Accreditation and Certification marking Schemes (ARAS), Metrology, Laboratory testing, Technical information and consultancy services in standardization and International liaison and participation. Other examples of regional standards include European Committee for Standardization (CEN), European Committee for Electro technical Standardization (ECETS), European Telecommunications Standards Institute (ETSI), Asian and Pacific Standard and Asian Consultative Committee on standards and quality (ACCSQ)
(http://www.ethiomarket.com/qsae/2016).

International standards: they are standards prepared for applicability at international level and have got International recognition and such standards include International Organization for Standardization (ISO)**,** Codex Alimentations Commission (CAC), International Electro-technical Commission (IEC), International Telecommunication Union (ITU), FAO, WHO, IDF etc. The exploitation right of copyright is automatically transferred to the national standard bodies which

comprises of memberships of the stated International Organization. At regional or international level, the work is conducted by technical committees for the secretariats of which responsibility is assumed by the national standards bodies. All national members are entitled to be represented within the international or regional committee dealing with a specific subject matter (http://www.ethiomarket.com/qsae/ 2016).

Other than the Ethiopian standards (ES) there are national and international standards available in the publication and documentation directorate of the Ethiopian Standard Agency (ESA), at the Head Office, Addis Ababa and they are listed below.
(http://www.ethiostandards.org/ESA/2016)

2.4.3. Role of standards and standardization

A standard is used as a reference document used in particular in the contexts of public contracts or in that of international trade on which the majority of commercial contracts rely. It is used by industrialists as the indisputable reference simplifying and clarifying the contractual relations between economic partners. A standard is a factor for rationalization of production, for clarifications of transactions, for innovating and developing products

(http://www.ethiomarket.com/qsae/2016). Standardization is recognized as being essential and discipline for all players within the economy who must strive to master its motivating forces and implications. Twenty years ago, standard was the reserved field of a few specialists. Today companies have integrated standardization as a major technical and commercial element. They are aware that they must play an active role in this field and prepared to accept standardization. Standards are drawn up at national, regional and international levels. The coordination of the work at these three levels is ensured by common structures and cooperation agreements. Countries in Africa have National standards for implementation within their own nations. There are also African or regional standards with head office centered in Nairobi, Kenya which is functionally valid among countries within the region. However, to perform export trade with other international nations in the world, countries in Africa are required to fulfill the standards of agricultural products set by the respective international setting organizations.

European countries have mainly developed international standards like the EU standard (EC 2013). Members of the United States of America have also established international grades and

standards for agricultural products for which United States Department of Agriculture (USDA) is responsible for this.

2.4.4. HACCP (Hazard Analysis and Critical Control Points)

HACCP is defined as Hazard Analysis and Critical Control Points System. It is the process of identifying, quantifying and controlling food safety hazards which addresses the farm to table safety and quality situations (Henok *et al.*, 2013). In the effort of meeting quality and safety of agricultural products, it is a task of inspecting and defining the critical points during the process not the final products (Cross, 1995). Hazard Analysis and Critical Control Points System (HACCP) is a scientific approach to quality control (Nurcan *et al*., 2005) and implementing HAACP system is useful for reduction, elimination, and or control of risks (John, 2001). Instead of analyzing and controlling the hazards at the endpoint which is too late to correct and/or prevent a problem, HACCP is applied and controlled along the production chain (Cross, 1995).

2.4.5. Implementation of HACCP in livestock feed in developed world

The HACCP (Hazard analysis Critical Control Point) approach for ensuring food safety was developed in 1960's in the USA by a food company as part of its efforts to produce foods for the space program. Developed countries have employed HACCP practices for food quality assurance system in the animal feed sector and they recognize that the feed industry is part of the food chain and they use the slogan "Feed for Food". The public, including people engaged in animal production and the consumers have awareness on bounded rules and regulations in relation to safety of animal products.

Application of HAACP allows control of feed production to assure that microbial, chemical and physical contaminants, additives and other undesirable substances that could contribute to food risks are controlled (Cross, 1995). Application of HACCP came into the picture after the feed manufacturing and livestock industries have experienced severe food safety incidents. The outbreak of Bovine Spongiform Encephalopathy (BSE), the Belgium Dioxin Crisis, which occurred in 1999 due to contaminated fat supplied to stock feed manufacturers, the 1993 Jack-in-the box hamburger *E. Coli* O157:H7 outbreak, the Mad cow disease and dioxin contamination of Brazilian citrus pulp (1998), food borne pathogens accounting up to seven million cases of food borne illness 7,000 deaths per year in the U.S.A. were some of the important impulses for implementation of HACCP programs (Cross, 1995).

Australia is another example that has adopted HACCP program in agricultural production by recognizing food safety assurance from farm to fork. For example, appropriate critical control points are developed and monitored for a calf at grazing/feeding, slaughter, and at the retail level to avail safe meat to consumers. Feed manufacturers and feed suppliers are certified for good feed manufacturing program (GMP) based on established standards (Cross, 1995).

Livestock farmers are also participating in the quality control program in which they are obliged to purchase feeds only from GMP-certified feed suppliers. The objectives of animal feed quality program in the countries mentioned above are to produce and supply animal feeds which are safe for consumers of animal products, for the animals and for the environment. The program is enforced by legislation and all stakeholders existing in the chain implement the program responsibly and transparently.

For example, microbial contaminants such as *Salmonella* and *E. coli* that can use feed ingredients as a vector and transfer to animal products are prevented by employing good animal husbandry practices by the use of clean water and prevention of fecal contamination of water and feedstuffs, cleaning and sanitizing of pens, feed bunks, and water troughs, avoidance of overcrowding, avoidance of commingling of sick and healthy animals, e.t.c. (Cross, 1995). By employing a comprehensive feed control program through HACCP, developed countries have been able to supply *E. coli* and *Salmonella*-free feed to animals in the overall food supply chain.

2.4.6. The need of feed and food safety assurance

Biological, chemical and physical hazards are the three broad categories of food safety hazards. Their origin can be from naturally occurring substances or agents in foods, from deterioration or decomposition of foods, or from contamination of the foods with the hazard at various stages of their production, harvesting, storing, processing, distribution, preparation, and utilization (Rhodehamel, 1992). For many hazards, government regulatory agencies have established an acceptable standard of the hazard in a food; the Codex Alimentarius has also established acceptable standards of certain hazards as part of its food standards program. For some hazards, such as pathogenic bacteria (e.g. *Salmonella* spp.), there is zero tolerance, which means that the presence or the detection of the hazard in the food is unacceptable (FAO, 2001). The broad categories of contaminants in foods and feeds include:

(1) Biological contaminants

a. Microbiological contamination

b. Parasites

c. Decomposition in feed and feed ingredients

(2) Chemical contaminants

a. Veterinary medications (drugs)

b. Mycotoxins

c. Pesticide

d. Toxic plants

e. Heavy metals

f. Excessive minerals

g. Unapproved feed ingredients

h. Unapproved food additives

(3) Physical contaminants (Foreign materials)

a. Stones

b. Wood

c. Metal

d. Glass

e. Plastic

Source: (Rhodehamel, 1992)

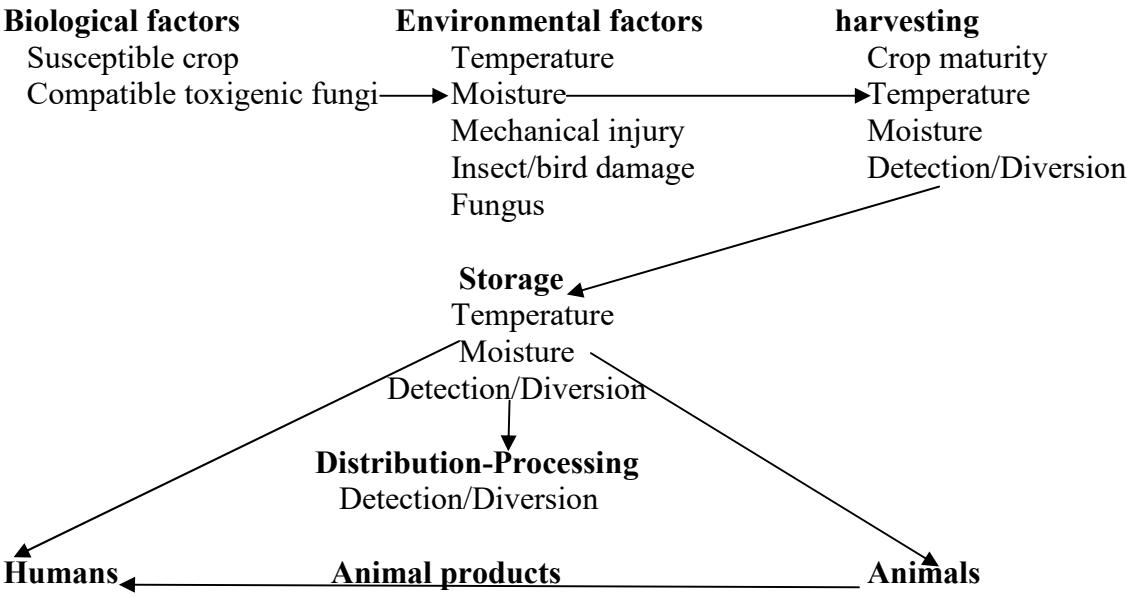

Fig 2.1. Factors affecting mycotoxin occurrence in the feed and food chain.

Source: (Bryden, 2012).

Factors that contribute to hazards in food include improper agricultural practice, poor hygiene at all stage of the food chain, lack of preventive control in food processing and preparation operations (stages), misuse of chemicals, contaminated raw materials, ingredients and water, improper storage agricultural products (Darwish *et al*., 2014) giving rise to food-borne health problems that do require intervention by regulatory bodies. Regulation in this case should aim at providing mechanisms in which the quality and safety of food and food products can be protected at appropriate stages. Hence maintaining safety of food measure is required not only in some end point of the food chain, but also in some starting point in farm production as agro-chemical residue in food is unmanageable at the point of consumption but it can be managed at the point of production by using good agricultural practice (EPA, 2002).

2.4.7. Safety standards of agricultural products in developing countries

People in developing countries are price-sensitive and few group of the society engaged in trading of agricultural products may adulterate the normal quality of agricultural products (Brufau and Tacon 1999). There are weak control systems towards safety and quality of feeds and foods. Two types of food safety control systems exist globally: traditional and science based.

Traditional food safety control system is a reactive approach, with the main responsibility lying on the government and does not involve structured risk analysis system and mainly relies on end product inspection (FAO/WHO, 1995). It is a practice of using enforcement tools for removing unsafe food from market and punishing responsible parties after the fact. Food safety control system in developing countries is mainly traditional in that it is a food control system mainly based on hygiene and adulteration and does not incorporate food laws and regulations, food control management, inspection laboratory services and enforcement system. Mechanisms for information communication, education and monitoring system of food supply are not strong (FAO, 2009). Science- based way of food safety control system is practiced in the developed world by incorporating good agricultural practices, good hygienic practices, good manufacturing practices and HACCP. The mechanism of risk analysis is structured and scientific in order to enhance the safety of feeds and foods throughout the food chain (FAO/WHO, 2003).

Ethiopian Animal Feed Industry Association (EAFA)

It is an association established in 2008 by feed factory owners, private dairy farmers and dairy cooperatives with a mission of improving the quantity, quality, and safety of livestock feed and provides services for its members. It is positioned to promote members and the public sector get cooperation among each other. The association provides technical assistance and trainings to members engaged in feed production, marketing and distribution and provide members with market information.

2.4.8. Factors affecting quality and safety of livestock products in Ethiopia

The safety aspects of agri-foods in Ethiopia are more of principles and are not fully practical, meaning the public are not enforced to practice and the enforcement mechanism is not strong. The quality control mechanism is traditional in which inspection is not comprehensive and does not consider the entire food chain to prevent the risks and networking and information communication among regulatory institutions is not strong (Tesfa, 2010).

The regulatory bodies involved in quality and safety of agri-foods are not equipped with the necessary laboratory facilities that can effectively respond to current international food regulation demands and there is lack of central analytical and microbiological laboratories to support regulatory activities (FAO/WHO, 2007). Though Consumers' Associations can play

substantial role in ensuring food safety, the existing association in Ethiopia both in power and in number is not adequate (Tesema, 2011).

The food control activities are scattered among various regulatory agencies such as Ministry of Health, Ministry of Agriculture and Rural Development and Ethiopian Standard Agency and the responsibilities given to these regulatory authorities are not adequately defined to avoid overlaps and duplication of tasks (Tesfa, 2010). The level of awareness on food regulation in most processing plants on HACCP, Good Agricultural Practices, Good Manufacturing Practices, Good Hygienic Practices, Good packaging practices & Good labeling practices is weak and the public knowledge towards food safety and quality aspects is limited and food regulation is considered as secondary issue to economic development (Melese and Melese, 2015).

Chapter 3.

Determination of Aflatoxin in Dairy Feeds and Milk in Selected Sites of Central Highlands of Ethiopia

Rehrahie Mesfin, Fassil Assefa and Getnet Assefa

3.1. Abstract

Human health is continuously influenced by food contaminations. Food safety is affected by different microbes and associated compounds and livestock feeds are the major routes of food contaminations that affect food safety. Aflatoxins are among the major contaminants that deteriorate the quality of feeds and can be transferred in to milk of lactating cows that consume aflatoxin contaminated feeds. Aflatoxins are secondary metabolites of toxigenic fungi which can affect human health when exposed to aflatoxin contaminated foods.

The objective of this study was to determine aflatoxin B1 (AFB1) in feeds and aflatoxin M1 (AFM1) in milk in West Shoa, East Shoa and Hawassa, Ethiopia. A total of 205 samples (115 concentrate feeds, 45 roughage feeds and 45 milk samples) were collected and quantified for aflatoxin using Enzyme-linked Immunosorbent Assay (ELISA). Concentrate feeds were more contaminated (7.67 ± 0.80 µg/kg) than roughage feeds (0.41 ± 0.14 µg/kg); hay was more contaminated (0.72 ± 0.25 µg/kg) than straw (0.05 ± 0.05 µg/kg) and oilseed cake based concentrate feeds were more contaminated (13.09 ± 1.12 µg/kg) than concentrate feeds without oilseed cake (2.78 ± 0.66 µg/kg). The AFB1 of feeds in this study was different from the level reported by Dawit et al. (2016). On average, the AFB1 level of feeds (5.63 µg/kg) was greater than the EU permitted level of 5 µg/kg and less than the permitted limit of the U.S.A. (20 µg/kg). On average, the AFB1 level of feeds was 5.63 µg/kg. Half of the feed samples were found to be free of aflatoxin and 69% and 31% of them was within the recommended limit of the EU (5µg/kg) and the USA (20 µg/kg) for feeds respectively. The average AFB1 of feeds in Bishoftu (9.76µg/kg) was higher than Holetta (6.33µg/kg) and this then was higher than Hawassa (1.19µg/kg). The AFB1 of feeds in dairy producers was higher (9.35 ± 1.04µg/kg) than in feed retailers (6.91 ± 1.09µg/kg) and this was higher than the AFB1 of feed in feed manufacturers (7.50 ± 1.43 µg/kg). The AFM1 of milk was in a range and avearage of 0–0.146 µg/L and 0.054

µg/L respetively and 29% of the milk samples had no aflatoxin, 58% of them had AFM1 level within the EU permitted limit of 0.05µg/L and 42% of the samples were less than the U.S.A. recommended limit of 0.5 µg/L. The AFB1and AFM1 level of milk samples collected from the study locations were in the order of Hawassa < Holetta < Bishoftu. Further studies of aflatoxins on dairy feeds and milk using other techniques such as HPLC, GC and multi-mycotoxin assay using LC-MS-MS should be carried out to obtain conclusive information.

Key words: Safety; Concentrate; Hay; Straw; Holetta, Bishoftu; Hawassa

3.2. Introduction

Livestock is one of the sectors that contributes to the national economy of the country (Mureja, *et al*., 2002) and is the basis for livelihoods of about 10 million pastoral communities (Little *et al*., 2001). Given that the country has the largest number of livestock species in Africa; it has the potential to further develop the dairy industries for local consumption and international market (Gian, 2006).

The supply of quality and safety feeds is one of the impediments for production of safe and good quality milk and milk products. The growth of fungi on grains and feeds and production of mycotoxins is the most serious problem in dairy industries (Ramesh and Siruguri, 2013). The level of mold growth and elaboration of aflatoxins is aggravated by damage of grains by insects, birds, rodents, bad storage and poor feed processing conditions in tropical and subtropical regions.

Aspergillus parasaticus and *Aspergillus flavus* are the known fungal species that produce aflatoxins (Michael, 2003), more now, *Aspergillus nomius* and *Aspergillus korhogoensis were* identified in Kot Devore (Campos, *et al*., 2017). The aflatoxin group found in feeds consists of B_1, B_2, G_1 and G_2, of which Aflatoxin B_1 (AFB1) is the most common, carcinogenic and potent mycotoxin. AFM1 is a hydroxylated AFB1 metabolite that is detected from milk within 12 hours after cows are fed with a contaminated feeds (Battacone *et al*., 2003).

Consumption of feeds contaminated with aflatoxin can lead to susceptibility to infectious diseases in livestock and poultry species and expresses in digestive, reproductive and respiratory health problems (Hasheminya and Dehghannya, 2013). Aflatoxins reduce the yield, nutritive value and organoleptic quality of grains and feeds (Akande *et al*., 2006). Aflatoxins can also reduce the production of meat, milk and egg industries (Bhat and Vasanthi 2003; Bennett and Klich, 2003) which can bring negative implications on domestic and export trades (Bhat and Vasanthi 2003).

Human beings that take up aflatoxins with contaminated grains and livestock products could be exposed to liver and kidney cancer and also to death in acute cases. In chronic cases, stunted growth and susceptibility to infectious diseases and immune suppression is apparent in children (Kiama *et al*., 2016; Bhat and Vasanthi, 2003).

Several studies in the past were undertaken to increase the quantity of feed resources (Samuel *et al*., 2009; Endale, *et al*, 2016; Mesfin *et al*., 2009; Getnet and Ledin, 2009). However, there was limited information on aflatoxin; *vs* cereals (Abdi and Alemayehu, 2014), dairy feeds and milk (Dawit *et al*., 2016). Since animal feeds are integral part of human food chain, they need to be assessed as potential sources of contaminants. Assessing the status of aflatoxin level in feeds from production to utilization helps to plan better feed management and utilization strategies. The current study was undertaken to determine aflatoxin contamination level of dairy feeds and milk following production, processing, and marketing.

3.3. Materials and methods

3.3.1 Study locations

The study covered different sampling sites of Central Ethiopia: West Shewa zone: Welmera (Holetta), Ejere (Addis Alem), Dendi (Ginchi), Ambo and Guder. East Shoa zone: Ada (Bishoftu), Addis Ababa (Kolfe-Keranyo, Yeka, Akaki-kaliti, and Lafto-Nfas Slk), and Tabour sub-city and Monopol areas in Hawssa, Sidama zone of southern Ethiopia (SNNP) (Table 3.1).

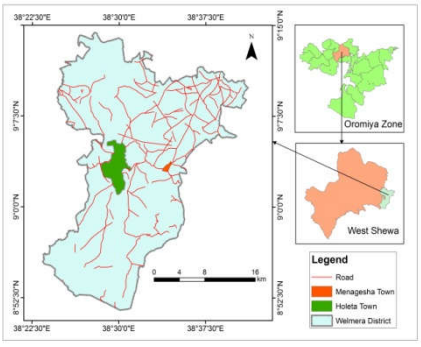

Fig 3. 1. Map of Western Shoa

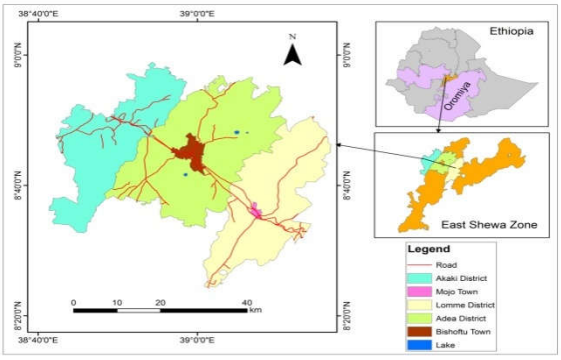

Fig 3. 2. Map of Eastern Shoa

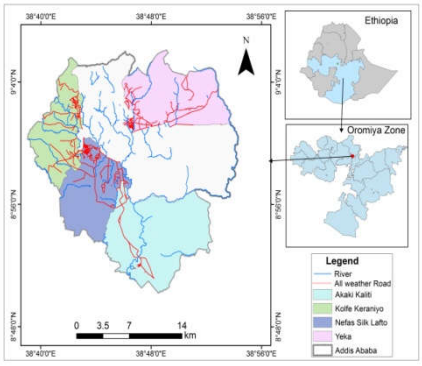

Fig 3. 3. Map of Addis Ababa

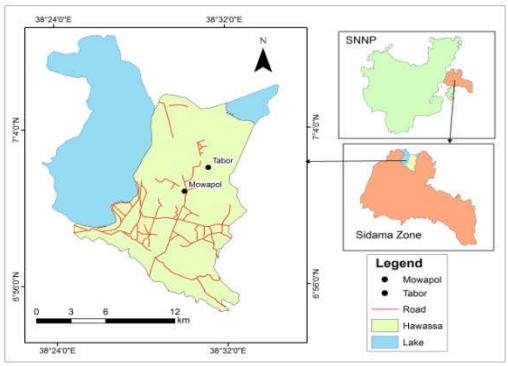

Fig 3. 4. Map of Hawassa

3.3.2. Sample collection and laboratory investigations

A total of 160 feed samples were collected from the study areas of which 115 and 45 were concentrate feeds and roughage feeds, respectively. The concentrate feeds were mainly compounded feeds composed of wheat bran, oilseed meals and byproducts of cereals, pulses of which some of them were a mixture of oilseed cake and the others were without oilseed cake. The oilseed cake based concentrates included, wheat bran, wheat middlings with either mixture of noug seed cake, linseed cake or cotton seed cake. The concentrate mixture feeds without oilseed cakes included wheat bran, wheat middling and other products of pulses and cereal by products. Concentrate feed with oilseed cake included either one or more than oilseed cakes mixed with wheat bran, wheat middlings, and bran of pulses and poultry liter, in which wheat bran was available in 73% of the feed samples. Whereas wheat middlings, bran of pulses and poultry liter were found in10%, 6%, 11% of feed samples respectively. Among the oilseed cake noug cake, linseed cake and cottonseed cake was available in 63%, 33% and 4% of the feed samples respectively. Those feed samples without oilseed cake included 75% pure wheat bran and the rest 25% included wheat middlings, bran of pulse and poultry liter. The other 45 samples were roughage feeds mainly of hay (56%), wheat straw (33%) and haricot bean straw (11%).

Sample preparations including drying, chopping, milling and packing and labeling processes were carried out at Holetta Agricultural Research Center.

3.3.2.1. Extraction of feed samples

Extraction process of feed samples was carried out based on the procedures mentioned in the manual of Enzyme-Linked Immunosorbent Assay (ELISA) for feeds (Helica biosystems Inc Aflatoxin B1 ELISA Quantitative Catalog ♯ 41BAFL01B1-96). Twenty g of each feed sample was transferred into 250ml Erlenmeyer flasks containing 100 ml of 70% methanol and allowed to digest for 30 minutes. The particulate matter was filtered with whatman ♯ 1 filter paper No 541 or 542 and the filtrate was transferred into 100 µl centrifuge tubes for determination of aflatoxin.

3.3.2.2. Determination of AFB1

Assay process was performed based on the protocol provided by the manufacturer (Helica biosytems Inc) using the commercial Enzyme-Linked Immunosorbent Assay (ELISA)-based test kits and micro plate ELISA reader of model Biotech USA. Hundred µl of the sample solutions or standards were added in to each dilution wells containing 200 µl of HRP conjugate (assay diluient) and the mixture was mixed using the multi-channel priming micro pipette. Then, hundred µl of the mixture was transferred in to each of the antibody coated micro wells and incubated for 15 minutes at room temperature. Then the liquid was poured out of the wells and wells were washed 5 times using distilled water and the bound substances was stacked to the antibody coated micro wells. Wells were tapped face down on a layer of paper towel to remove the residual wash solution. Next, hundred µl of tetramethylbenzidine (TMB) as enzyme substrate solution was added in to each well and incubated for 5 minutes at room temperature in the dark and a blue color was developed. Then 100 µl of acidic stop solution was added to stop the reaction which changed the chromagen color from blue to yellow. The optical density (OD) or absorbance of each sample in the micro well was read using ELISA reader at 450 nm filter. A calibration curve was drawn using the aflatoxin concentrations of 0µg/kg - 20µg/kg in the X-axis and absorbances in a range of 0.2% -3.12% in the Y-axis of the standards (Fig 3.5) to measure the aflatoxin concentration of each sample. Then the absorbance of each sample was compared with the absorbance of the standards and the concentration of AFB1 in µg/kg and AFM1 in µg/L was directly measured from the standard curve.

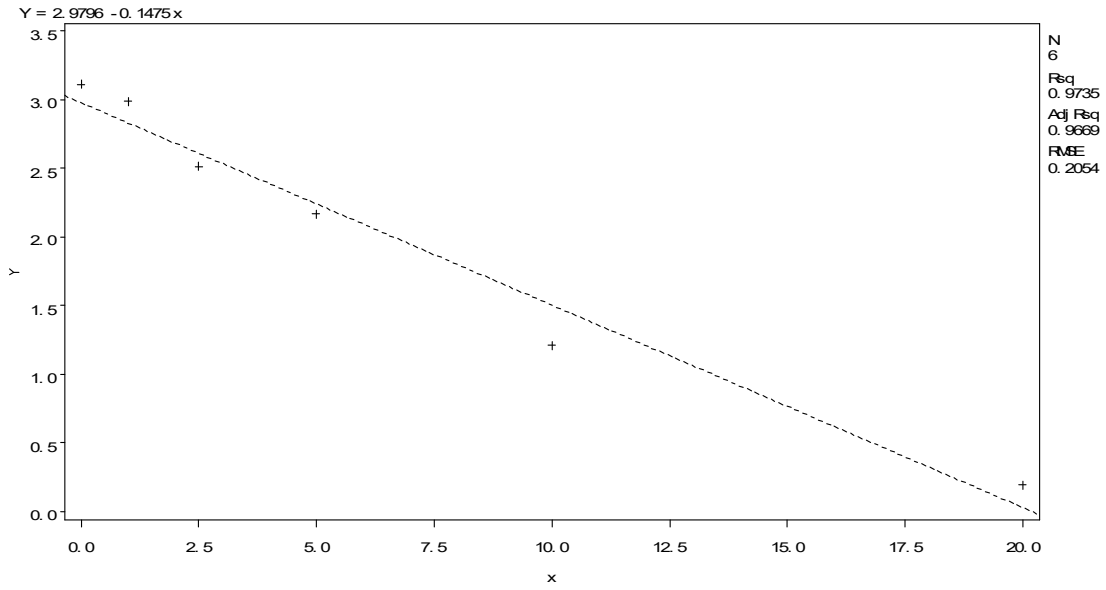

Fig 3.5. Standard curve of different concentrations of Aflatoxin B1

3.3.2.3. Collection of milk samples

Milk samples were collected from 45 smallholder urban dairy producers in the study locations, 15 samples per each study location. Hundred ml of fresh milk samples were collected in ice box from each household from the morning milking and stored in a refrigerator until analysis at Holetta Agricultural Research Center.

3.3.2.4. Determination of AFM1 in milk samples

The laboratory investigation for AFM1 in milk was carried out at Naivashsa Dairy Research Coordination Center, Nairobi, Kenya. The standards were presented in homogenized skim milk (milk plasma). An aliquot of unprocessed raw fatty milk was placed at refrigerated temperature overnight to allow the fat globules to rise to the surface in a natural "creaming" effect. The upper fatty layer was removed by aspiration and the lower plasma layer was used for the assay. The aflatoxin standards were: 0.0pg/ml, 5.0pg/ml, 10.0pg/ml, 25.0pg/ml and 100.0pg/ml. The reagents were brought from refrigerator temperature to room temperature before starting the assay. Using a new pipette tip for each, 100 µl aliquots of standards and samples were dispensed into the dilution wells containing 200 µl conjugate. This was incubated at room temperature for 2

hours. The contents of the wells were discarded into an appropriate receptacle. The micro wells were washed by filling each with PBS-Tween wash buffer and were decanted into an appropriate receptacle. This was repeated for three washings. The wells were tapped face down on a layer of absorbent towel to remove residual wash buffer. Then hundred µl of enzyme substrate was placed to each antibody coated micro-well and incubated at room temperature for 5 minutes in the dark. To stop the reaction, hundred µl of stop solution was added in to the antibody coated micro wells. The optical density (OD) of the standards and the samples were read on each well with a micro plate reader/ELISA reader using a 450nm filter. A graph was plotted based on the aflatoxin concentration and absorbance of the standards and the absorbance of each milk sample were compared with the absorbance of the standards and then the concentration of aflatoxin M1 in pg/ml was directly measured from the standard curve.

3.3.3. Study design and statistical analysis

The study was undertaken based on Completely Randomized Design (CRD) and the statistical model was $Yi= \mu + Li + ei$ Where,

Yi = level of aflatoxin

μ = the overall mean

Li = effect of location

ei = the error term

The data was subjected to Analysis of variance using the General Linear Model Procedures of Statistical Analysis System (SAS, 2002). Different statistical analysis including descriptive statistics and t-test were employed to determine the differences.

3.4. Results and discussions

3.4.1. Level of aflatoxin in feeds in this study & other countries in comparison with EU and USA permissible limits

In this study 81 feed samples (50.6%), did not contain any toxin; whereas 29 of the feed samples (18.1%) and 50 samples (31.3%) contained aflatoxin levels which were within the EU permitted level of 5µg/kg, and the U.S.A. permitted level of 20 µg/kg AFB1 for feeds, respectively (Table 3.1). This result was similar to the AFB1 level of feeds reported in Portugal almost all (98%) of the feed samples fulfilled the EU permissible level of aflatoxin in feeds (5µg/kg) and the U.S.A. permitted level of 20 µg/kg AFB1 for feeds (Martins *et al*., 2007).

The aflatoxin status and level of feeds in this study was much lower than the previous report in the country where the contaminated level was in a range of 7-419 µg/kg (Dawit *et al*., 2016). The results of this study are in contrast to the AFB1 level of feeds reported in India, Kenya and previous authors in Ethiopia in which 17% and 15%, 46% and 45% of the feed samples did not fulfill the U.S.A AFB1 level recommended for feeds (Table 3.1) respectively. The reason for the difference of AFB1 in feeds between Ethiopia and Kenya; as well as Ethiopia and India could be due to the difference in feed type used in Ethiopia (wheat by-products based feeds) and other African countries and India (by-products of maize and groundnut based feeds) which are the most susceptible crops to AFB1 contamination as compared to wheat-based feeds (Idris *et al*., 2010).

Table 3.1. AFB1 (µg/kg) in feeds in this study & other countries in comparison with EU and USA permissible limits (%)

Country	N	Range (µg/kg)	0 level	EU standard	USA standard	> USA Standard	Reference
Ethiopia	160	0-20	50.6	68.7	31.3	0	This study
Portugal	312	5.1-74	0	84	14	2	Martin *et al*., 2007
India	40	1.8-245	28	13	55	17	Vijayasamundeeswari *et al*., 2009
Kenya	144	1-9661	0	27	54	46	Senerwa *et al*., 2016
India	356	-	46	NA	39	15	Karthikeyan *et al*., 2013
Ethiopia	156	7-419	0	5	50	45	Dawit *et al*., 2016

N= number of samples; NA= not available; Standard = permissible limit

3.4.2. AFB1 mean, range and percent of feed samples belonging to different levels of aflatoxin

The distribution of AFB1 showed variations among the different feed types (Table 3.2.). Accordingly, concentrate feeds contained significantly (P<0.05) higher AFB1 (7.67µg/kg) contents than roughage feeds (0.41µg/kg). Among the concentrate feeds, oil seed cake based concentrate feed contained significantly (P<0.05) higher AFB1 (13.09µg/kg) than those without oilseed cake and grass hay showed significantly (P<0.05) higher AFB1 (0.72 µg/kg) level than the other roughage feed types.

Table 3.2. AFB1 mean, range and percent of feed samples belonging to different aflatoxin categories

Type of feed	N	Level of aflatoxin (µg/kg)			Range (µg/kg)	Mean (µg/kg)
		0	0.1 - 5	5.1 – 20		
		Percent				
Roughage feeds	45	21.9	6.3	0.63	0-0.55	0.41 ± 0.14^{f}
Grass Hay	25	33.3	22.2	2.2	0-5.5	0.72 ± 0.25^{e}
Wheat straw	15	31.1	2.2	0	0-1	0.05 ± 0.05^{g}
Straw of haricot bean	5	11.1	ND	ND	ND	ND
Concentrate feeds	115	6.3	2.5	22.5	0-20	7.67 ± 0.8^{b}
With oil seed cake	47	8.7	3.5	31.3	0-20	13.09 ± 1.12^{a}
Without oil seed Cake	68	33.9	8.7	14.8	0-20	2.78 ± 0.66^{c}
Wheat bran	44	26.1	6.1	4.4	0-20	2.15^{d}
Mean						5.63

N= number of samples; ND= non detectable; LS-means with different superscripts among three consecutive feeds in the last columns were significantly different (P<0.05).

The lowest AFBI was recorded from wheat straw (roughage feed) and wheat bran (concentrate feed) with AFBI of 0.05µg/kg and 2.15µg/kg, respectively. Interestingly, no aflatoxin was detected in haricot roughage feed with the limited number of samples tested for AFBI. The pattern of AFB1 contamination in roughage feeds and concentrate feeds was similar to the report of Makau *et al*. (2016) who showed that concentrate feeds (47.84 1 µg/kg), contained almost 10 times more AFBI than the roughage feeds (5.14 g/kg) indicating concentrate feeds are more susceptible to AFB1 contamination than roughage feeds.

This may be due to the fact that concentrate feeds are rich in nutritional content than roughage feeds which can provide adequate substrate for growth and reproduction of fungus. This is

justified by the fact that, compared to grass hay which contains 59 g CP/kg feed, noug and noug based concentrate feeds contained CP contents of 312 g/kg and 251.3g/kg, respectively (Rehrahie and Ledin 2001). Ephrem (2015) also reported that food stuffs with high fat content are good substrates for aflatoxin producing fungi and they are more susceptible to AFB1 contamination as compared to other dairy feeds (Makun *et al*., 2012; Dawit *et al.*, 2016). It was reported that the first effect of mold on feeds is utilization of nutrients for their metabolism and propagation which results in decreased nutritional value of feeds. The energy, crude protein and crude fat values of moldy feeds decreased by 5%, 6% and 63% respectively (Dicostanzo, 2012) and dietary fats were affected more extensively than proteins or carbohydrates and they decreased 37% to 40% after 25 days of storage or 52% to 57% after 50 days of storage respectively (Dicostanzo, 2012).

The data showed that higher proportion (22%) of the roughage feed samples contained 0µg/kg of AFB1 with fewer samples (0.63%) with AFB1 level in the range of 5.1 - 20µg/kg. On the contrary, higher proportions (22.5%) of the concentrate feed samples contained AFB1 in the range of 5.1 - 20µg/kg, and fewer proportions (6.3%) of the concentrate feed samples were free from aflatoxin contamination

Similarly, higher proportions (31.3%) of AFB1 was detected in the range of 5.1 - 20µg/kg, from concentrate feeds containing oilseed cake compared to the lower proportions (14.8%) detected from the same concentrate without fortification with oil seed cake (Table 3.2.). The overall mean and range of AFB1 in feeds in this study was 5.63 µg/kg and 0-20 µg/kg respectively which was lower than the mean and range AFB1 of feeds (25.53 µg/kg and 0.54 - 204.72 µg/kg) reported in Chennai, India (Ramesh *et al*., 2013). The reasons for difference of AFB1 in feeds in Ethiopia and India could be due to the difference in feed types commonly utilized in Idia (by-products of maize and groundnut grains), which are the most susceptible commodities to AFB1 contamination (Oyebangi and Efiyuvwevvvere, 1999) as compared to the common Ethiopian dairy concentrate feeds, mainly by-products of wheat (wheat bran) which is relatively less susceptible to aflatoxin infestation **(**Idris *et al*., 2010**).** It was also reported that Aflatoxin synthesis is higher when feeds are stored under warmer temperature ranging 24^0C - 37^0C (Ramesh *et al*., 2013) and in humid environmental conditions (Amer *et al*., 2010). The relatively higher environmental temperature of around 29^0C and humidity of 41% prevailing in Chennai, India (https://www.google.com/search?q=chennai+india+weather, 2017) seems more favorable

to mold proliferation and aflatoxin contamination as compared to the central highlands of Ethiopia having relatively lower environmental temperature ranging 16°C - 22°C and humidity of 24% (https://journeysbydesign.com/destinations/Ethiopia, 2017).

3.4.3. Levels of aflatoxin in different feeds sampled from the different study locations

The concentration of AFB1 in different feed types is shown in table 3.3. Though there was no significance difference (P>0.05) among roughage feeds across the study locations, AFB1 level of wheat straw in Bishoftu had the lowest concentration (0.07µg/kg) as compared to AFB1 content of hay in Holetta (1.07µg/kg) and Hawassa (0.13µg/kg). The lowest AFB1 level in wheat straw could be attributable to its poor nutritional composition (Schiere and Ibrahim, 1989) which makes it less vulnerable to fungal contamination.

Table 3.3. Levels of aflatoxin (µg/kg) in different feeds by study locations

Type of feed	Study location					
	Holetta	% contamination	Bishoftu	% contamination	Hawassa	% contamination
No of samples	58		48		54	
Roughage feeds						
Grass hay	1.07 ± 1.4^{a}	13.8			0.13 ± 1.7^{a}	1.9
Wheat straw			0.07 ± 1.4^{a}	2.1		
Concentrate feeds						
With oil seed cake	10.29 ± 1.1^{b}	29.3	17.5 ± 1.3^{a}	35.4	8.13 ± 2.8^{b}	7.4
Without oil seed cake	5.24 ± 1.3^{b}	24.1	9.64 ± 1.5^{a}	20.8	2.0 ± 1.6^{b}	11.1
Wheat bran	3.61 ± 2.1^{ab}	10.3	6.43 ± 2.1^{a}	8.3	0.81 ± 1.0^{b}	9.3

%= percentage of aflatoxin contamination for each type of feed under each study location
LS-means with different superscripts between columns were significantly different (P<0.05)

The results also showed that the AFB1 level in concentrate feeds fortified with oilseed cakes was significantly (p<0.05) higher (17.50 ± 1.3 µg/kg) in Bishoftu as compared to the same type of feed in Holetta with AFB1 content 10.29 ± 1.1 µg/kg and AFB1 of 8.13 ± 2.8 µg/kg at Hawassa. Similarly, the highest AFB1 level of concentrate feeds without oilseed cakes was observed in samples from Bishoftu (9.64 ± 1.5µg/kg) when compared with Holetta (5.24 ± 1.3µg/kg) and

Hawassa (2.0 ± 1.6µg/kg). Similar pattern of AFB1 contamination was also observed in other feed types from Bishoftu sampling sites (table 3.3). It was reported that aflatoxin synthesis is highest when feeds are stored under hot environment particularly in temperatures between 24^0C and 37^0C (Ramesh *et al*., 2013). The highest AFB1 content in feeds observed in Bishoftu might be due to the relatively higher temperature which could be aggravated by the relatively longer storage duration (temperature 25^0C and storage duration 3-6 month) prevailing in this area as compared to Holetta (temperature 22^0C and storage duration 3-6 month) and Hawassa (temperature 27^0C and shorter storage duration of < 1 month).

3.4.4. Storage duration of aflatoxin in feeds sampled from the different study locations

The storage duration of feeds collected from Holetta, Bishoftu and Hawassa along with the AFB1 contamination level is indicated in table 3.4. The average AFB1 content of feeds in Bishoftu (9.76µg/kg**)** was significantly ($p<0.05$) higher than the AFB1 content of feeds in Holetta (6.33µg/kg) and this was significantly ($p<0.05$) higher than that of Hawassa (1.19µg/kg), in which case AFB1 of feeds in Hawassa fulfilled the EU standard of 5µg/kg (FAO, 2004). AFB1 level of feeds observed in Hawassa in this study was mainly due to the short storage duration (<1 month) of dairy feeds practiced in that area. The roughage feeding system of urban dairying in Hawassa during the sampling time of was mainly based on cut and carry in which roughage feeds were collected from protected areas like universities and prison stations and was not stored for more than 10 days. In addition, about 80% of the concentrate feeds available in feed manufacturers and feed retailers in Hawassa was based on wheat bran with some mixture of linseed cake and other home processed grain by products from barley and pulses, these factors altogether makes AFB1 in feeds the lowest as compared to other study locations. Our findings agree with the report of Adebayo-Tayo and Ettah (2010) in which wheat offal was found to be less susceptible to aflatoxin contamination.

Table 3.4. Storage duration (month) of feeds in different study locations

	Study location					
	Holetta	% contamination	Bishoftu	% contamination	Hawassa	% contamination
No of samples	58		48		54	
Time of storage	3-6	77.5	3-6	66.6	< 1 month	29.7
Mean AFB1	6.33 ± 1^b		9.76 ± 1.3^a		1.19 ± 1.1^c	

The level of AFB1 contamination of feeds in Bishoftu (9.76 µg/kg) was significantly ($p<0.05$) higher than the average AFB1 of Holetta and Hawassa (3.8 µg/kg). The trend was similar to the report of Dawit *et al*. (2016) in that AFB1 level in Bishoftu (156 µg/kg) was higher than the average AFB1 level of other study locations (Sendafa, Sululta and Addis Ababa) which was 133 µg/kg .

3.4.5. Aflatoxin contamination level of feeds across the feed value chain

The AFB1 contamination levels of feeds belonging to dairy producers were significantly ($P<0.05$) higher (9.35 ± 1.04µg/kg) than feed retailers (6.91 ± 1.09 µg/kg) and were also significantly ($p<0.05$) higher than the AFB1 levels of feeds in feed manufacturers (7.50 ± 1.43 µg/kg) (Table 3.5).

Table 3.5. AFB1(µg/kg) contamination of feeds across the feed value chain

Dairy feed value chain	N	Study location			Mean AFB1
		Holetta	Bishoftu	Hawassa	
Feed manufacturers	29	7.92 ± 1.84^{ab}	12.45 ± 3.25^{a}	2.14 ± 2.42^{c}	7.50 ± 1.43^{ab}
Feed retailers	41	5.33 ± 2.02^{b}	15.40 ± 2.01^{a}	0.00^{c}	6.91 ± 1.09^{bc}
Dairy producers	45	11.37 ± 1.88^{ab}	15.18 ± 1.88^{a}	1.5 ± 1.30^{c}	9.35 ± 1.04^{a}
Overall	115				

LS-means with different superscripts between rows were significantly different (last column); LS-means with different superscripts between columns were significantly different (P<0.05) (excluding last column); N=number of samples

Regarding the study locations, the AFB1 levels in feeds produced by feed manufactures in Bishoftu (12.45 ± 3.25µg/kg) was significantly ($p<0.05$) higher than those of Holetta (7.92 ± 1.84µg/kg) and Hawassa (2.14 ± 2.42 µg/kg), respectively. Similarly, the AFB1 level in feeds handled by feed retailers in Bishoftu (15.40 ± 2.01µg/kg) was significantly ($p<0.05$) higher than that of Holetta (5.33 ± 2.02µg/kg) and Hawassa (0µg/kg), respectively. The trend was also the same for AFB1 level in feeds handled by dairy producers across the study locations (table 3.5).

The high AFB1 contamination levels of feeds across the feed value chain were in the order of dairy producers > feed manufacturers > feed retailers (table 3.5). The reason for the higher level of AFB1 in concentrate feeds handled by urban smallholder dairy producers might be due to the

poor storage situation employed by them. The lower contamination level of AFB1 of concentrate feeds administered by feed retailers could be related to the small scale of holding and shorter duration of storage because of high market demand. For example in Hawassa, concentrate feeds belonging to feed retailers were not stored for more than a week. Kang and Lang (2009) in their study of AFB1 in animal feeds in Kenya reported that contamination levels for urban smallholder dairy, feed manufacturers and feed retailers were 20.48 ± 29.8µg/kg, 20.62 ± 15.62 and 22.38 ± 17.78 respectively and these amounts were higher than the results obtained in this study.

3.4.6. Level of aflatoxin M1 (AFM1) in milk across study locations

The overall (average) AFM1 contamination of milk in this study was in the range of 0-0.146; with a mean AFM1 of 0.054 µg/L which was different from the average and range of AFM1 (0.41µg/L and 0.028-4.98 µg/L) in milk samples reported in Ethiopia by Dawit *et al*. (2016). The data also showed variations in AFM1 content of milk samples at different experimental sites (Table 3.6). Accordingly, milk samples collected from Bishoftu contained significantly ($p<0.05$) higher AFM1 (0.088 µg/l), followed by milk samples with AFM1 contents of (0.057 µg/L and (0.017 µg/L collected from Holetta and Hawassa sampling sites, respectively (Table 3.6). Although all samples were within the EU and USA permitted levels, the quality of milk seemed to be better in Hawassa than the other experimental locations which may be associated with the lower AFB1 content of feeds in the area (table 3.4).

Table 3.6. Pattern of aflatoxin contamination of milk at different study locations

Sampling site	Mean (µg/L)	Range (µg/L)	Free from AFB1 (n & %)	EU standard (0.05 µg/L) (n & %)	USA standard (0.5µg/L) (n & %)	Ethiopian standard (ES) (0.05 µg/L) (n & %)
Bishoftu	0.088	0-0.1403	2 (13)	4 (27)	11 (73)	4 (27)
Holetta	0.057	0.0015-0.146	0 (0)	4 (27)	11 (73)	4 (27)
Hawassa	0.017	0-0.11	11(73)	15 (100)	0 (0)	15 (100)
Level of AFM1 (oveall)	0.054	0-0.146	29%	58%	42%	58%

3.4.7. Level of aflatoxin in milk in comparison with other countries

The quality of milk in terms of aflatoxin contamination in this study was compared with other countries (Table 3.7) and the AFM1 contamination level was slightly higher than the AFM1 of

milk studied in Turkey where, 39% of the samples were free from aflatoxin, and 61% of the samples were within the EU stringent standard reported by Aksoy *et al.* (2010).

It is interesting to note that the aflatoxin level of milk in this study was in the "low " category (within the Stringent EU standard) similar to most reports from different countries, especially from Egypt (Amer, 2010; Ghareeb *et al*., 2013), Iran (Rokhi 2013; Norian *et al*., 2015), Kenya (Kang and Lang 2009), India (Iqbal, *et al*., 2011) and Sri Lanka (Pathirana, *et al*., 2010) (Table 3.7). The AFM1 content of milk in this study was lower than the previous results reported in Ethiopia (Dawit *et al*., 2016), in Pakistan (Jawaid, *et al*., 2015) and in India (Jawaid, *et al*., 2015), and others (table 3.7). However the quality of milk in terms of the level of AFM1 in milk was lower than the milk samples reported from Iran (Norian *et al*., 2015) and Tukey (Aksoy *et al*., 2010) (table 3.7). The difference of AFM1 in milk in Ethiopia and Iran and Tukey could be due to the difference in feed type used in Ethiopia which is mainly by-products wheat as compared to Iran and Tukey mainly used by-products of maize and groundnut feeds that are the most susceptible commodities to aflatoxin contamination as compared wheat (Oyebangi and Efiyuvwevvvere, 1999; Idris *et al*., 2010**)**. The other reason could be the relatively lower environmental temperature (16°C - 22°C) and humidity (24%) in the central highlands of Ethiopia (https://journeysbydesign.com/destinations/Ethiopia, 2017) which is lower than that of Gilan, Iran having humidty of 70% (https://www.google.com/search?q=weather+Gilan+Iran, 2017) and Samsun, Turkey, with prevailing humidity of 45% (https://www.google.com/search?q=weather+Samsun+Turkey, 2017) in which warmer temperature and higher humidity are favorable conditions for mould proliferation and aflatoxin synthesis (Amer *et al*., 2010).

Table 3.7. Level of AFM1 in milk in comparison with other countries (2009-2016)

Country	AFM1 in milk (µg/L)			Reference
	Mean	Range	Status	
Ethiopia	0.054	0–0.146	Low	This study
Ethiopia	0.41	0.028-4.98	Medium	Dawit *et al*., 2016
Pakistan	0.38	0.01–0.76	Medium	Jawaid, *et al*., 2015
India	0.5	-	Medium	Lunden, 2015
Iran	-	0.0021 - 0.131	Low	Rokhi, 2013
Egypt	0.05	-	Low	Amer, 2010
Egypt	0.063	0.002-0.11	Low	Ghareeb *et al*., 2013
Sudan	0.5	-	Medium	Suliman and Abdalla, 2013
Thailand	0.070	-	Low	Ruangwises *et al.*, 2013
Kenya	0.064	-	Low	Kang and Lang 2009
India	0.046	-	Low	Iqbal, *et al*., 2011
Sri Lanka	0.04	-	Medium	Pathirana, *et al.*, 2010
Iran	0.0004	-	Low	Norian *et al.*, 2015
Turkey	0.0023	-	Low	Aksoy *et al.*, 2010

Low= within the EU standard (0.05 µg/L); Medium= Within the USA standard (0.5µg/L)

Overall the AFB1 and AFM1 levels in feeds and milk in the current study were 5.63µg/kg and 0.054µg/L respectively and the transfer rate of 1-2 % of AFB1 in feeds to AFM1 in milk was 0.054/5.63 x 100 = 0.96% which was similar to the findings of Van Egmond (1989).

3.5. Conclusions

In this study, half of the feed samples were free from aflatoxin contamination; whereas the remaining were within the stringent EU level of 5 μg/kg and the more permissible USA standards of 20 μg/kg. The level of aflatoxin contamination among the different feed sources, showed that concentrate feeds such as oil seed cakes contained more AFB1, followed by cereal byproducts, roughage feeds (grass hay and straws) respectively. The data obtained in this study also showed differences in aflatoxin levels among sampling sites in that feeds from Bishoftu had more aflatoxin, followed by feeds from Holetta and Hawassa, respectively.

The aflatoxin contamination level in feeds followed the order: those belonging to dairy producers contained significantly higher AFB1 than feed retailers, followed by feed manufacturers. Variations in aflatoxin levels in feeds and sampling sites were due to different nutritional contents of feeds and the storage conditions in different study areas.

With regards to milk samples, aflatoxin was not detected in some samples (29%) while 58% of the samples examined were within the stringent EU standard of (0-0.05 μg/L); and 42% were within the USA standard (5 μg/L) set for milk indicating that the milk samples were within the permissible level of aflatoxin in milk. The data obtained in this study also showed the conversion rate of afltoxin from feeds to milk which was within the values computed for other milk samples elsewhere.

The AFM1 level of milk in this study was in the "low category" which was comparable to those AFM1 levels obtained elsewhere. In general, the AFM1 contamination level of feeds and milk samples collected from the study locations were in the order of Hawassa < Holetta < Bishoftu. Further studies on aflatoxins in dairy feeds and milk should be carried out using other techniques such as HPLC, GC and multi-mycotoxin assay using LC-MS-MS to obtain conclusive information.

Chapter 4.
Determination of heavy metals in feeds, water and milk from some parts of Central Ethiopia

Rehrahie Mesfin, Fassil Assefa and Getnet Assefa

4.1. Abstract

Human being is continuously exposed to various pollutants and chemicals through foods that can lead to serious health problems. Heavy metals are among the many pollutants that are released from effluents of different industries, agrochemicals and weathering of rocks to the environment. Soils and water are the major reservoirs of heavy metals. Livestock water and feeds are the major routes of food contamination by toxic heavy metals, vital inputs for livestock products and are important component of human food chain.

The study was conducted to investigate toxic heavy metals: cadmium (Cd), lead (Pb), arsenic (As) and chromium (Cr) in livestock feed, livestock water and cow milk in West Shoa zone (Holetta area) and East Shoa zone (Akaki-Mojo) Ethiopia. A total of 90 feeds, water and milk samples were collected from the study areas. Quantification of heavy metals was determined using Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS). Results showed that concentrations of heavy metals in teff straw in Holetta and Bishoftu were 1543.54 ± 318.70 µg/kg and 1486.92 ± 279.73 µg/kg respectively. The overall concentration of heavy metals in teff straw in the study locations was in the order of Cr > As > Pb > Cd. The concentrations of heavy metals in livestock water in Bishoftu (28.08 ± 7.02 µg/L) was significantly higher ($p<0.05$) than the concentration of water collected from Holetta (1.96 ± 0.28 µg/L) and the levels in both study locations were in the order of Cr > As > Pb > Cd. Water sample taken from the effluent discharged from Modjo Tannery had the significantly ($p<0.05$) highest concentration of heavy metals (86.89 µg/L) with an elevated concentration of Cr (300.56 µg/L) which was followed by water sampled from Mojo Lake (67.89 µg/L) and a river near Mojo town (43.64 µg/L). With the exception of pH of water from Mojo Lake (10.37) and Gelan dye factory (8.9), the rest of water samples collected from Eastern shoa were within the legal pH limit of 6.5-8.5 for livestock drinking. All water samples collected from Holetta had an average pH of 7.0 ranging 6.4–7.7 and were within the permissible limit. The overall concentration of heavy metals in cow milk in

the study locations were in the order of Cr > Cd > Pb > As. The concentrations of Cd and As in milk in both study locations were within the permissible limits. However, the concentrations of Pb in 60% and 73% of the milk samples collected from Holetta and Bishoftu respectively and the concentration Cr in all milk samples collected from both study locations were above the permissible limit which is an indication of environmental pollution. This preliminary study showed elevated concentrations of Cr and As in teff straw in both study locations suggesting the need of further investigation of heavy metals from soil samples and feeds.

Key words: Lead, Cadmium, Chromium, Arsenic, teff straw, Holetta, Bishoftu.

4.2. Introduction

Heavy metals are one of the hazardous chemicals that enter into the environment from weathering of rocks, and as pollutants coming from the development of modern technologies and rapid industrialization (Kaplan *et al*., 2011), aggravated by discharge of industrial effluents, sewage sludge and agrochemicals because of poor planning in waste disposal and management (Swarup and Dwevedi, 1998; Pavlovic, 2004). Heavy metals are extremely persistent in the environment where they are non-biodegradable and non-thermo-degradable and thus they get accumulated in the environment (Akan *et al.*, 2009).

Cadmium (Cd), lead (Pb), Arsenic (As) and Chromium (Cr) are some of the most toxic heavy metal pollutants that get accumulated in soil and water and are known for their toxicity even when taken in small amount, and they were rated with varying toxicity degree: Cd (highly toxic), Pb (toxic), As (moderately toxic) and Cr (slightly toxic) (FAO, 1999).

Heavy metals are absorbed by plants from soil and subsequently channeled in to livestock and livestock products (milk, meat and egg) and human being via the food chain (Kaplan *et al.*, 2011; Ogundiran, *et al*., 2012). They are capable of causing serious health problems and have the capacity of damaging vital organs like the kidney and liver in chronic cases and can also lead to acute poisoning in situations of exposure to higher concentration (Rajaganapathy *et al*., 2011).

With the growing of industries, heavy metals becomes a challenge to agricultural production because they contaminate grains, feeds and water sources that compromise the quality of dairy products (Kaplan *et al.*, 2011). Long term dietary intake of food contaminated with heavy metals results in their accumulation in the body which contribute to the development of various disorders of growth, biological functions and hormonal system (Orisakwe *et al.*, 2012). Heavy metals have the capability of interfering with the normal process of milk synthesis and affect milk yields in dairy cattle (Khan *et al,* 2012). They are also capable of displacing other essential minerals from where they do vital biochemical and enzymatic reactions in the metabolism process (Dawd, *et al*., 2010). Feeds containing excess cadmium, lead and arsenic appear in toxic levels and their toxicity interferes with copper and zinc absorption, resulting in decreased milk production and in acute cases. It is known that lead toxicity in cattle results to death within 24 hours (Mukesh, *et al*., 2008).

In Ethiopia, many research activities were undertaken to improve the quantity of feed resources and increase dairy production. However, research on feed, water and milk quality in relation to heavy metals was limited. Dawd (2010) reported that samples of cow milk from Addis Ababa contained 100 mg/L, 40 mg/L and 998mg/L, for Cd, Cr and Pb respectively. Tasew *et al*. (2014) also reported 870µg/ml of Cr and un-detectable level of Cd and Pb in cow milk in Borena zone. A study undertaken in Hawassa around Tikur-Wha textile industry on heavy metal content of water and milk showed that Pb and Cd content of water was 400mg/L and 20mg/L respectively and that of milk were 600 mg/L, 150 mg/L and 150 mg/L for Pb, Cd and Cr respectively which were reported to be unsafe (Muluken, 2014). All **t**aken together, the few studies mentioned above water and milk samples were highly contaminated with heavy metals beyond the acceptable standards.

The objective of this study, therefore, was to undertake a comprehensive study of Cd, Pb, Cr and As in livestock feeds, water and cow milk in selected areas of West and East Shoa zone.

4.3. Materials and Methods

4.3.1. Study locations

The study was carried out in Oromiya regional state of West and East Shewa zones and the detailed description of the study sites are presented in the table 4.1.

Holetta (Welmera) is located 45 km west of Addis Ababa at an altitude of 2400 m.a.s.l. with annual rainfall of 1066 mm and average minimum and maximum temperatures of 6° and 22°C, respectively. The soil type was reported to be predominantly Eutric Nitosols, pellic and chromic vertisols (Getachew *et al*., 2014), Majority of the soils was derived from lava flows and the rest were developed from volcanic rocks. The characteristics of the soils vary from sandy to clay soils and the pH of the soils is mainly acidic, 5.24 (Gemechu, 2007). Holetta is a potential place for agricultural production where both crop and livestock farming has been undertaken. Holetta have been hosting the prominent research center named as Holetta Agricultural Research Center and many improved agricultural technologies of crop varieties, pesticides and fertilizers have been applied for verification and demonstration purposes for the last 50 years. Since 2004, Holetta is hosting as an investment place for many flower production farming.

Bishoftu area (Eastern Shoa) is located at 45 km East of Addis Ababa at an altitude of 1950 m. a.s.l. with a mean minimum and maximum temperature of 10.6 and 25.0 °C, respectively (Samuel *et al.*, 2009). The soil type towards Akaki area is predominately vertisol and fluvisol in which the Fluvisol is more contaminated with polluted waste water than the vertisol (Fisseha *et al.*, 2003).

The direction to Akaki is one of the industrialized places in Ethiopia where it hosts over half of the industries of Addis Ababa. Large and medium industrial establishments like foods, beverage, furniture, detergent, dye, leather, paper, metallic products and textiles industries are running in this area. The polluted Akaki River also flows through this area after passing Addis Ababa (Ellen *et al.*, 2015). Gelan (the direction to Bishoftu) also has accommodated different factories from which effluents are released and commonly meet in Gerbecha River (the River that connects Gelan area and the Awash River). Since neighboring farmers are experiencing water shortages for livestock drinking, they use the River as alternative source of water supply for livestock production.

4.3.2. Collection and preparation of samples

Fifteen teff straw samples were randomly collected in plastic bags from each study location. Feed samples were chopped, milled, labeled and packed at Holetta Agricultural Research Center.

Similarly, fifteen samples of water from each study locations were randomly collected in clean 0.5 capacity plastic bottles from surface waters mainly from streams, surface runoff of and Rivers used for livestock drinking. PH of water samples was measured using PH/ion-meter, WTW, Inolab (Germeny). Similarly, fifteen milk samples were collected from farmers having local milking cows from each study location immediately after milking. They were collected in 100 ml capacity plastic bottles cleaned with 20% HNO_3 and rinsed with de-ionized water. They were immediately placed in an ice box and delivered to Holetta Agricultural Research Center and preserved in refrigerator at -20 °C.

All samples were delivered to the laboratory of the Environmental Protection Office in Addis Ababa for the determination of Cd, Cr, Pb and As.

4.3.3. Laboratory analysis of feeds, water and milk samples

Feed samples were dry-ashed in a muffle furnace at 450^0c followed by wet digestion using nitric acid (HNO3) and the extract was subjected for determination of Cd, Cr, and Pb using Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS) of model AAA-NOVAA 400 analytrekjena. Water samples previously observed for pH was directly subjected to GFAAS for determination of Cd, Cr, Pb and As. (AOAC, 1990).

For milk digestion, 10 ml of HNO3 (65%) and HCLO4 (72%) in a ratio of 9:1 mixture was added into beakers containing 3ml milk samples. Then 3 ml of H_2O_2 (30%) was added and placed in a hot plate at 90ºC for oxidation of organic matter for overnight until a clear extract was recovered and this was nebulized into GFAAS for reading of Cd, Cr, Pb and As (AOAC, 1990). Before determination the concentration of each metal, the GFAAS instrument was calibrated with standard solutions by establishing calibration curve (concentration vs. absorbance).

The stock standard solutions used for calibration were salts of nitric acid (Pb, Cd and Cr) and salts of hydrochloric acid for As. To prepare standard solution of chromium, chromium solution of 1000mg/L in Ca. M nitric acid was used. To prepare standard solution of lead, traceable to SRM from NIST Pb (NO_2) in HNO_2 0.5mol/L was used. To prepare standard solution of cadmium, cadmium solution of 1000mg/L in Ca. M nitric acid was used. To prepare standard solution of arsenic, arsenic solution of 1000mg/L in Ca. M hydrochloric acid was used.

The working standard solution for each metal was obtained by dilution of the stock solution (1000mg/L, Merck Germany) using de-ionized water. To prepare 100mg/L of working standard solution, 10mg of the 1000mg/L individual metal stock standard solution was diluted with 100ml de-ionized water (AOAC, 1990). It was nebulized in to GFAAS instrument which was fitted in to computer and a calibration curve (concentration vs. absorbance) was established to verify if the GFAAS instrument was working perfectly. Following this procedure, each sample, obtained after the digestion process, was nebulized into GFAAS and the elemental concentration of each element was read from the computer which was interfaced with the GFAAS. The accessory hydride was fitted for reading the concentration of As whereas, graphite was fitted to the apparatus for reading the concentration of Pb, Cd and Cr (AOAC, 1990).

4.3.4. Study design and statistical analysis

The study was undertaken based on Completely Randomized Design (CRD) and the statistical model was $Yi= \mu + Li + ei$ Where,

Yi = level of heavy metals

μ = the overall mean

Li = effect of location

ei = the error term

The data was subjected to analysis of variance using Statistical Analysis System (SAS, 2002); and GLM and t-test were used to analyze the data.

4.4. Results and discussions

4.4.1. PH of livestock water in different sampling sites of Eastern shoa

The pH of livestock water in different sampling sites of Eastern shoa is indicated in table 4.1. and their pH were within the acidic range of 6.51 for water samples from Akaki River to pH 10.4 for Mojo Lake with mean pH value of 7.59 indicating a slightly higher pH of the water samples. With regards to site specific distribution of heavy metals, the lowest pH of below 7.0 was recorded from Akaki Dello river (Akaki sampling site), followed by water samples from Bishoftu (near East African factories) (pH 6.57). Even though there was no significance difference ($p > 0.05$) among the mean pH value of water from the sampling sites, the mean pH value of water from Mojo area were the highest (pH 8.34) as compared to other mean pH values of sampling sites such as Gelan (pH 7.68), Akaki (pH 7.19), Bishoftu (pH 6.57) and Awash River (pH 7.50). The higher nature of pH in Mojo areas has similarity with the findings of Dejene (2011) in that the pH of waste water discharged from Tanneries in Mojo area on average was 9.55 which was due to the use of alkaline compounds such as sulfides and lime in hair removal process of hides and skins (Dejene, 2011).

4.1. PH values of livestock water in Eastern shoa

Sampling site	Water source	pH (Mean ± SE)
Gelan	Surface water	7.52
	Gerbechena River	7.71
	Water nearby dye and ceramic factories	7.52
	Near dye factories	8.90
	Garment factory	7.03
	Galvanized sheet/steel factory	7.37
Mean		**7.68 ± 0.39[a]**
Akaki	Akaki Dellolo River	6.51
	Akaki Beseka	7.58
	Akaki 06 kebele	7.22
	Near University of South Africa (UNISA)	7.46
Mean		**7.19 ± 0.48[a]**
Bishoftu	Near Eastern African factories	**6.57 ± 0.95[a]**
Mojo	River near Mojo town	7.60
	Mojo lake	10.37
	Effluent discharged from Mojo Tannery	7.05
Mean		**8.34 ± 0.55[a]**
Awash	Awash River	**7.50 ± 0.95[a]**
Overall mean		**7.59 ± 0.95**

4.4.2 Concentration of heavy metals in livestock water in Eastern shoa

Among the sampling sites, water sample taken from the effluent discharged from Mojo tannery had the highest concentrations of heavy metals (86.89 μg/L) with high level of chromium (300.56 μg/L) which was followed by water samples from Mojo Lake (67.89 μg/L) and next was water collected from the River near Mojo town (43.64 μg/L). The very high concentration of chromium in water samples collected from Mojo areas could be attributable to the discharges released from the tanneries in Mojo to the surrounding water bodies (Dejene, 2011). Likewise, water samples from Eastern Shoa had the highest mean concentration of chromium (92.8 μg/L), followed by lead (15.2, μg/L) and mean arsenic content of 11.1μg/L respectively. Among the heavy metals, the lowest mean value of 0.80 was recorded for cadmium (Table 4.2).

Table 4.2. Heavy metals from livestock water samples from Eastern Shoa (Gelan, Akaki, Ada and Mojo)

Sampling site	Water sources	Concentration of heavy metals (µg/L)				
		Pb	Cr	Cd	As	Mean ± SE
Gelan	Surface water	26.78	43.58	0.84	2.02	18.31
	Gerbecha River	0.0001	34.73	0.88	0.0001	17.80
	Water nearby dye and ceramic factories	0.0001	45.07	0.67	0.0001	22.87
	Near dye factories	2.2	132.21	1.39	9.69	36.37
	Garment factory	2.27	79.45	0.30	6.74	22.19
	Galvanized sheet/steel factory	0.0001	69.67	0.44	8.22	26.10
Mean		**5.21**	**67.45**	**0.75**	**4.45**	**23.94 ± 5.36^{b}**
Akaki	Akaki Dellolo River	4.0	9.99	0.75	15.41	7.54
	Akaki Beseka	0.10	25.4	0.17	15.11	10.19
	Akaki 06 kebele	11.55	105.8	2.89	9.78	32.50
	Near University of South Africa (UNISA)	0.0001	85.74	0.16	10.44	32.11
Mean		**3.91**	**56.73**	**0.99**	**12.69**	**20.59 ± 6.57^{c}**
Bishoftu	Near Eastern African factories	**0.0001**	**34.26**	**0.54**	**15.82**	**16.87 ± 13.14^{e}**
Mojo	River near Mojo town	0.0001	116.03	0.29	14.61	43.64
	Mojo lake	36.26	225.55	1.28	8.47	67.89
	Effluent discharged from Mojo Tannery	37.72	300.56	0.44	8.83	86.89
Mean		**24.66**	**214.05**	**0.67**	**10.64**	**66.14 ± 7.58^{d}**
Awash	Awash River	16.18	83.52	0.92	19.04	**29.92 ± 13.14^{a}**
Overall mean		**9.14 ± 3.54^{b}**	**92.77± 20.33^{a}**	**0.80± 0.18^{c}**	**9.61±1 .50^{b}**	**31.41± 13.14**

LS-means with different superscripts among rows holding mean values were significantly different (last column)
LS-means with different superscripts among cells were significantly different (last row)

The lead contents of water samples significantly varied from a negligible quantity of 0.0001 µg/L from several sampling sites to the very high values of 36.26 (Mojo Lake) and 26.76 (Gelan surface water samples), showing more than 30-fold difference between the extreme sampling points.

Among the heavy metals examined in this study, values for cadmium were within the range of 0.17 µg/L in Akaki Beseka River and 2.89 µg/L from surface water from Akaki Kebele 06 showing a 16 fold difference between the highest and lowest cadmium contents recorded from the water samples (Table 4.2).

The arsenic contents of water samples examined also showed differences among the sampling sites and these ranged from negligible quantity of 0.0001 µg/L from Gerbecha River (Gelan) and water samples collected from nearby dye and ceramic factories of Gelan area to 19.04 µg/L from water samples collected from Awash River (Mojo) showing a 20- fold difference between the two extreme levels of arsenic. The data showed that the mean concentration of all heavy metals in water from Mojo (66.14 ± 7.58**)** significantly ($p<0.05$) differed from Awash (29.92 ± 13.14), Gelan (23.94 ± 5.36), Akaki (20.59 ± 6.57) and Bishoftu (16.87 ± 13.14).

The average concentrations of lead (9.14 µg/L), chromium (92.77 µg/L) and cadmium (0.80 µg/L) in water samples for livestock in Eastern Shoa in this study were 74, 10 and 80 times lower than the heavy metal content in livestock water from Pakistan where the values were for lead (670µg/L), chromium (950µg/L) and cadmium (80µg/L) respectively (Khan *et al.*, 2012). With the exception of chromium, the concentrations of other heavy metals in samples from Eastern Shoa were also lower than the heavy metal levels of livestock water from Hyderabad where the values were for lead (37.5 µg/L), chromium (62.25 µg/L) and cadmium (3.75 µg/L) (Ramchander *et al.*, 2015). The mean concentration of chromium (92.77 ± 20.33) in livestock water in Eastern shoa significantly ($P<0.05$) differed from arsenic (9.61 ± 1.50), lead (9.14 ± 3.54) and cadmium (0.80 ± 0.18) and it was in the order of Cr > As > Pb > Cd which were in similar order with heavy metal levels of waste water discharged from Hawassa textile factory as reported by Solomon *et al.* (2015).

Interestingly, the chromium contents of the different water samples were higher than all the tested heavy metals (mean = 92.77 µg/L) ranged, 9.99 µg/L sampled from Akaki Dellolo River (site where no Tannery and dye factory is existed) to 300.56 µg/L (water samples collected from near Mojo tannery effluent discharge points) with a 30-fold difference between the two sampling points.

The higher level of chromium recorded in effluents released from factories of Eastern Shoa and Hawassa textile factory (Solomon, *et al.*, 2015) was due to the use of a chemical chrome used as raw material for tanning process in tanneries and textile industries (Solomon *et al*., 2015).

In general, water samples from Mojo area were higher in chromium (mean 214.04 µg/L), and over all mean of heavy metals (66.14 µg/L); followed by water samples from Awash River with higher levels of overall heavy metals (29.92 µg/L). Except water samples from Akaki Dellolo River and Akaki Beseka, the other water samples from surfaces and rivers were highly contaminated with heavy metals arising from heavy pollution from urban and industrial activities around the sampling sites. Overall, the contamination levels with heavy metals in livestock water in the sampling sites of Eastern Shoa were in the order of Mojo > Awash > Gelan > Akaki > Ada (Bishoftu). Research studies on heavy metal content of livestock water in Eastern Shoa were very scarce, and among the scanty studies, Prabu (2009) showed that samples of waste water from the polluted Akaki River had higher chromium and cadmium levels as compared to the level of heavy metals found in unpolluted water. Studies on heavy metals in soils and on vegetables in these areas particularly around the polluted Akaki River (Fiseha *et al*., 2003; Prabu, 2009) and in Zeway (Amare, 2007) showed that soils and vegetables might have been irrigated with industrial waste water contaminated with heavy metals.

4.4.3. Concentration of heavy metals in water used for livestock by location

The levels of heavy metals in livestock water in Bishoftu and Holetta are shown in table 4.3. With the exception of cadmium, the levels of heavy metals in livestock water were significantly different ($P<0.05$) between Holetta and Bishoftu study sites. Accordingly, higher concentrations of heavy metals were observed in livestock water in Bishoftu area (28.08 ± 7.02µg/kg) than in Holetta (1.96 ± 0.28 µg/kg) which was associated with various factories running in the study locations and effluents being released from the factories to nearby water bodies. As a result, water in these areas has become the primary reservoir for heavy metals.

Table 4.3. Concentrations of heavy metals (µg/L) in water used for livestock

Element	N	Holetta	Bishoftu	Average
Cadmium	30	0.72 ± 0.09^{a}	0.80 ± 0.18^{a}	0.76 ± 0.1^{d}
Lead	30	0.84 ± 2.84^{b}	9.14 ± 5.80^{a}	4.99 ± 1.93^{c}
Arsenic	30	4.03 ± 0.41^{b}	9.61 ± 1.5^{a}	6.82 ± 0.92^{bc}
Chromium	30	2.26 ± 0.25^{b}	92.77 ± 20.33^{a}	47.52 ± 13.05^{a}
Overall mean	120	1.96 ± 0.28^{b}	28.08 ± 7.02^{a}	15.0 ± 12.34
pH (mean and range)	120	7 (6.4-7.7)	7.6 (6.51-10.37)	

Ls-means with different superscripts between columns were significantly different (P<0.05) (column 3 & 4); Ls-means with different superscripts between rows were significantly different (P<0.05) (column 5); N= number of samples

The concentrations of cadmium, chromium and lead in water samples from Bishoftu were lower than 30µg/L (cadmium), 640µg/L (chromium) and 160µg/L (lead) found in untreated water in Pakistan (Farid and Baloch, 2012). With the exception of lead and arsenic, the elemental difference between the heavy metals in Holetta and Bishoftu was significant (P<0.05) and the levels of heavy metals in livestock water in the study locations were in the order of Cr > As > Pb > Cd (table 4.3). The average concentrations of Pb (4.99 µg/L) and Cd (0.76 µg/L) observed in this study were also lower than the Pb (270 µg/L) and Cd (70 µg/L) in water collected from shallow wells in Egypt (EI-Bassiony, *et al.*, 2016).

4.4.4. Concentrations of heavy metals in feeds by location (teff straw)

The concentrations of heavy metals on teff straw from Holetta and Bishoftu are shown in table 4.4. Higher levels of As (2205.3µg/kg) and Cr (3204 µg/kg) were observed in teff straw when compared with the relatively lower concentrations of Cd (125.6-µg/kg) and Pb (526.1µg/kg) in this study, indicating that there were significant (p<0.05) variations among individual elements and the concentrations of heavy metals in teff straw were in the order of Cr > As > Pb > Cd (table 4.4).

Table 4.4. Levels of heavy metals (µg/kg) in feeds (teff straw)

Element	N	Holetta	Bishoftu	Average
Cadmium	30	168.81 ± 18.13^{a}	82.41 ± 10.80^{b}	125.61 ± 13.11^{d}
Lead	30	608.34 ± 103.16^{a}	443.86 ± 68.76^{a}	526.1 ± 62.80^{c}
Arsenic	30	1979.51 ± 986.35^{b}	2431.00 ± 797.95^{a}	2205.3 ± 624.73^{b}
Chromium	30	3417.50 ± 516.16^{a}	2990.40 ± 483.48^{b}	3204.00 ± 349.72^{a}
Overall	120	1543.54 ± 318.70^{a}	1486.92 ± 279.73^{a}	1515.23 ± 295

Ls-means with different superscripts between columns were significantly different (P<0.05) (column 3 & 4); Ls-means with different superscripts between rows were significantly different (P<0.05) (column 5); N= number of samples

With the exception of cadmium (Holetta 168.81 ± 18.12 µg/kg *vs* Bishoftu 82.41 ± 10.79 µg/kg), the levels of the other heavy metals in teff straw did not show significant ($p > 0.05$) variation between Holetta (1543.54 ± 318.70 µg/kg) and Bishoftu (1486.92 ± 279.73 µg/kg). In this study, cadmium (125.6 µg/kg) and lead (526.1µg/kg) levels in teff straw were lower than the concentrations of similar elements studied in forage grasses grown on road side of Botswana which were found to be 129 µg/kg and 3689 µg/kg respectively (Moreki *et al*., 2013).

The cadmium and lead content of teff straw in the present study was also lower than the cadmium (155µg/kg) and lead (563µg/kg) content studied in wheat fodder and the cadmium (305 µg/kg) and lead (844 µg/kg) content studied in maize fodder in Pakistan (Iftikhar, *et al.*, 2014).

Similarly, the cadmium (125.6 µg/kg) and lead (526.1µg/kg) content of teff straw found in this study were lower than 160 µg/kg and 2090 µg/kg of the same elements in maize fodder (livestock feed) grown in unpolluted area of Pakistan was and these levels were also lower than the cadmium (873.3 µg/kg), lead (24317 µg/kg) and chromium (29250 µg/kg) levels in fodder feed grown in polluted area of Pakistan (Farid and Baloch, 2012). Teff straw is categorized in graminae (grasses) as well as in cereals which makes is comparable with wheat and maize fodder. The fact that cadmium, lead and chromium levels in teff straw in this present study were lower than the results reported in Pakistan by Farid and Baloch (2012) is an indication that the areas where teff were grown was not relatively highly polluted.

The chromium content of teff straw in the present study (3204 µg/kg) was 15-fold higher than the chromium contents of forage grasses (208 µg/kg) grown on the road side of Botswana (Moreki *et al.*, 2013) and 2-fold higher than the chromium content of maize fodder studied in Pakistan which was 1340 µg/kg (Farid and Baloch, 2012).

4.4.5. Concentration of heavy metals in cow milk

The levels of heavy metals in milk samples from the different study sites are presented in table 4.5. Accordingly, the concentration of chromium (95.35 ± 14.16 µg/L) was significantly ($P < 0.05$) higher than the concentrations of cadmium 34.77 ± 4.41 µg/L, lead 33.01 ± 2.63 µg/L and arsenic 6.70 ± 1.15 µg/L. With the exception of chromium, the concentrations of cadmium, lead and arsenic in the milk samples did not show significant ($P>0.05$) difference between Bishoftu and Holetta sites.

Table 4.5. Concentration of heavy metals (µg/L) in cow milk

Element	N	Holetta	Bishoftu	Average
Cadmium	30	37.69 ± 3.49^{a}	30.78 ± 9.36^{a}	34.24 ± 4.95^{b}
Lead	30	34.77 ± 4.41^{a}	31.24 ± 2.95^{a}	33.01 ± 2.63^{bc}
Arsenic	30	7.03 ± 2.24^{a}	6.36 ± 0.68^{a}	6.70 ± 1.15^{d}
Chromium	30	139.73 ± 23.00^{a}	50.97 ± 4.56^{b}	95.35 ± 14.16^{a}
Overall mean	120	54.81 ± 8.76^{a}	29.84 ± 3.35^{b}	42.32 ± 35.79

Ls-means with different superscripts between columns were significantly different ($P<0.05$) (column 3 & 4); Ls-means with different superscripts between rows were significantly different ($P<0.05$) (column 5); N= number of samples

The overall levels of heavy metals in cow milk samples in the study locations were in the order of Cr > Cd > Pb > As. It is interesting to note that cadmium (34.24 ± 4.95) and lead contents (33.01 ± 2.63) of milk in this study were lower (3 and 30 times) than the cadmium (100.25 µg/L) and lead (998.25 µg/L) contents of milk collected from high yielding crossbred cows that were fed grass hay and agro-industrial byproducts in Addis Abba (Dawd, 2010).

The lead content of milk in the present study was 3 times lower than the lead level of milk (91µg/L) collected from cows reared within 2kms of industrial zone of India (Roy *et al.*, 2009), and that of Pb level of (110µg/L) milk samples studied in Pakistan (Farid and Baloch, 2012). Similarly, the cadmium (34.24 µg/L) and chromium (95.35 µg/L) levels of milk samples in this study were 2 and 3 times lower than the cadmium (60 µg/L) and chromium (290 µg/L) level of milk studied in Pakistan respectively (Farid and Baloch, 2012). The lead, cadmium, and chromium content of milk of cows grazed around an industrial estate in Nigeria were 550µg/l, 163 µg/l and 1756 µg/l respectively (Ogabiela *et al.,* 2011) which was higher than the lead (33.01 µg), cadmium (34.24 µg) and chromium (95.35 µg) contents recorded in this study.

The level of chromium in milk in this study was higher (95.35g/L) and this could be attributed to the high concentration of chromium in teff straw collected from the study locations.

4.4.6. Comparative levels of heavy metals in milk collected from different sites

When the levels of heavy metals in milk of the sampling sites were compared with the permissible standard set by WHO (Table 4.6), cadmium and arsenic levels in milk samples examined in this present study were within the permissible limits. While only 27% and 40% of the milk samples collected from Bishoftu and Holetta respectively were within the acceptable standard for lead (25 µg/l), none of the milk samples met the standard set for chromium (17 µg/l) by WHO/JECFA (1989).

Table 4.6. Comparative levels of heavy metals in milk collected from different sites

Site	Heavy metal	Mean heavy metal (µg/L)	Permissible standard (µg/L)	Within standard (%)	Beyond standard (%)
Bishoftu	Cd	30.78	71	100	0
	Pb	31.24	25	27	73
	As	6.36	156	100	0
	Cr	50.97	17	0	100
Holetta	Cd	37.69	71	100	0
	Pb	34.77	25	40	60
	As	7.53	156	100	0
	Cr	139.74	17	0	100

Source: WHO/JECFA 1989

4.5. Conclusions

Results showed that the pH values of water collected from all the study locations were within acidic and alkaline range meaning that mean pH value satisfied the acceptable level for livestock water. Water samples collected from Mojo area had the highest concentration of chromium, when compared with the means of other heavy metals. The overall contamination level of heavy metals in livestock water along the major sampling sites in Eastern Shoa were in the order of Mojo > Awash river > Gelan > Akaki > Bishoftu.

There was no significant difference between the concentrations of heavy metals in teff straw obtained from Holetta and Bishoftu. However, the concentration of heavy metals in samples of water from Bishoftu was more than ten times higher than that from Holetta. The concentrations of heavy metals in animal feeds and water in the study locations were in the order of Cr > As > Pb > Cd and those for milk was in the order of Cr > Cd > Pb > As. Despite the overall high concentration of heavy metals in feeds and livestock water, cadmium and arsenic contents of all milk samples were within the permissible limits, whereas the concentrations of chromium and lead in majority of the milk samples examined in this study were above the permissible limit which is an indication of environmental pollution and poor milk quality. This preliminary study has shown that the levels of chromium and arsenic in teff straw in Holetta and Bishoftu are very high; therefore, there is the need for further investigation of heavy metals from soil and fodder samples grown in these areas.

Chapter 5.

Perceptions and practices of farmers, feed processors and feed trades on risks of contaminations of feeds and water for livestock production

Rehrahie Mesfin, Fassil Assefa and Getnet Assefa

5.1. Abstract

The perception, experience and indigenous knowledge of stakeholders in the feed value and market chain of milk production, together with the technical knowhow, and safety issues, is very vital to ensure the safety of milk and milk products. The study was conducted in Western Shoa, Easter Shoa and SNNP regional states of Ethiopia with the objective of assessing the processing, handling, and storage practices of feeds by peri-urban farmers, feed processors, feed retailers and urban dairy producers using semi-structured questionnaires and field observations. A total of 180 individuals from feed manufacturers (33), feed retailers (51) and smallholder dairy producers (96) were participated in the interview. The study showed that 91% of the farmers encountered mold formation in roughage feeds due to lack of good harvesting and stacking practices. Most of the farmers admitted to feed light moldy feeds to their livestock by diluting with uncontaminated ones. Most of the respondents (67%) used extreme moldy feeds for firewood; and 33% of the interviewees damped into landfills. Farmers recognized two causes of water contaminants that caused health and production problems in livestock. Accordingly farmers from Eastern Shoa (100%) were aware of the effect of the industrial effluent as the most important hazard for dairy production; whereas 66% of the farmers from Eastern Shoa and 34% of the respondents from Western shoa identified leech problems in water bodies. Farmers also had indigenous knowledge to tackle the leach problem in that 69% of the farmers used bucket for selectively scooping water from the water body to exclude the Leech from being consumed by animals; whereas 50% of the respondents treated animals with chopped tobacco and onion. The majority of the feed processors (64%), feed retailers (82%) and dairy producers (56%) responded that they did not use palate for placing their concentrate feeds implying that the probability of mold infection during storage. Among the respondents, 88% of feed processors, all feed retailers and most (96%) of the dairy producers recognized that wheat bran was the most mold susceptible feed ingredient. Majority of the feed processors (67%), feed retailers (73%) and dairy producers (58%) stored their concentrate feeds for a short period of 1 month. To

overcome mold formation in concentrate feeds, 64% of the feed processors spared enough space between the stored feed and the wall. Majority of the feed processors (74%), feed retailers (87%) and most dairy producers (91%) did not encounter mold formation in their concentrate feed because of the small amount of concentrate feed they keep and shorter storage time.

Key words: Concentrate feed, dairy producers, questionnaire, storage time, value chain

5.2. Introduction

In Ethiopia livestock has a 40% share of agricultural production and contributes to 13-16% of the total GDP (Zinash and Alemu, 2001). At household level, livestock production has become important for the livelihood of pastoralists, agro-pastoralists, and smallholder farmers (Negassa, *et al*. 2011).

The economic benefits gained from export of livestock and livestock products in 2015/2016 were estimated 37.5 million US dollar (IGAD, 2017). Although the country poses the highest number of livestock species in Africa, the annual per capita consumption of milk in 1996 for example was 17 kg which was lower than the per capita milk consumption of other South and Central African countries and developed countries which was 26kg and 200 kg respectively (Azage and Alemu, 1998).

Despite some improvements on export of livestock and livestock products in recent years, productivity and commercialization of the livestock sub-sector is still very low (Negassa *et al.*, 2011; Alemie and Lemma, 2015).This is because livestock production is mainly undertaken by small-holding rural producers and pastoralists using inefficient local breeds depending on poor quality pastures and lack of veterinary services (Azage and Alemu, 1998). Moreover, the peri-urban and urban livestock production system is suffering from shortage of animal feeds due to the underdeveloped agri-food chain and other structural problems (Azage and Alemu, 1998).

Livestock production in the country is mainly dependent upon basal roughage feeds resources including natural pasture, hay and crop residues whereas peri-urban and urban dairy producers use supplemental feed including agro-industrial by-products such as wheat bran, noug seed meal, linseed meal, cottonseed meal and non-conventional concentrate feeds such as poultry liter and home produced by products of cereals and pulses (Rehrahie, unpublished data, 2016).

For many years now, a lot of attention has been given to increase the quantity of feed through research in Ethiopia (Dereje *et al.,* 2014; Menbere, 2014; Abdirahin *et al.,* 2015; Endale, *et al.,* 2016). However, there is a dearth of information on the quality and safety status of feed stuffs that determine the quality, safety of livestock products (milk, meat and egg). The ever-growing movement of food through international trade also necessitates ensuring the quality and safety of imported, exported and locally produced food products (FAO, 2006).

The main factors that affect feed and food safety are categorized into biological, chemical and physical contaminants (Rhodehamel, 1992). Feed and water sources are contaminated with chemicals such as heavy metals, and toxins and compromise the quality and safety of dairy products through the food chain. The biological contaminants, mainly the filamentous fungi grow on agricultural products and cause postharvest deterioration in cereals, oilseeds and legumes and elaborate the chemical contaminants known as mycotoxins (Mold fact sheet, 2001).

The fungus that is grown in the field further flourishes at harvest, storage and processing (Iheshiulor *et al.*, 2011; Suleiman *et al.*, 2013). Under optimum condition (moisture of > 13% and temperature of 40^0F-100^0F), mold spores germinate, increase in number and consequently utilize the nutrients in grains and feeds and can reduce the nutritional quality of grains and feeds (Tarr, 2006). Mashilla (2004) reported that storage fungi had adverse impact in reducing the carbohydrate content of sorghum grain in Eastern Harerghie, Ethiopia. Apart from that, supply of moldy feeds results in reduced digestibility (Tarr, 2006), reduced feed intake, nutrient intake, weight gains and milk production (Thomas *et al.*, 1983). A production performance loss of 5-10% was also observed with feeding moldy feeds to livestock even in the absence of mycotoxins (Tar, 2006).

There are different stakeholders in the value chain of dairy production systems which include small-scale peasant farmers, peri-urban and urban milk producers, feed processors, and distributors. The experience and indigenous practices of these partners, together with the monitoring and quantitative analysis of physical, chemical, and biological contaminants, is very vital to improve the handling and management of feeds in order to ensure the safety of dairy products. Such studies can be used as benchmarks to scale up dairy production in the country. In this study attempt was made to assess feed storage practices, and problems associated with safety of feeds and water on livestock species based on the experiences and perception of stakeholders including feed processers, feed retailers, smallholder urban dairy producers and farmers.

5.3. Materials and Methods

5.3.1. Study location

The study was carried out in Western s Shoa (Welmera/Holetta), Addis Ababa, Eastern Shoa (Akaki, Gelan and Ada) and Hawassa. Addis Ababa and Holetta are located at 38° 30`E, 9° 3`N. Ada and Hawassa are located in the Great Rift Valley at latitude of $8^{O}50$ to $8^{O}53$ and longitude of $38^{O}55$ to $38^{O}59$ at an altitude of 1708 m.a.s.l and 1600-2400 m.a.s.l respectively. Hawassa is located 285 km of Addis Ababa, South Ethiopia. The average annual rainfall was 1100 mm and average low and high temperature of 12.6 ^{o}c and 27.3 ^{o}c respectively (https://en.wikipedia.org/wiki/Awasa 2017).

5.3.2. Data collection and analysis

The study was conducted using a semi-structured questionnaire involving a total of 180 feed processors, feed retailers, urban smallholder dairy producers and rural farmers in Central Ethiopia (Welmera, Addis Ababa, Akaki, Gelan, Ada and Hawassa) on feed storage methods and observed risks on livestock species that have been exposed to contaminated feeds and water. The collected data (qualitative) was organized and analyzed using descriptive statistics (SPSS, 2011)

5.4. Results and discussions

5.4.1. Problems of mold in animal feeds

The experiences of farmers on occurrence of moldy feeds, suitable seasons for mold formation and risks encountered due to offering of moldy feeds to livestock species are presented in table 5.1. According to the interviewed respondents 91% (78) of them recognized the presence of mold problem mainly on roughage feeds including crop residues and hay. According to these respondents, mold formation occurred mainly during the rainy season and/or due to unseasonal rainfall when roughages feeds are stacked in situations of inadequate drying during harvest or due to bad baling style that can promote transfer of rain water.

Table 5.1. Response of farmers on occurrence of mold in feeds, severe moldy season and problems of offering moldy feeds to livestock

Occurrence of moldy feeds	Farmer's view		Suitable season for mold formation	Farmer's view		Problems of offering moldy feeds to livestock	Farmer's view		Management of moldy foods	Farmer's view	
	N	%		N	%		N	%		N	%
There was mold problem	78	91	Rainy season	32	100	Illness	52	59	Damping	9	33
No mold problem	8	9	Dry season	0	0	Feed rejection	22	25	Firewood	18	67
Total	86	100	Total	32	100	Abortion	13	15	Total	27	100
						Death	1	1			
						Total	88	100			

Those farmers who encountered mold in their feeds admitted that they offered lightly contaminated moldy feeds to livestock species by diluting it with fresh feeds that are commonly practiced in many countries (Shewmaker, 2009) and 33% of farmers interviewed completely discarded/dumped the extremely moldy feeds in landfills, and 67% of the respondents used moldy feeds for firewood implying that farmers have recognized the disadvantage of feeding moldy feeds to livestock and discarding the extremely moldy feeds. In situations when animals were fed moldy feeds, farmers observed problems on their livestock such as Illness (59%), feed rejection (25%), abortion (15%) and death (1%). The observation of farmers was similar with the bad effect of mold reported in literature that feed rejection and reduced feed intake were some of the associated problems of offering moldy feeds to livestock species (Akande *et al*., 2006). It was also reported that moldy feeds affected the production performance of cattle due to deterioration of nutrients in the mold contaminated feeds and apart from any toxic effects, the mold itself

caused production losses (Dicostanzo, 2012). Livestock producers in Minoseta reported that animals offered mold contaminated feeds encountered health problems such as reduced feed intake, diarrhea, reduced weight gain, abortions or death (Dicostanzo, 2012).

5.4.2. Farmer's perception on causes and effects of contaminated water

The experience of farmers in relation to the causes and effects of contamination/pollution of livestock water is presented in table 5.2. Majority (80%) of the interviewed farmers knew contamination of livestock water with undesirable substances and parasites in their surroundings and recognized two major contaminants of livestock water. One of them was the effluents discharged from industries particularly in Eastern Shoa, and the other was the problem of the parasitic leech (*Lymnatis nilotica*) that naturally infest water sources during dry season which was commonly observed in Eastern and Western Shoa. Scarcity and poor quality of water in relation to leech infestation have been reported by farmers as third most important problems (27%) of livestock production in Dendi area, Ethiopia next to feed and animal diseases (Belay *et al.,* 2013). High prevalence of leech infestation was observed in the dry season due to reduced flow, quantity and run of water in to water sources as well as due to washing and bathing closer to livestock watering points (Belay *et al.*, 2013). Report from interview of farmers in Sodo district SNNP, also revealed that 95% of the respondents rated leech as a major animal health problem (Tadesse *et al.*, 2010). Whereas, in Northern Ethiopia, Alamata, infestation of livestock water with leech was identified as a last risk among the major cattle health problems and verified, leech, as blood sucking parasite attach the pharynx part of the oral cavity while animals drink water and was responsible for loose of large amount of blood within short period of time resulting in anemia (Yohannese, 2007). Negm-Eldin *et al.* (2013) in Libya also reported the exposure of different livestock species to leech infestation with increased rate observed in cattle (Negm-Eldin *et al.*, 2013).

Table 5.2. Farmers' perception on causes and effects of contaminated water

Problem related to livestock water	View of farmers		Causes of water contamination by location	View of farmers	Problems of effluent contaminate water	View of farmers		Problem of leech contaminated water	View of farmer	
	No	%		%		No	%		No	%
There was problem	72	80	Leech in Holetta	34	Diaharrea	6	20	Illness and water rejection	37	76
No problem	18	20	Leech in Bishoftu	66	Bad smell, Water rejection, skin itching, emaciation	22	73	Death	8	16
Total	90	100	Effluent in Holetta	0	Coughing	2	7	Milk reduction	4	8
			Effluent in Bishoftu	100	Total	30	100	Total	49	100

Almost all of the farmers interviewed from Eastern Shoa have observed that the effluents released from different factories to the surrounding water bodies have become a risk to health of livestock species. About 66% the farmers interviewed from Eastern Shoa admitted that the parasite leech (*Lymnatis nilotica*) was the second important contaminant in livestock water. However, the farmers interviewed from Holetta did not perceive pollution of water due to effluents released from factories; but 34% of the respondents from Holetta identified that leech was the major contaminant of livestock water.

Almost all of the farmers in Eastern Shoa had the perception of the different risks related to drinking of effluent contaminated water to livestock species, and 73% of them associated the contamination with commonly observed problems of bad smell, water rejection, emaciation and skin itching, followed by diarrhea (20%) and coughing (7%). Likewise, the majority (76%) of the farmers in both locations agreed that illness (nose bleeding), reduced body weight and water rejection were commonly associated with leech contamination of water followed by death (16%) and reduction of milk yield (8%). The health problems mentioned by the interviewed farmers interviewed in the present study was similar with the reports of Bahmani *et al.* (2012) in that

bleeding and reduced appetites were the commonly observed problems in cows that consumed leech contaminated water in Iran.

5.4.3. Indigenous practices employed to overcome leech contaminated water

The farmers interviewed had traditional knowledge and experience of applying different traditional practices to control contamination of leech in water bodies and from the mouths of animals (Table 5.3). Among the farmers interviewed the highest proportion (69%) of them used bucket for selectively scooping water from the water body given to animals which helped to exclude the ingestion of leech by animals. Farmers also employed immersion of Endod in water bodies as a second (13%) important leech minimizing practice. And this has been scientifically proved by a study undertaken in Sodo, Guragie zone of Ethiopia, in that application of Endod (*Phytolacca dodecandra)* in water bodies resulted in 97-100% reduction in streams used for livestock drinking (Tadesse *et al.*, 2010).

Farmers also practiced different strategies to remove leech from the mouths of animals of which 50% of them used to drench chopped tobacco and or onion to livestock. According to the respondents, 32% of them physically removed the leech from the animal by hand which was similar with the practices and experience of leech removal employed in Iran (Bahmani *et al.,* 2012). In this study, 10% and 8% of the respondents respectively solved the problem by drenching tablet and restriction of animals from drinking water for one day to facilitate release of leech to the mouth of the animal to be able easier to remove from the mouth. To prevent contamination of livestock water with leech, farmers in West Shoa (Dendi), Ethiopia employed different practices such as separating human and livestock water points, washing and bathing areas and fencing livestock water points (Belay *et al.,* 2013).

Table 5.3. Indigenous practices to overcome leech contaminated water

Practice to reduce leech from water body	View of farmers		Practice to remove leech from animal	View of farmer	
	No	%		No	%
Immersion of stalk of noug and linseed	1	6	Drenching chopped Tobacco and/or onion	20	50
Immersion of Endod (*Phytolacca dodecandra*)	2	13	Drenching tablet	4	10
Bucket watering	11	69	Withdrawal of water	3	8
Use of other alternative water sources	2	12	Taking away from mouth	13	32
Total	16	100	Total	40	100

5.4.4. Storage methods of concentrate feeds in feed processing factories

In this study, different stakeholders involved in the feed production, marketing and utilization chain were interviewed to evaluate the storage methods of concentrate feeds. Accordingly, the majority of the feed manufacturers (64%) stored their concentrate feed on cement floor without using palate (table 5.4). Palate is any material (minimum of 50 cm height) placed on a floor over which concentrate feed is stored to avoid contact between the concentrate feed and the floor to minimize the transfer of moisture.

It was observed that some feed manufacturers left some space between two adjacent stored concentrate feeds and between the stored feed and the wall and the roof to allow ventilation implying they have good understanding on benefit of air circulation in stored feeds. They have similar understanding with people in developed countries in that grain storage facilities in the U.S. have fans installed for ventilation and temperature control of stored grain (Thomas *et al.*, 1983).

Table 5.4. Storage methods of concentrate feeds in feed processing factories (% and n)

Storage method	Study location			
	Holetta	Bishoftu	Hawassa	Total
On cement floor with no palate	61 (11)	50 (3)	78 (7)	64 (21)
On cement floor with palate	33 (6)	17 (1)	11 (1)	24 (8)
On soil floor with no palate	0 (0)	17 (1)	0 (0)	3 (1)
On soil floor with palate	6 (1)	0 (0)	0 (0)	3 (1)
Missing	0 (0)	17 (1)	11 (1)	6 (2)
Total	100 (18)	100 (6)	100 (9)	100 (33)

Consecutive figures in each cell are percent and number of respondents respectively

Among the feed manufacturers interviewed in this study, majority (72%) of them preferred use of palate to store only wheat flour whereas, 28% of the interviewees used palate equally for both wheat flour and wheat bran.

5.4.5. Storage duration of concentrate feeds in feed manufacturers

The storage duration of concentrate feeds by feed manufacturers is presented in table 5.5. Majority (67%) of the feed manufacturers interviewed stored their concentrate feeds for shorter duration (a maximum of 1 month) which was related to the higher market demand for concentrate feeds in the country. The result also showed that 24 % of the feed manufacturers

kept their concentrate feed for duration of 6-8 months, particularly during the rainy seasons when alternative feed resources such as green grass were available and resulting in decreased market demand and increased storage time of concentrate feeds.

Table 5.5. Storage duration of concentrate feeds by feed manufacturers (% and n)

Storage duration (month)	Study location			
	Holetta	Bishoftu	Hawassa	Total
1	61 (11)	50 (3)	89 (8)	67 (22)
2 - 3	6 (1)	0 (0)	0 (0)	3 (1)
4 - 6	6 (1)	0 (0)	0 (0)	3 (1)
6 - 8	28 (5)	33 (2)	11 (1)	24 (8)
Missing	0 (0)	17 (1)	0 (0)	3 (1)
Total	100 (18)	100 (6)	100 (9)	100 (33)

Consecutive figures in each cell are percent (%) and number of respondents (n) respectively

5.4.6. Practices to overcome mold formation on feeds in feed manufacturers

The data on the practices of preventing mold growth in concentrate feeds by feed manufacturers is presented in table 5.6. Under these circumstances, majority of the feed manufacturers (64%), and all feed manufacturers in Hawassa left some space between the stored feed and the wall as well between the stored feed and the roof. Only 12% of the feed manufacturers used palate for placement of concentrate feeds.

Table 5.6. Practices to overcome mold formation on feeds by feed manufacturers (% and n)

Mold minimizing practices	Study location			
	Holetta	Bishoftu	Hawassa	Total
Using palate over floor for feed placement	17 (3)	17 (1)	0 (0)	12 (4)
Leaving space between feed and wall	6 (1)	0 (0)	0 (0)	3 (1)
Placing the feed in dry and aerated place	17 (3)	0 (0)	0 (0)	9 (3)
Leaving space on wall and roof	44 (8)	67 (4)	100.0 (9)	64 (21)
Missing	17 (3)	17 (1)	0 (0)	12 (4)
Total	100 (18)	100 (6)	100 (9)	100 (33)

Consecutive figures in each cell are percent (%) and number of respondents (n) respectively

5.4.7. Storage methods of concentrate feeds by feed retailers

The storage style of concentrate feeds by feed retailers is presented in table 5.7. Majority of the feed retailers (82%) in the study locations stored their concentrate feed on cement floor without

using palate. The situation was worse than that of feed manufacturers and feed processors where 64% of the owners stored their concentrate feed on cement floor without using palate. Majority of the feed retailers were in Hawassa (93%) followed by Holetta (84%) and Bishoftu (71%).

Table 5.7. Storage methods of concentrate feeds by feed retailers (% and n)

	Study location			
Feed storage styles	Holetta	Bishoftu	Hawassa	Total
On cement floor with no palate	84 (16)	71 (12)	93 (14)	82 (42)
On cement floor with palate	5 (1)	6 (1)	0 (0)	4 (2)
On soil floor with no palate	0 (0)	24 (4)	7 (1)	10 (5)
On soil floor with palate	5 (1)	0 (0)	0 (0)	2 (1)
Missing	5 (1)	0 (0)	0 (0)	2 (1)
Total	100 (19)	100 (17)	100 (15)	100 (51)

Consecutive figures in each cell are percent (%) and number of respondents (n) respectively

5.4.8. Storage duration of concentrate feeds in feed retailers

The storage durations of concentrate feeds in feed retailers are shown in table 5.8. Majority of them who -specify (73%) in the study locations on average stored their feed for a shorter period of time (1 month). This was because of the higher demand of concentrate feeds by livestock producers. The feed retailers that stored their concentrate feeds for very short duration of time (1 month) was lower in Holetta (47%) than the feed retailers from Bishoftu (82%) and Hawassa (93%).

Table 5.8. Storage duration of concentrate feeds in feed retailers (% and n)

Feed storage duration (month)	Study location			
	Holetta	Bishoftu	Hawassa	Total
1	47 (9)	82 (14)	93 (14)	73 (37)
1 - 2	0 (0)	6 (1)	0 (0)	2 (1)
2 – 3	5 (1)	0 (0)	0 (0)	2 (1)
Missing	47 (9)	12 (2)	7 (1)	24 (12)
Total	100 (19)	100 (17)	100 (15)	100 (51)

Consecutive figures in each cell are percent (%) and number of respondents (n) respectively

5.4.9. Storage methods of concentrate feed by dairy producers

The storage methods of concentrate feeds by smallholder dairy producers are shown in table 5.9. Majority (56%) of the smallholder dairy producers stored concentrate feeds on cemented floor with no palate, followed by 16% of dairy producers that stored their feed samples on soil

floor lined with certain sheet such as plastic or cloth of which the majority were dairy producers from Holetta (27%).

Majority of the dairy producers from Bishoftu (76%) and Hawassa (73%) stored their concentrate feeds on cemented floor without palate. The present study has also shown that 64% of the feed processor (table 5.4), 82% of feed retailers (table 5.7) and 56% of dairy producers (table 5. 9) have not used palate for placing their concentrate feeds thus there was the possibility of mold growth in stored concentrate feeds.

Although no information was available showing the negative effect of traditional storage and handling methods of livestock feeds in Ethiopia and abroad, few survey studies were conducted in the country to evaluate traditional grain storage practices and structures (differing from region to region) on nutrient quality of cereal grains thus storing sorghum for longer duration could cause deterioration in nutritional composition of grains (Mashla, 2004; Abebe and Bekele, 2006). About 16% loss in grains (by weight) in the pastoralist areas of Ethiopia have been reported to be due to deteriorations in nutrient composition of stored sorghum in relation to mold contamination (Hodgson, 1990). According to Dubale (2014), quality and nutrient deteriorations was were observed in stored maize in farmers employing traditional grain storage practices. The author identified mold and insects to be the two major factors causing nutrient deterioration in grains with moisture and temperature as enhancing mold growth. According to Chemeda *et al*. (2007), 50% loss in the yield of sorghum grain was recorded due to insect pests in South Western Ethiopia.

Table 5.9. Storage methods of concentrate feeds in dairy producers (% and n)

Feed storage methods	Study location			
	Holetta	Bishoftu	Hawassa	Total
On cemented floor with no palate	21 (7)	76 (25)	73 (22)	56 (54)
On cemented floor with palate	0 (0)	0 (0)	10 (3)	3 (3)
On soil floor with no palate	3 (1)	15 (5)	7 (2)	8 (8)
On soil floor lined with certain sheet	27 (9)	9 (3)	10(3)	16 (15)
Missing	49 (16)	0 (0)	0 (0)	17 (16)
Total	100 (33)	100 (33)	100 (30)	100 (96)

Consecutive figures in each cell are percent (%) and number of respondents (n) respectively

5.4.10. Storage methods of roughage feed by dairy producers

The storage methods of roughage feeds including hay and straw belonging to smallholder dairy producers are presented in table 5.10. Majority (41%) of the dairy producers stored their feed on soil floor without slate and of which 88% of the respondents were dairy producers from Bishoftu. The rest of the dairy producers stored their roughage feeds on raised floor and among them 45% were from Holetta. Even though no research was undertaken on the effect of traditional storage practices of roughage feeds in the country, there were few research results showing the negative effect of elevated moisture on quality of stored grains. Amare *et al*. (2006) estimated a maximum of 25.9µg/kg AFB1 in sorghum grain stored under the traditional way of underground pit storage system due to elevated moisture in grains. Boxall (1998) indicated a 5%-30% post harvest grain loss (weight) that was associated with handling and storing of wet grains due to occurrence of unseasonal rainfall in Ethiopia (Boxall, 1998).

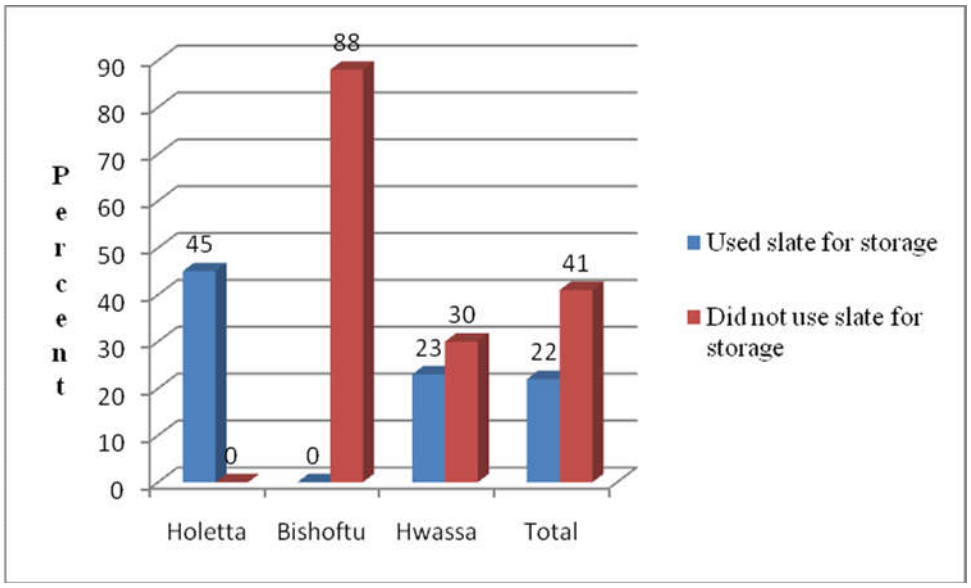

Fig.5.3.Storage methods of roughage feed by dairy producers

5.4.11. Storage duration of concentrate feeds in dairy producers

The storage durations of concentrate feeds in smallholder dairy producers are presented in table 5.11. Due to increased price of concentrate feeds these days, the majority of dairy producers (58%) stored their concentrate feed for a shorter period of about 1-2 weeks.

Table 5.10. Storage duration of concentrate feeds in dairy producers (% and n)

Storage duration (week)	Study location			
	Holetta	Bishoftu	Hawassa	Total
1	0 (0)	0 (0)	25 (10)	10 (10)
1 – 2	30 (10)	33 (11)	40 (16)	58 (37)
3 - 4	24 (8)	42 (14)	20 (8)	12 (30)
Missing	46 (15)	24 (8)	15 (6)	31 (29)
Total	100 (33)	100 (33)	100 (40)	100 (106)

Consecutive figures in each cell are percent (%) and number of respondents (n) respectively

5.4.12. Occurrence of mold in concentrate feeds across the feed value chain

The status of mold occurrence in concentrate feeds amongst the feed production and marketing chain is presented in Fig 5.1. Little proportion of the respondents (26%, 13% and 5%) of feed processors, feed retailers and dairy producers respectively encountered mold in their concentrate feeds. The occurrence of mold was not severe in these stakeholders' feeds not because of the good storage practice they employed but due the small amount of concentrate feed they held.

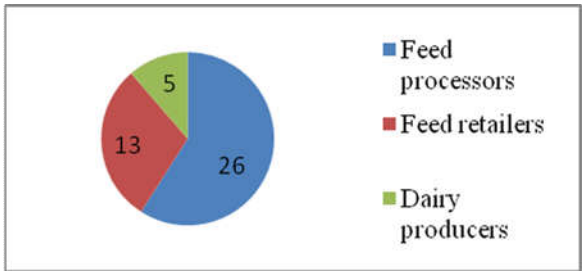

Fig.5.4. Status of mold occurrence in concentrate feeds

5.4.13. Status of mold occurrence in wheat bran across the feed value chain

Majority of the feed processors (88%), all the feed retailers (100%) and majority of the dairy producers (96%) reported that wheat bran could easily develop mold (Fig 5.2). Some of respondents of feed processors from Addis Ababa (25%) also observed that wheat bran can easily absorb moisture from the surroundings and consequently develop paste. Their observation was similar to other reports elsewhere that the moisture in the air which clings to the surface area of concentrate feeds can provide sufficient moisture for mold growth (Solomon *et al*., 2016).

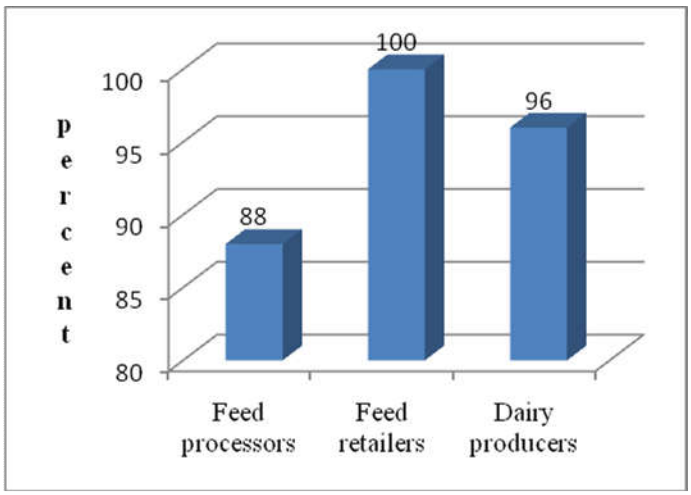

Fig.5.5 Status of wheat bran to mold contamination

5.5. Conclusions

In this study, majority of farmers had the knowledge on the occurrence, causes and effect of mold in feeds and, farmers dwelling in the industrial zone of Eastern Shoa mainly in Gelan area were aware of the pollution problem of livestock water due to effluents released from different factories. They were also aware of leech problem in livestock water use, and some also had indigenous knowledge on how to protect and treat their livestock from the scourge of the leech. They were also conscious of the pollution problems on the health and productivity of their livestock. Majority of the dairy producers, feed retailers and feed manufacturers did not use palate for placing concentrate feed giving rise to possibility of mold occurrence in concentrate feeds. Further research needs to be undertaken along the feed production, marketing and utilization chain targeting on effect of different storage conditions by considering factors such as use of palate, ventilation and time of feed storage on mold formation and nutrient quality of feeds using larger sample size.

6. General conclusions

In this study, attempts were made to evaluate the quality and safety of milk on the basis of aflatoxin and heavy metal contents along the feed and milk production and distribution chain as well as water sources in some parts of central Ethiopia. The data obtained in this study showed that, half of the feed samples were free from aflatoxin, and the remaining were within the EU permitted level of 5µg/kg, and the U.S.A. permitted level of 20 µg/kg AFB1, respectively.

Concentrate feeds were more contaminated with AFB1 than roughage feeds; hay was more contaminated than straw; and oilseed cake based concentrate feeds were more contaminated than concentrate feeds without oilseed cake. The data obtained showed duration of feed storage greatly contributed to increased level of aflatoxin. With regards to milk, aflatoxin was not detected from a reasonable number of samples (29%); whereas 58% of the samples were within the stringent EU standard of (0-0.05µg/L); and 42% were within the USA standard set for milk (5µg/L) indicating that the milk samples were within the permissible level of aflatoxin in milk. The AFM1 contamination levels of feeds and milk samples collected from the study locations were in the order of Hawassa < Holetta < Bishoftu. The aflatoxin contamination levels of milk samples in this study were within the medium level similar to most developing countries. With regards to contamination with heavy metals, the concentration of heavy metals in teff straw from Holetta and Bishoftu did not show significant difference. However, the levels of heavy metals in livestock water in Bishoftu were more than ten times higher than water from Holetta. The concentrations of heavy metals in animal feeds and water in the study locations were in the order of Cr > As > Pb > Cd. Due to shortage of clean water for livestock drinking, farmers in communities dwelling around Gelan area used the waste water discharged from different factories. Despite the overall high concentration of heavy metals in feeds and water, the Cd and As contents of all milk samples were within the permissible limits, whereas the concentrations of Pb in majority and Cr in all milk samples were above the permissible limits which are indicative of environmental pollution.

Majority of dairy producers, feed retailers and feed manufacturers did not use palate for storing concentrate feeds indicating that mould could occurr in their concentrate feed.

7. General recommendations

Further research needs to be undertaken along the feed and milk production and distribution chains using other techniques such as HPLC, GC and multi-mycotoxin assay using LC-MS-MS taking into account different storage conditions such as use of palate, ventilation, and duration of feed storage on aflatoxin and nutrient compositions of feeds. The effect of mold growth in feeds on nutrient composition also needs to be investigated. The effect of cooking, roasting and boiling of agricultural products on reduction of aflatoxin level should be studied. In general, proper handling and management is a key issue for minimizing the incidence of aflatoxins on grains and feeds; and storage for longer time is known to predispose grains and feeds to aflatoxin infestation. Therefore, there is a need to educate, train and supervise farmers, feed traders and feed manufacturers on the importance of producing grains and feeds with low levels of, or exempt from, aflatoxin by implementing good agricultural practices and good harvesting practices especially during rainy season. Feed manufacturers should employ good feed manufacturing practices such as proper screening to isolate spoiled grains before milling and processing and employ good storage practices. Dairy producers should follow better feed storage and feeding practices such as screening moldy feeds before availing to livestock species.

With regard to heavy metals, there is a need of further investigation of toxic heavy metals from soils and fodder feed samples grown in similar study locations. Owners of different industries in Ethiopia should employ proper waste treatment practices for the waste water their discharge in to fields and water bodies and concerned regulatory institutions should do regular surveillances to check up if industries implement appropriate waste disposal practices.

8. References

Abdirahin, S., Ali, H., Kefyalew Gebeyehu and Kibru Beriso (2015). Assessment of Dairy Cattle Feed Resources in Fafan Town Eastern Ethiopia. European Journal of Biological Sciences, 7 (2): 50-54.

Abebe, H.G. and Bekele, H. (2006). Farmers post harvest grain management choices under Liquidity constraints and impending risks. Implications for achieving food security objectives in Ethiopia. Poster paper prepared for presentation at International Association for Economists Conference, Gold Coast, Australia.

Adebayo-Tayo, B.C. and Ettah, A.E. (2010). Microbiological quality and aflatoxin B1 level in poultry and livestock feeds. Nigerian Journal of microbiology, 24(1):2145-2152.

Abdi Mohammed and Alemayehu Chala (2014). Incidence of *Aspergillus* contamination of groundnut *(Arachis hypogaea* L.) in Eastern Ethiopia. African Journal of Microbiology Research, 8(8):759-765.

Abdullahi, M. (2011). Assessment of Metal Levels in Fresh Milk from Cows Grazed around Challawa Industrial Estate of Kano, Nigeria. J. Basic. Appl. Sci. Res.1(7):533-538.

Adugna Tolera (2008). Feed resources and feeding management. A manual for feed lot operators and development workers. Ethiopian Sanitary and Phyto-sanitary Standards and livestock and meat marketing program (SPS-LMM), Texas Agricultural Experiment Station (TAES)/Texas A & M University. USAID, Addis Ababa, Ethiopia.

Adugna Tolera (2009). Livestock Feed Supply Situation in Ethiopia. In: Commercialization of Livestock Agriculture in Ethiopia, (Tamrat Degefa and Fekede Feyissa, eds). Proceedings of the 16thAnnual Conference of the Ethiopian Society of Animal production (ESAP) held in Addis Ababa, Ethiopia, October 8-10, 2008.

http://abtassociates.com/Aflatoxin- Stakeholders-Conference-Related-Matrie.aspx (2017). Consensus Findings from the Aflatoxin Stakeholder Workshop held November 5-6, 2012 in Abuja, Nigeria at the Agricultural Research Council of Nigeria (ARCN).

Akan, J.C., Abdulrahman, F.I., Ogugbuaja, V.O., Ayodele, J.T. (2009). Heavy metals and anion levels in some samples of vegetable grown within the vicinity of Challawa Industrial area, Kano State, Nigeria. American Journal of Applied Sciences,6 (3):534-542.

Ahmad, K., Khan, Z. I., Rizwan, Y., Sher, M., Mukhtar, M. K., Nawaz, R., Shaheen, M., Mirzaei, F.

(2013). Nickel, lead and manganese content of forages irrigated with different sewage water treatments: a case study of a semiarid region (Sargodha) in Pakistan. Agricultural Sciences, 4(4): 180-184.

Ahmed, M.A.M., Ehui, S. and Yemeserach, A. (2002). Dairy development in Ethiopia. ILRI working paper, Addis Ababa, Ethiopia.

Akande, M. M., Abubakar, T.A., Adegbola, S.E. and Bogoro (2006). Nutritional and Health Implications of Mycotoxins in Animal Feeds: A Review. Pakistan Journal of Nutrition,5(5):398-403.

Aksoy, A., Yavuz, O., Guvenc, D., Das, Y.K., Terzi, G. and Suleyman Celik, S. (2010). Determination of Aflatoxin Levels in Raw Milk, Cheese and Dehulled Hazelnut Samples Consumed in Samsun Province, Turkey.

Alberta (2017). Moldy feeds and reproductive failure in cows. Alberta Agriculture and Forestry.

Alemayehu Mengistu (2002). Forage production in Ethiopia. A case study with implications for livestock production. Ethiopian Society of Animal production (ESAP), Addis Ababa, Ethiopia.

Alemayehu Mengistu (2005). Feed resource base of Ethiopia. Status, limitations and opportunities for integrated development. In: Participatory innovation and research lessons for livestock development, pp. 377-386, (Asfaw Yimegnuhal and Tamrat Degefa, eds). Proceedings of the 12th Annual conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia, August 12-14, 2004 Part II: Technical papers.

Alemayehu Mengistu, Gezahegn Kebede, Getnet Assefa and Fekede Feyisa (2016). Improved forage crops production strategy in Ethiopia: A review. Academic Research Journal of Agricultural Science and Research,4(6):285-296.

Alemie Asressie and Lemma Zemedu (2015). Contribution of Livestock Sector in Ethiopian Economy: A review. Advances in life Science and technology, 29:79-90.

Alganesh Tola, Ofodile, L.N., Fekadu Beyene (2007). Microbial quality and chemical composition of raw whole milk from Horro cattle in East Wollega, Ethiopia. Ethiopian Journal of Education and Sciences,3(1):1-10.

Alloway, B. J. (1995). Soil processes and the behavior of metals. In: Heavy Metals in Soils. 2nd ed. (Alloway, B. J., ed). Kluwer Academic Publ., Dordrecht, the Netherlands.

Amare Ayalew, Hartmut F., Johnn, L., Robert, B. and Dawit Abate (2006). Natural

occurrence of mycotoxins in staple cereals from Ethiopia. Mycopathologia,162:57-63.

Amare Hailu (2007). Metal concentration in vegetables grown in Northern Addis Ababa and part of rift valley (Ziway), Ethiopia. MSc Thesis, Department of Environmental Sciences, Addis Ababa University, Addis Ababa, Ethiopia.

Amer, A.A. and Ibrahim, M.A.E. (2010) Determination of aflatoxin M1 in raw milk and Traditional cheeses retailed in Egyptian markets Journal of Toxicology and Environmental Health Sciences,2(4):50-53.

AOAC (1990). Official methods of analysis, 15th ed. (Kerlich, K., ed). Association of Official Analytical Chemist INC, Arlington, Virginia, U.S.A.

Azage Tegegne and Alemu Gebrewold (1998). Prospects for peri-urban dairy development in Ethiopia. Available at: http://hdl.handl.net/10568/50997/.

Bandyopadhyay, R., Ortega-Beltran, A, Akande, A, Mutegi, C. Atehnkeng, J. Kaptoge, L. Senghor, A.L. Adhikari, B.N. and Cotty, P.J. (2016). Biological control of aflatoxins in Africa: Current status and potential challenges in the face of climate change. World Mycotoxin Journal online. Review article. ISSN 1875-0796 online, DOI 10.3920/WMJ2016.2130

Bankole, S. A., Adenusi, A. A., Lawal, O. S. and Adesanya, O. O. (2010). Occurrence of aflatoxin B1 in food products derivable from 'egusi' melon seeds consumed in southwestern Nigeria. Food Control,21:974-976.

Bahmani, M., Efekhari, Z., Mohsezadeghan, A., Ghotbian, F., Alighazi, N. (2012). Leech (Limnatis nilotica) causing respiratory distress in a pregnant cow in Ilam province in Iran. Comp Clin. Pathom.21:501-503.

Battacone, G., Nudda, A., Cannas, A., Cappio Borlino, A., Bomboi, G., Pulina, G. (2003). Excretion of aflatoxin M1 in milk of dairy ewes treated with different doses of aflatoxin B1. J. Dairy Sci.86:2667-2675.

Battacone, G., Nudla, A., Palomba, M., Pascale, M., Nicolussi, P., Pulina, G. (2005). Transfer of aflatoxin B1 from feed to milk and from milk to curd and whey in dairy sheep fed artificially contaminated concentrates. J.Dairy Sci, 88(9):3063-3069.

Belay Duguma, Azage Tegegne and Hegde, B.P. (2011). An Assessment of Availability of

Livestock Drinking Water Resources, Patterns of Exploitation and Management Strategies at Ginchi Watershed, Ethiopia. American-Eurasian Journal of Agronomy,4(3):38-45.

Belay, D., Getachew, E., Azage, T. and Hedge, B. (2013). Farmers perceived livestock production constraints in Ginchi watershed area: Results of participatory rural appraisal. International Journal of livestock production,4(8):128-134.

Bennett, J.W. and Klich, M. (2003). Mycotoxins. Clin. Microbiol. Rev.16:497-516.

Berhanu Kuma, Fekede Feyissa and Kedir Nesha (2006). Gender Based Analysis of Livestock Production Systems at Kuyu wereda in North Shao zone, Ethiopia. In: Institutional arrangements and challenges in market-oriented livestock agriculture in Ethiopia, pp. 88-100, (Tamrat Degefa and Fekede Feyissa, eds). Proceedings of the 14th Annual Conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia, September 5-7, 2006 Part II: Technical papers.

Benin, S., Ehui, S. and Pender, J. (2003). Policies for livestock development in the Ethiopian highlands. Environment Development and Sustainability,5:491-510.

Betran, E.J. and Isakeit, T. (2003). Aflatoxin Accumulation in Maize Hybrids of Different Maturities**.** A-Agronomy Journal,96(2):565-570.

Bhat, R.V. and Vasanthi, S. (2003). Mycotoxin food safety risk in developing for food, agriculture and environment. Food safety in food security and food trade. International Food Policy Research Institute (IFPRI).

Brankove, T. P., Jovanovic, M., Grujic, B. (2013). Aflatoxin standards and maize trade. Economics of agriculture,60(3):595-607.

Bryden, W.L. (2012). Mycotoxin contamination on the feed supply chain: Implication of animal productivity and food security. Animal feed Science and technology.

Brufau, J. Tacon, A. (1999). Animal feeding and food safety. Report of a FAO Expert consultation. Feed manufacturing in the Mediterranean region: Recent advances in research and technology. Zaragoza: CIHEAM:155-193.

Boxall, R. (1998). Grain post-harvest lose assessment in Ethiopia: Final report (Working paper). Greenwich Academic Literature Archive (GALA).

Campos, A.C., Manizan, A.L.,Tadris, S., Akaki, D., Koffe-Nevry, R., moore, G.G., Fapohunda,

S., Bailly, S., Montet, D., Oswald, I.P., Lorber, S., Brabet, C.nand Puel, E. (2017). Aspergillus korhogoensis, a novel aflatoxin producing species from the Cote d' Ivoire. 9(11):353. doi:10.3390/toxins9110353

Carlson, M.P. and Ensley, S.M. (2003). Use of feed contaminated with fungal (mold) toxins (mycotoxins). University of Nbraska, Linkoln Extension, Institute of Agriculture and natural resources.

Cassel, K.E., Campbell, B. Draper, M., Epperson, B. (2011). Aflatoxins: Hazard in grain/aflatoxicosis and livestock. Cooperative Extension Services.

Chandra, H., Bahuguna, J. and Singh, A. (2013). Detection of aflatoxin in zea mays L. from Indian markets by competitive ELISA. Octa.J.Biosci.1(1):62-68.

Chemeda, A., Abush, T., Zekarias, S. and Habte, J. (2007). Survey on farmer's perception and management practices of insect pests on traditional stored sorghum in South Western Ethiopia. Crop protection 26,1817-1825.

Cruz, P.S. (1996). Feed quality problems and management strategies. In: Feeds for Small-Scale aquaculture. pp. 64-73, (Santiago, C.B., Coloso, R.M. Millamena, O.M. and Borlongan, I.G., eds). Proceedings of the National Seminar-Workshop on Fish Nutrition and Feeds, Tigbauan, Iloilo, Philippines: SEAFDEC Aquaculture Department.

Cruz, G.C, Din Z, Feri C.D, Balaoing, A.M, Marie Gonzales E, Navidad, H.M. (2009). Analysis of toxic heavy metals (arsenic, lead, and mercury) in selected infant formula milk commercially available in the Philippines by AAS. E-International Scientific Research Journal,1(1):45-51.

CSA (Central Statistics Agency) (2015). Agricultural Sample Survey, volume II, Report on livestock and livestock characteristics (private peasant holding). Statistical Bulletin 578, Addis Ababa, Ethiopia.

Cornea, C. P., Ciuca, M., Voaides, C., Gagiu, V. and Pop, A. (2011). Incidence of fungal Contamination in a Romanian bakery: a molecular approach. Romanian biotechnological letters,16:5863-5871.

Cross, R.H. (1995). HACCP: what it is and why should I care. Presented to the Texas Cattle Feeders Association Board of Directors. Institute of food science and engineering center for food Ssfety, Texas A and M University.

Dawd Gashu, (2010). Determination of the level of essential and toxic metal constituents in cow's whole

milk from selected sub-cities in Addis Ababa, Ethiopia. A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements of the Degree of Masters of Science in Food Sciences and Nutrition.

Dawit D. (2010). The role of Regulatory Bodies in ensuring food safety in Ethiopia. Status and achievements of MOH (Ministry of Health).

Dawit Gizachew, Barbara S., Azage Tgegne, Hanson, J. and Grace, D. (2016). Aflatoxin analysis of dairy feeds in the greater Addis Ababa milk shed, Ethiopia. Food Control, 59:773-779.

Darwish, W.S., Ikenaka, Y., Nakayama, S.M.M., Ishizuka, M. (2014). An overview of mycotoxin contamination in foods in Africa. J. vet. Med. Sci.76(6):789-797.

Dejene, (2011). Effectiveness of oxidation ponds in industrial waste water treatment: the case of Mesaco Global and Ethiopia Tanneries, Modjo area. MSc Thesis, Addis Ababa University, Addis Ababa, Ethiopia.

Dereje Duressa, Debela Kenea, Wakgari Keba, Zelalem Desta, Gutema Berki, Gera Letta and Adugna Tolera (2014). Assessment of livestock production system and feed resources availability in three villages of Diga district Ethiopia. Research program on integrated systems for the humid tropics. ILRI.

Derek, F. (2010). Mycotoxin in cereals: Sources and risks. European Mycotoxin, Awareness Network. Leatherhead Food Research, Randalls Road, Leatherhead, Surrey KT22 7RY UK.

Dereje Fekadu, Seyoum Bediye and Zinash Sileshi (2010). Characterizing and predicting chemical composition and *in vitro* digestibility of crop residue using near infrared reflectance spectroscopy (NIRS). Livestock Research for Rural Development, 22(2).

Dicostanzo, A. Murphy, M. (2012). Strategies for feeding mycotoxin and mold contaminated grains to cattle. Beef cattle feed lot nutrition, University of Minoseta Extension.

Dhanasekaren, D., Shanmugapiriya, S., Thajuddin, N., Panneerselvam, A. (2011). Aflatoxins and Aflatoxicosis in Humans and Animals. Aflatoxins-Biochemistry and Molecular Biology, (Ramon G. Gueivara-Gonzalez, ed), ISBN, 978-953-307-395-8,

Dubale Befikadu (2014). Factors affecting quality of grain stored in Ethiopian traditional

storage structures and opportunities for improvement. International Journal of Sciences: Basic and applied Research (IJSBAR),18(1):235-257.

Ellen Yared, Tesfaye Bayleyegn, Almaz Abebe, Andualem Mekonnen, Murphy, M., Kathleen, L., Luce, C.R., Hunt, D.R., Kirubel Tesfaye, Moa Abate, Tsigereda Assefa, Firehiwot Abera, Kifle Habte, Feyissa Chala, Lewis, L. and Amha Kebede (2015). Metals exposure of residents living near the Akaki River in Addis Ababa, Ethiopia: A cross-sectional study. Journal of Environmental and Public Health,5:8. doi: 10.1155/2015/935297.

El-Bassiony, Tawfik A., Amin, Wallaa F., Ahmed, Ebtsam, O. (2016). Impact of heavy metal contamination on milk and underground water of the new valley, Egypt. IOSR Journal of Environmental Science, Toxicology and Food Technology,10(8):23-29.

Enb, A., Abou, M.A., Abd-Rabou, N.S., Abou-Arab, A.A.K. and El-Senaity, M.H. (2009). Chemical composition of raw milk and heavy metals behavior during processing of milk products. Department of Dairy Science, National Research Center, El-Behoos St., Dokki, Giza, Egypt. Global veterinarians,3:268-275.

Endale Yadessa, Abule Ebro, Lemma Fita and Getnet Aseffa (2016). Feed resources and its utilization practices by smallholder farmers in Meta-Robi district, West Shoa zone, Oromiya regional state, Ethiopia. Academic Research Journal of Agricultural Science and Research,4(4): 124-133.

Ephrem Guchi (2015). Implication of Aflatoxin Contamination in Agricultural Products. American Journal of Food and Nutrition,3(1):12-20.

EPA (2002). Pesticide usage estimation and use in estimating dietary risk. Biological and economic analysis division, Office of pesticide program.

ESA (Ethiopian Standard Agency), (2013). ES ISO 14501:2013, Milk and milk powder-Determination of aflatoxin M1 content-Clean-up by immunoaffinity chromatography and determination by high-performance liquid chromatography second edition, ©ESA.

FAO (1990b). Pesticides as water pollutants. Chapter 4.

FAO/WHO (1995). Hazard Analysis and Critical Control Points system concept and application, FAO food and nutrition paper 76, Rome, Italy.

FAO (2001). The state of food and agriculture in 2001. Produced by Economic and Social

Development Department, Food and Agriculture Organization of the United Nations, Rome.

FAO/WHO (2003). Assuring Food Safety and Quality: Guidelines for Strengthening National Food Control Systems, FAO food and nutrition paper 76, Rome, Italy.

FAO (2004). Worldwide Regulations for Mycotoxins in Food and Feed in 2003. Food and Nutrition Paper 81, Rome.

FAO/WHO (2005). Practical actions to promote food safety. Regional conference on food safety for Africa. Harare, Zimbabwe.

FAO/WHO (2006). Food safety risk analysis a guide for national food safety authorities. FAO food and nutrition paper 87, Rome, Italy.

FAO/WHO (2007). Joint FAO/WHO food standards program FAO/WHO coordinating committee for Africa, 17th session, Rabat, Morocco, 23-26.

FAO/WHO (2009). The state of food and agriculture, Livestock in the balance, Rome, Italy.

Farid, S. and Baloch, M.K. (2012). Heavy metal ions in milk samples collected from animals feed with city effluent irrigated fodder. Greener Journal of Physical Sciences,2 (2): 36-43.

FAOSTAT (2015). Faostat.fao.org

Feddern, V., Dors, C., Tavernari, F.C., Mazzuco, H., Cunha, J. R. A., Krabbe, E.L. and Scheuermann, G.N. (2013). Aflatoxins: Recent Advances and Future Prospects, Aflatoxins Importance on Animal Nutrition.

Fekede Feyissa, Getinet Asefa, Lulseged G/Hiwot, Muluneh Minta and Tadesse T/Tsadik (2004). Evaluation of Napir grass-vetch mixture to improve total herbage yield in the central highlands. In: The Role of Agricultural Universities/Colleges in Transforming Animal Agriculture in Education, Research and Development in Ethiopia: Challenges & Opportunities, pp. 155-164, (Tamrat Degefa and Fekede Feyissa, eds). Proceedings of the 13th Annual Conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia, August 25-27, 2004 Part II: Technical papers.

Fisseha Itanna, Anderson, D., Stahr, K. (2003). Influence of soil type differences on the distribution of DTPA extractable heavy metals in soils. *SINET*: Ethiop.J.Sci.26 (1):47-54.

Finnegan, D. (2010). Mycotoxins in cereals: sources and risks. MERIDEN animal health.

Letherhead Food Research. European Mycotoxin Awareness Network.

Food Business Africa (2017). Africa food and beverage industry Megazine. Posted in industry special reports, Nairobi, Kenya.

Francesconi, G.N. (2006). Promoting milk quality in smallholders' cooperatives: Evidence from Ethiopia. In: Institutional arrangements and challenges in market-oriented livestock agriculture in Ethiopia, pp. 85-96, (Tamrat Degefa and Fekede Feyissa, eds). Proceedings of the 14th annual conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia, September 5-7, 2006 Part I: Plenary Session. Addis Ababa, Ethiopia.

Funte, S, Negesse, T., Legesse, G. (2010). Feed resources and their management systems in the Ethiopian highlands. The case of Umbulo Wach water shade in Southern Ethiopia. Tropical and subtropical agro ecosystems,12(1):47-56.

Galvano, F., Galofaro, V., DeAngelis, A., Galvano, M., Bognanno, M., Galvano, G. (1998). Survey of the occurrence of aflatoxin M1 in dairy products marketed in Italy. J.Food protection,61:738-741.

Gemechu Keneni (2007). Phenotypic diversity for biological nitrogen fixation in Abyssinian field pea (Pisum sativum var. abyssinicum) germplasm accession. Report on independent study for PhD study. Addis Ababa University. Science Faculty, Addis Ababa, Ethiopia.

Getachew Agegnehu, Berhane Lakew and Nelson, P.N. (2014). Cropping sequence and nitrogen fertilizer effects on the productivity and quality of malting barley and soil fertility in the Ethiopian highlands. Archives of Agronomy and Soil Science 60 (9):1-15.

Getnet Assefa, and Ledin, I. (1999). Feed resource assessment and evaluation of forage yield, quality and intake of oats and vetches grown in pure stands and in mixtures in the highlands of Ethiopia. Available feed resources and the role of cultivated forage crops in the small holder farming systems in the central highlands of Ethiopia, MSc Thesis. Swedish University of Agricultural Sciences, Uppsala, Sweden.

Ghareeb, K., Elmalt, L.M., Awad, W.A., Bohm, J. (2013). Prevalence of aflatoxin M1 in raw milk produced in tropical state and imported milk powder. J.vet. Anim. Sci.3(1-2):1-4.

Gian N., Francesconi (2006). Promoting Milk Quality in Smallholders' Cooperatives: Evidence from Ethiopia. In: Institutional arrangements and challenges in market-oriented livestock agriculture in Ethiopia, pp. 69-82, (Tadelle Dessie ed.). Proceedings of the 14th annual

conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia, 5-7 September 2006.

Guo, B., Yu, J., Holbrook, C.C., Cleveland, T.E., Nierman, W.C. and Scully, B.T. (2009). Strategies in Prevention of Pre-harvest Aflatoxin Contamination in Peanuts: aflatoxin Biosynthesis, Genetics and Genomics. Peanut Science,36:11–20.

Hasheminya, S.M. and Dehghannya, J. (2013). Strategies for decreasing aflatoxin in livestock feed and milk. International Research Journal of Applied and Basic Sciences (IRJABS),4(6):1506-1510.

Helica biosystems Inc (2015). Aflatoxin B1 ELISA Quantitative Catalog ♯ 41BAFL01B1-96 www.helica.com, California, USA.

Helica biosystems Inc (2015). Aflatoxin M1 ELISA Quantitative Catalog # 961AFLM01M-96 www.helica.com California, USA.

Henok Ayalew, Amare Birhanu, Biruhtesfa Asrade (2013). Review on food safety system: Ethiopian perspective. African journal of food science,7(12):431-440.

Hodgson, R.J., (1990). The Southern Sidamo Rangeland Project 1985-1988. Perspectives on development interventions and extension. Unpublished monograph.

IARC (International Agency for Research on Cancer) (2002).Traditional herbal medicines, some mycotoxins, Napthalene and styrene. Monographs on the evaluation of carcinogenic risks to humans, pp. 82-171.

https://en.wikipedia.org/wiki/Awasa (2016). Geographical location of Hawassa.

http://www.ethiostandards.org/ESA/(2016). Types of standards and Ethiopian standards.

(http://www.ethiomarket.com/qsae/(2016). Situation of standards in the Ethiopian context.

http://abtassociates.com/Aflatoxin- Stakeholders-Conference-Related-Matrie.aspx (2017). Consensus Findings from the Aflatoxin Stakeholder Workshop held November 5-6, 2012 in Abuja, Nigeria at the Agricultural Research Council of Nigeria (ARCN).

https://www.google.com/search?q=chennai+india+weather (2017). Weather condition of hennai, India.

https://journeysbydesign.com/destinations/Ethiopia (2017). Weather condition of Ethiopia.

https://www.google.com/search?q=weather+Gilan+Iran (2017). Weather condition of Gilan, Iran.

https://www.google.com/search?q=weather+Samsun+Turkey, (2017). Weather condition of Samsun, Turkey.

Idris, Y. M. A., Mariod, A. A., Elnour, I. A. and Mohamed, A. A. (2010). Determination of aflatoxin levels in Sudanese edible oils. Food Chem. Toxicol.48(8-9):2539-2541.

Iftikhar, B., Arif, S., Siddiqui, S. and Idris, Y. M. A., Mariod, A. A., Elnour, I. A. and Mohamed, A. A. (2010). Determination of aflatoxin levels in Sudanese edible oils. Food and Chemical Toxicology,48:2539-2541.

IGAD (2017). The contribution of livestock to the Ethiopian economy-part II. IGAD livestock policy initiative, IGAD LPI working papers, No 02-11.

Iqbal, S.Z., Asi, M.R. and Ariño, A. (2011) Aflatoxin M1 contamination in cow and buffalo milk samples from the North West Frontier Province (NWFP) and Punjab provinces of Pakistan, Food Additives & Contaminants: Part B,4(4):282-288.

Iheshiulor, O. M., Esonu, B.O., Chuwuka, O.K., Omede, A.A., Okoli, I.C., Ogbuewu, I.P. (2011). Effects of mycotoxins in animal nutrition: A review. Asian J. Anim. Sci.5(1):19-33.

Iriksson, S. (2013). Water quality in the Koga irrigation project, Ethiopia: A snapshoot of general quality parameters. Department of Earth Sciences, Geotryckeriet Uppsala University, Uppsala, Sweden.

Järup, L. (2016). Hazards of heavy metals contamination. http://bmb.oxfordjournals.org/

Jawaid, S., Talpur, F.N., Nizamani, S.M., Afridi, H.I. (2015). Contamination profile of aflatoxin M1 residues in milk supply chain of Sindh, Pakistan. Toxicology reports,2:1418-1422.

John, E. E., Marcel C. A., Collins N. U., Ebere C. A., Kasimir, M. I. & Michael O. O. (2001). Bulletin of the World Health Organization,79:423–433.

Kang, E.K. and Lang, K.A. (2009). Aflatoxin B1 and M1 contamination of animal feeds and milk from urban centers in Kenya. African Health Sciences,9(4):219-226.

Kaplan, O, Yildirim, N.C, Yildirim, N, and Cimen, M. (2011). Toxic elements in animal products and environmental health. Asian J Anim.6: 228-232.

Kefena Iffa, Mengistu Alemayehu, Zewdie Wondatr, Driba Hundie, Getnet Assefa, and Getu Ktaw (2016). Achievements, status and prospects in dairy research and development in Ethiopia: review article. EIAR 50th year Jubilee Anniversary special Issue: 51-65.

Keskin, E. (2006). Could cooperatives be a panacea for Ethiopian livestock sector? In: Institutional arrangements and challenges in market-oriented livestock agriculture in Ethiopia, pp. 97-102, (Tadelle Dessie ed.). Proceedings of the 14th annual conference of

the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia, September 5-7 2009.

Khan, R., Qureshi, M.S., Mushtaq, A., Guhfranullah and Naveed, A. (2012). Effect of quality and frequency of drinking water on productivity of dairy buffaloes. The Journal of animal and Plant Sciences,22(2): 96-101.

Kiama, T.N., Lindahl, J.F., Sirma, A.J., Senerwa, D.M., Waithanji, E.M., Ochungo, P.A., Poole, E.J., Kang'ethe, E.K., and Grace, D. (2016). Kenya dairy farmer perception of moulds and mycotoxins and implications for exposure to aflatoxins: a gendered analysis. African Journal of Food, Agriculture, Nutrition and Development,16(3):11106-11107.

Kirui, C.C. (2016). Assesement of the traditional and improved processing methods in the reduction of aflatoxin levels in maize and maize products. MSc Thesis in Food science and Technology. University of Nairobi, Kenya.

Kudo, H., Cheng, K.J., Rode, L.M., Abdullah, N. H., Hussain, H.Y., Jalaludin, H.Y. (1994). Effects of chemical treatments of barley straw on leaching and digestibility by rumen fluid and cellulolytic bacteria. Asian-Australian J. Anim. Sci.7:389-398.

Khan, R., Qureshi, M.S., Mushtaq, A., Ghufranullah, A. and Naveed, A. (2012). Effect of quality and frequency of drinking water on productivity and fertility of dairy buffaloes. The Journal of Animal and Plant Sciences,22(2):96-101.

Lars, J. (2003). Hazards of heavy metal contamination. Department of Epidemiology and Public Health, British Medical Bulletin,68:167–182.

Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., luber, G., Nyamongo, S.J., Baker, L., Dahiye, A.M, Misore, A, Kevin, D.R, and the Kenya aflatoxin investigating group (2005). Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in Eastern and Central Kenya. Environmental Health Perspective,113(12):1763-1767.

Little, P., Smith, K., Cellaruis, B., Coppock, D., Barrette, C. (2001). Avoiding Disaster: Diversification and Risk Management among East African Herders. Development and Change,32(30):401-433.

Lunden, H. (2015). What is milk? Aflatoxin and antibiotic residues in cow's milk in Assam, North East India. MSc Thesis, Swedish University of Agricultural sciences, Uppsala, Sweden.

Makun, H.A., Dutton, M.F., Njobeh, P.B., Gbodi, T.A. and Ogbadu, G.H. (2012).

Aflatoxin Contamination in Foods and Feeds: A Special Focus on Africa. In: Trends in Vital Food and Control Engineering, (Ayman, A.E. ed), ISBN: 978-953-51-0449-0.

Martins, H.M., Mendes Guerra, M.M., Almeida Bernardo, F.M. (2007). Occurrence of Aflatoxin B1 in dairy cows' feed over 10 years in Portugal (1995-2004). Rev. Iberoam micol,24:69-71.

Masoero, F., Gallo, A., Moschini, M., Piva, G., Diaz, D., (2007). Carryover of aflatoxin from feed to milk in dairy cows with high or low somatic cell counts. Animal, 1(9):1344-1350.

Mashlla Dejene (2004). Grain storage method and their effects on sorghum grain quality in Harerghe, Ethiopia. Department of ecology and crop production Science, Doctoral Thesis, Swedish University of Agricultural University, Uppsala, Sweden.

Mazumder D N, G. (2008). Chronic arsenic toxicity and human health. Indian J Med Res, 128(4):436-447.

Matthews-Amune, O. C. (2012). Determination of forage grasses (Carpet grass (Axnopus Ompresus), Guinea grass (Panucum maximum) and Elephant grass (Pennisetum Purpureum)) in the vicinity of Itakpe Iron Ore mine, Nigeria. Int.J.Pure Appl.Sci.Technol.13(2):16-25.

Matt, H. and Sonja, C. (2012). Water nutrition and quality considerations for cattle. University of Florida IFAS (Institute of Food and Agricultural Sciences) extension.

Mekonnen Haileselassie, Habtamu Taddele, Kelali Adhana, and Shewit Kalayou (2013). Food safety knowledge and practices of abattoir and butchery shops and the microbial profile of meat in Mekelle City, Ethiopia. Asian Pac.J.Trop.Biomed.3(5):407–412.

Melese Temesgen and Melese Abdisa (2015). Food Standards, Food Law and Regulation System in Ethiopia: A Review. Public Policy and Administration Research,5(3):58-72.

Mesfin Dejene, Seyoum Bediye, Getu Kitaw and Aemiro Kehaliw (2009). On-farm evaluation of lactating local (*Bos indicus*) dairy cows fed urea-treated wheat (*Triticum aestivum*) straw supplemented with soluble protein source-based concentrate mixture during the dry season in crop-livestock production system of south west shoa, Ethiopia. In: Climate change, livestock and people: Challenges, opportunities, and the way forward, pp. 239-252, (Zelalem Yilma and Aynalem Haile, eds). Proceedings of the 17th Annual conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia September 24-26, 2009.

Michael, P.C. (2003). Use of feed contaminated with fungal (mold) toxins (mycotoxins).

Neb guide Institute of Agriculture and natural Resource (IANR) available at: http://extension.unl.edu/publcations

Mor, F., (2005). Cadmium and lead in livestock feed and cattle manure from four agricultural areas of Bursa, Turkey. In: Dawd Gashu, (2010). Determination of the level of essential and toxic metal constituents in cow's whole milk from selected sub-cities in Addis Ababa, Ethiopia. MSc Thesis, Addis Ababa University, Addis Ababa, Ethiopia.

Mold fact sheet (2001). Cornell Cooperative Extension, New York Times Magazine, Cornel University.

Moreki, J.C., Woods, T.O. and Nthoiwa, P.G. (2013). Estimation of the concentration of heavy metals in forages harvested around Dibete area, Botswana. International Journal of Innovative Research in Science, Engineering and Technology,2(8):3060-4071.

Mosweu, S. and Letshwenyo, M. (2013). Is roadside grass suitable for use as livestock? Forage in Botswana. Journal of environmental chemistry and toxicology, 5(10):265-268.

Mukesh, K. R., Puneet, K., Manoj, S. and Anand, S. (2008). Toxic effects of heavy metals in livestock health: A review. Veterinary World, 1(1):28-30.

Muluken Mekuyie (2014). Heavy metal concentration in effluents of textile industry, Tikur-Wha River and milk of cows watering on this water source, Hawassa, Southern Ethiopia. Journal of Natural Resource Research,4(20):47-56.

Mureja Shiberu, Eskil Brannang and Kano Banjaw (2002). Milk Production Characteristics of Holstein Friesian Cattle at Holetta Government Dairy Farm, Ethiopia, Eth. J. Anim. Prod.2(1):1-10.

Mazumder, G. (2008). Chronic arsenic toxicity and human health. Indian J. Med. Res.128(4): 436-447.

Nabarro, D. and Wannous, C. (2014). The potential contribution of livestock to food and nutrition security: the application of the one health approach in livestock policy and practice. Rev. Sci. tech. Off. int. Epiz.33(2):475-485.

Negassa, A., Rashid, S. and Gebremedhin, B. (2011). Livestock production and marketing in Ethiopia. Ethiopian Support Strategy Program II (ESSP II) Working Paper 26. Washington, D.C. IFPRI.

Negm-Eldin, M.M., Abdraba, A.M., Benamer, H.E. (2013). The first reported leech infestation by Limnatis nilotica (Savigny 1822) of farm animals in Libya. Rabat Serie Zoologie,4:33-36.

Norian, R., Mahmoudi, R., Porfarzaneh, A., Mashatian, F., Ata Kaboudari, A., Faezeh, S., Mahalleh, R.P.,

Katiraee, F. (2015). Determination of aflatoxin M1 levels in raw milk samples using ELISA and high-performance liquid chromatography in Qazvin, Iran Journal of Mycology Research,2(1):41-48.

NRC (National Research Council) (1980). Mineral Tolerance of Domestic Animals. Washington, D.C., National Academy Press.

NRC (National Research Council) (2001). Toxic Minerals. Washington, D.C., National Academy Press. pp:145-150.

Ogundiran, M.B., Ogundele, D. T., Afolayan, P.G.and Osibanjo, O. (2012). Heavy Metals Levels in Forage Grasses, Leachate and Lactating Cows Reared around Lead Slag Dumpsites in Nigeria .

Orisakwe, O. E., Nduka, J. K., Amadi, C. N., Dike, D. O., and Bede, O. (2012). Heavy metals health risk assessment for population via consumption of food crops and fruits in Owerri, South Eastern, Nigeria. Chemistry Central Journal,6: 77.

Ogabiela, E.E, Udiba, U.U, Adesina, O.B, Hammuel, C, Ade-Ajayi, F. A., Yebpella, G.G, Mmereole, U.J. and Abdullahi, M. (2011). Assessment of Metal Levels in Fresh Milk from Cows Grazed around Challawa Industrial Estate of Kano, Nigeria. J. Basic. Appl. Sci. Res.1(7):533-538.

Oyebangi, A.O. and Efiyuvwevvvere, B.J.O. (1999). Growth of spoilage mold and aflatoxin B1 production in naturally contaminated or artificially inoculated maize as influenced by moisture content under ambient tropical condition. Inter Biodeter Biod.44:209-217.

Paddock, M. (2017). Arsenic poisoning: causes, symptoms and treatment. Medical News Today. Healthline Media UK. Ltd. Brighton, UK

Patra, R.C., Swarup, D., Kumara, P., Nandi, D., Naresh, R., Ali, S.L. (2008). Milk trace elements in lactating cows environmentally exposed to higher level of lead and cadmium around different industrial units. Science of the total environment,404:36-44.

Pathirana, U.P.D., Wimalasiri, K.M.S., Silva, K.F.S.T. and Gunarathne, S.P. (2010). Investigation of farm gate cow milk for aflatoxin M1. Tropical Agricultural Research, 21(2):119-125.

Pavlovic, M., Sikiric, J. L., Havranek, N., Plavljanic, N., Brajenovic, (2004). Lead and cadmium levels in raw cow milk from an industrialized Croatian region determined by electro thermal atomic absorption spectrometry. Czech J. Anim. Sci.49:164-168.

Prabu, P. C. (2009). Impact of heavy metal contamination of Akaki River of Ethiopia on soil and metal toxicity on cultivated vegetable crops. Electronic Journal of Environmental, Agricultural and food chemistry,8(9):818-827.

Rajaganapathy, V., Xavier, F., Sreekumar, D., and Mandal, P.K. (2011). Heavy metal contamination in

soil, water and fodder and their presence in livestock and products: A review. Journal of Environmental Sciences and Technology,4(3):234-249.

Raji, B.G., Patnaik, M.C., Babu, M.C., Kalakumar, B., Singh, M.V. and Shylaja, J. (2006). Heavy metal contaminants in soil, water and plant-animal continuum due to pollution of Musi River around Hyderabad in India. Indian J. Anim. Sci.76:131-133.

Ramchander, J., Rajitha, B., Sunitha, G., Praveen, E., Anjaneyulu, A., Sunitha, J. and Rao, S. (2015). Quantitative determination of heavy metals in the water samples of four areas of Hyderabad in Telangana state. IQSR. Journal of Applied Chemistry,8(7):18-19.

Ramesh, V.B. and Siruguri, V. (2003). Mycotoxin food safety risk in developing countries. Food safety in food security and food trade 2020 vision for Food, agriculture and the environment.

Ramesh, J., Sarathchandra, G. and Sureshkumar, V. (2013). Analysis of feed samples for aflatoxin B1 contamination by HPTLC - a validated method. International Journal of Current microbiology and applied science,2(5):373-377.

RASFF (2011). RASFF portal. In DG SANCO available at: http://webgate.ec.europa.eu/rasff. windows/portal/.

Reddy, S.V, Waliyar, F. (2000). Properties of Aflatoxin and its Producing Fungi. Aflatoxin. Int. Crops Res. Inst. for the Semi-Arid Tropics.

Rehrahie Mesfin and Ledin, I. (2001). Biological and economical evaluation of feeding urea treated teff and barley straw based diets to crossbred dairy cows in the highlands of Ethiopia. MSc Thesis. Swedish University of *A*gricultural Sciences, Department of Animal Nutrition and Management, Uppsala, Sweden.

Reilly, C. (1991). Metal Contamination of Food. The Metals We Consume. 2nd ed. Elsevier Science Publishers Limited:1-20.

Rios, L.D., Gokah, I.B., Kauma, B.C., Matumba, L., Njoroge, S. and Chinseu, A. (2013). Advancing Collaboration for Effective Aflatoxin Control in Malawi. Malawi Program for Aflatoxin Control (MAPAC), Lilongwe, Malawi.

Rhodehamel E.J. (1992). Overview of Biological, Chemical, and Physical Hazards. In: Pierson M.D., Corlett D.A. (eds) HACCP. Springer, Boston, MA. Online ISBN978-1-4684-8818-0.

Rokhi, M.L. (2013). Determination of Aflatoxin M1 levels in raw milk samples in Gilan, Iran. Advanced studies in Biology,5(4):151-156.

Roy, D., Bharathidhasan, S., Mani, V., Kaur, H., Kewalramani, N. (2009). Heavy Metal Contents in cow and buffalo milk samples from haryana, India. Indian Journal of Animal Nutrition,26(1):29-33.

Rozso, K., Varhegy, J., Mocsenyi, A.R. and Fugli, K. (2003). Lead content of forages and the effect of lead exposure on plants. Vet. Bull.73:510-510.

Ruangwises, S., Saipan, P., Ruangwises, N. (2013). Occurrence of Aflatoxin M1 in raw and pasteurized goat milk in Thailand. Aflatoxins-Recent advances and future prospects,207-219.

Samuel Menbere, Azage Tegegne and Hegde, B.P. (2009). Feed resources availability, utilization and management in smallholder livestock production system in Yerer watershed of Ada Liben district. In: Commercialization of livestock agriculture in Ethiopia, pp 61-74, (Tamrat Degefa and Fekede Fetissa, eds). Proceedings of the 16th Annual Conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia. October 8-10, 2008. Part II Technical papers.

Sarah D. E., Michael J. B. and Thomas K. M. (2008). Fungal and fungal like disease of plants. Fact sheets agriculture and Natural resources, Ohio State University.

SAS (Statistical Analysis Systems) (2002). SAS Institute Version 9.0. SAS Inc., Cary, North Carolina, USA.

Schiere, J. B. and Ibrahim, M. N. M. (1989). Practical methods of urea treatment. Feeding of urea ammonia treated rice straw, a compilation of miscellaneous reports produced by the straw utilization project (Sri Lanka), Pudoc, Wageningen, pp 19-20.

Schweihofer, J. (2013), Biological, chemical and physical hazards assessed with HACCP. Michigan State University Extension and Sarah Wells, MSU departments of Animal Science & Food Science and Human Nutrition. Available at: http://msue.anr.msu.edu/news/biological chemical and physical hazards assessed withhaccp, 2017).

Senerwa, D.M. (2016). Prevalence of aflatoxin in feeds and cow milk from five countries in Kenya. African Journal of Food, Agriculture, Nutrition and development,16(3):11005-11021.

Sharma, P., and Dubey, R. S. (2005). Lead toxicity in plants. Braz. J. plant physiol.17(1):35-52.

Smith, J. (2015). Feeding Africa. An action plan for African livestock transformation.

United Nations for Economic Commission Africa, Senegal.

Solomon, S.G., Tiyamiyu, L.O., Okomoda, V.T. and Adga, K. (2016). Effect of storage condition on quality characteristics of commercial aqua feeds and growth of African cat fish Clarias Gariepinus, Croatian Journal of Fisheries,30-37.

Solomon Sorsa, Yadessa Chibsa, Girma Tilahun and Daniel Fitamo (2015). Heavy metal concentrations and physicochemical characteristics of effluent along the discharge route from Hawassa textile factory, Ethiopia. J. Environ. Anal. Toxicol.5: 285.

Speijers, G.J.A and Speijers, N.H.M. (2004). Combined toxic effects of mycotoxins. Toxicoll. Let. 158(1). 91-98.

SPSS (2011). SPSS for windows version 20, Statistical Package for Social Sciences, US.

Shewmaker, G. (2009). What do you do with moldy hay? Extension dairy specialist, University of Idaho Extension.

Suleiman, S. E., and Abdalla**,** M. A. (2013). Presence of Aflatoxin M1 in Dairy Cattle Milk In Khartoum State-Sudan. International Journal of Scientific & Technology Research,(2)4:2277-8616.

Swarup, D. and Dwevedi, S.K. (1998). Research on effects of pollution in livestock. Indian J. Anim. Sci.814-824.

Tadesse Eguale, Getnet Abie, Mesfin Sahile (2010). Control of aquatic leeches (*Lymantis nylotica*) using *Phytoloca dodecandra* (Endod) in Sodo district, Guragie zone, Southern Nations, Nationalities and Peoples Region, Ethiop. Vet. J.14(2):125-135.

Tarr, B. (2006). Managing the effects of molds and mycotoxins in ruminants. Nutrition news and information update, Shur-Gain, Nutrico Canada Inc.

Tassew Belete, Ahmed Hussen, Rao, V. M. (2014). Determination of Concentrations of Selected Heavy Metals in Cow's Milk: Borena Zone, Ethiopia, Journal of Health Science,4(5):105-112.

Tessema Elias (2011). A Critical Analysis of the Enforcement Framework of Consumer Protection in Ethiopia: Challenges and Prospects. Addis Ababa University, School of graduate studies faculty of law.

Tesfa Tolla (2010). Food Quality Regulations in Ethiopia. MSc Thesis submitted to Addis Ababa University, Faculty of law.

Tesfaye, L.T., Azage, T., Banjitha, P., Dirk, H. (2009). Moving Ethiopian Smallholder

dairying along a Sustainable Commercialization Path: Missing Links in the Innovation System. In: Commercialization of Livestock Agriculture in Ethiopia, pp: 39-50, (Tamrat Degefa aand Fekede Feyissa, eds). Proceedings of the 16th Annual Conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia. October 8-10, 2008. Part II Technical papers.

Thomas J.W., Hjubik, J.G., Patric Hart, L., Meerdink, G. and Marczewski, A.E. (1983). how to handle mold feed problems. MSU AG Facts, Extension Buletin E, 1670.

Thornton, P. K. (2010). Livestock production: recent trends, future prospects. Phil. Trans. R. Soc. B.365:2853-2867.

Thürmer, K., Williams, E., Rfobbey, J. R., (2002). Autocatalytic oxidation of lead crystallite surface. Science,297(5589):2033-2035. [doi: 10.1126/science.297.5589.2033]

Ulfina, G., Habtamu, A., Jiregna, D. and Chala, M. (2013). Utilization of brewer's waste as replacement for maize in the ration of calves. http//www//researchwebpub.org/wjar (2016).

Upadhaya, S.D., Park, M.A and Jong, K.H (2010). Mycotoxins and their Biotransformation in the Rumen: A Review. Asian-Aust. J. Anim. Sci.23(9):1250-1260.

USDA (1999). Grain Fungal Diseases & Mycotoxin Reference. Grain Inspection, Packers and Stockyards Administration Technical Services Division. Fungi and Fungal Diseases of Plants.

Van Egmond, H.P. (1989). Aflatoxin M1: Occurrence, Toxicity and Regulation. In: Mycotoxins in Dairy Products, (Van Egmond H. P., ed). London and New York: Elsevier Applied Science.

Vijayasamundeeswari, A., Mohankumar, M., Kartikeyan, M. Vijayanandrja, S., Paranidaharan, V., Velazhahan, R. (2009). Prevalence of aflatoxin B1 in pre and post harvest maize kernels, food products, poultry and livestock feeds in Tamil Nadu, India. Journal of plant protection research,49(2):220-224.

WHO, (1995). Guidelines for drinking water quality. World Health Organization, Geneva, Switzerland, pp:121.

WHO (1996). Guidelines for drinking water quality. World Health Organization, Geneva, Switzerland, pp:121.

WHO (2008). Food Safety Issues, Terrorist Threats to Food, Guidance for Establishing and

Strengthening, Prevention and Response Systems. Department of Food Safety, Zoonoses and Food borne Disease Cluster on Health Security and Environment.

Wise, S.S., Holmes, A.L., Wise, J.P. (2008). Hexavalent chromium-Induced DNA damage and repair mechanisms. Jan-Mar,23(1):39-57.

Yitay Alemayehu, Azage Tegegne and Mohammod Yususf Kurtu (2000). Chemical composition and nutritive values of major available feed resources in three peasant associations of the Awassa woreda. In: Proceedings of the 8th annual conferences of ESAP, Ethiopian Society of Animal Production, Addis Ababa, Ethiopia

Yohannese Melesse and Zelalem Zemede (2015). Consumer Seminar Program for ARSO/COCO- ISO/COPOLCO. Consumer Participation, Food safety and Service Issues in Ethiopia. 21st ARSO General Assembly, Addis Ababa, Ethiopia.

Yohannese Tekle (2007). Major animal health problems of market oriented livestock development in Alamata wereda. Doctor of Veterinary Medicine (DVM) Thesis, Debre Zeit, Ethiopia.

Zewdie Wondatr (2010). Livestock production system in relation with feed availability in the highlands and central rift valley of Ethiopia. MSc Thesis submitted to the school of animal and range science, school of graduate studies Haramaya University, 160 pages.

Zaidi J., Fatima, I., Arif, M., Qureshi, I.(2006). Determination of Trace Elements in Coffee Beans and Instant Coffee of Various Origins by INAA. J. of Radio analytical and Nuclear Chemistry,267:109–112.

Zinash Sileshi and Alemu Yami (2001). Contribution of animal science research to food security. Proceedings of the Ethiopian Society of Animal production (ESAP). Addis Ababa, Ethiopia.

Zodape, G.V., Dhawan, V.L., Wagh, R.R. (2012). Determination of metals in cow milk collected from Mumbai City, India. Bionano Fronter ISSN. 0974-0678.

9. APPENDIXES

9.1. Questionnaires used to collect data

Research title: Stakeholder's knowledge on risks of contaminations of feeds and water for livestock production: views of farmers, feed processors and distributors

Section one: General information (household characterization)

Date of interview_____

Name of the respondent_____

Type of actor _____

Gender of the respondent _____

Region_____

Town _____

District_____

Kebele/village_____

Section two: technical information (Questionnaire)

1. What feed resources do you have?

   _____

2. In what season of the year is mold more prevalent?

   A) Wet season b) dry season c) in both seasons

3. Have you ever been gave moldy feeds to your livestock?

   a) Yes b) no

4. What storage methods do you employ to your concentrate feeds?

   _____

5. If you have ever been gave moldy feeds to livestock species, what problems have you observed in your livestock?

   _____

6. For what duration do you store your feeds before marketing or before you feed to your livestock?
7. Dou you use palate for storing concentrate feeds?

a) Yes b) no

8. For what agro-industrial product do you give priority regarding use of palate for storage?
   a) Wheat flour b) wheat bran
9. What practices do you employ to overcome mold formation in your feeds?

   _____
10. Among the concentrate feed ingredients, which one is/are susceptible to mold?
    a) Wheat bran b) noug cake c) cottonseed cake d) linseed cake d) concentrate mixture
11. For what purpose do you utilize if feeds become extremely moldy?

    _____
12. Have you ever been observed illness problems on livestock due to drinking of contaminated water?
    a) Yes b) no
13. What are the possible causes for contamination of livestock water?

    _____
14. What are the health problems faced in your livestock due to drinking of effluent contaminated
    Water?

    _____
15. What traditional practices do you employ to overcome problem of effluent contaminated water?

    _____
16. What are the problems faced in your livestock due to drinking of leech contaminated water?

    _____
17. What traditional practices do you employ on the water body containing leech to overcome problem?

    _____
18. What traditional treatments do you practice on your animals to treat the animal that drinks leech contaminated water?

    _____

9.2. List of interviewed individuals from feed manufacturers, feed retailers and dairy producers

Table 9. 1. Interviewed feed processing factories across location

Study locations		
Holetta	Bishoftu	Hawassa
Ambo (Kebede) flour factory	ADA dairy union	Tateme flour factory
Ambo (Samuel) flour factory	Alema feed processing factory	Aklan flour factory
Ambo farmers' cooperative and food processing complex	Awash feed manufacturing factory	Martha Wendu flour factory
Afya flour factory*	Bora feed processing factory	Getu Metaferya flour factory
KOJJ flour factory*	Alfa fodder and dairy farm	Abdulhakim Nasr flour factory
Akaki feed processing enterprise*	JJK dairy farm	Admas flour factory
DHGDA flour factory*		Nasr Usman flour factory
FAFA food complex*		Sani Umer flour factory
Kaliti feed processing enterprise*		Barkot flour factory
Kebron food complex*		Hawassa flour factory
Kaliti food complex (Cheralia) *		St. Georgis brewery factory
Kokeb flour factory*		
NAS foods*		
President flour factory*		
Meta-Abo brewery factory*		
St, Georgis brewery factory (BGI) *		

Factories with * are found in Addis Ababa

Table 9. 2. Interviewed edible oil extracting factories by study locations

Holetta	Bishoftu
Ginchi (Werkie G/ebremeskel) oil extracting plant	Modjo edible oil extracting factory
Ginchi (Asegd Alemayehu) oil extracting plant	
Guder (W/o Keneni) oil extracting plant	
Addis-Modjo edible oil extracting factory*	
Modjo edible oil extracting factory	
Kana edible oil extracting factory*	

Factories with * are found in Addis Ababa

Table 9. 3. Interviewed feed retailers by study location

Holetta	Bishoftu	Hawassa
Abera Werku	Abdi Seyid	Aklilu Peltessa
Abebe Sisay	Abnet	Dengisa Kasa
Alemayehu Takele	Adane	Fkrie
Ambo farmers' cooperative	Ayele	Feed retailer 1
Aseffa	Derbew	Feed retailer 2
Asegid	Fikrie	Feed retailer 3
Aster Aseffa	Frehiwot	Feed retailer 4
Demisie Balcha	Getachew	Hussien
Genet	Habtamu Ngussie	Mortie Girma
Holetta Biftu Berga union	Mohammed	Neiredin kemal
Kibru	Serkalem	Reshad Faris
Lemma Feyissa	Sofya	Samya Abdela
Mekdesu	Tadesse 1	Sani Umra
Tisigereda	Tadesse 2	Shemsu Kedir
Werkie Gebremeskel	Tariku	Temesgen Basa
	Wondu Rufie	Zeyba Seyid

Table 9. 4. Interviewed dairy producers by study location

Holetta	Bishoftu	Hawassa
Almaz Hailu	Abera Mengistu	Edlias Chebo
Balcha G/tsadik	Alemu Ayeno	Bekele Mukale
Berhanie Zegie	Amelewerk	Biru Bimbessa
Dejene Shiferaw	Aselefech	Dagnachew Altaye
Feyisie	Asfaw	Elshaday dairy cooperative
Gebrehiwot Wami	Demeku	Elyasie Teamu
Gelana Achaltu	Engda	Ermyas Daferssa
Getachew Gudeta	Etesgent	Esayas
Getachew Kebede	Gebre	Genet Gabara
Gutema Begi	Genesis dairy farm*	Gmbo Daimu
Hailemichael	Getaneh Yimer	Leteshe Jemole
Kemil Mehammed	Girma	Nigussie Tadesse
Meseret Werku	Habtamu	Rhobot dairy coperative
Tadelech Dubale	JJK dairy farm	Sisay Abebe
Tafa Demisie	Kefeni	Hawassa dairy producer 1
Weinshet Beyicha	Legesse	Hawassa dairy producer 2
	Ngussie	Hawassa dairy producer 3
	Regassa Bedada	Hawassa dairy producer 4
	Seyifu	
	Yeshi kasa	

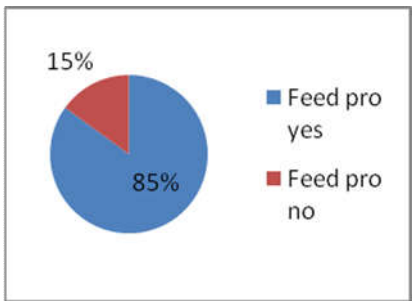

Fig 9.1. Training given to feed processors

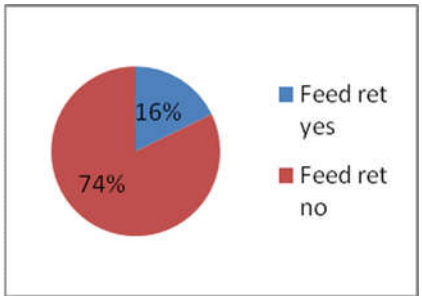

Fig 9.2. Training given to feed retailers

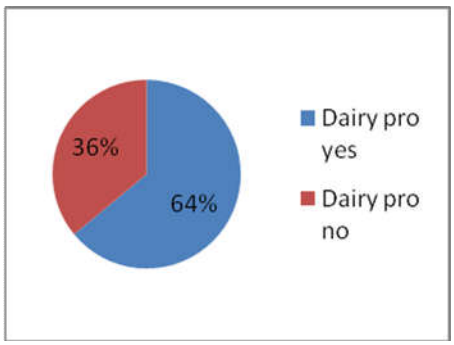

Fig 9.3. Training given to dairy producers