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AFLATOXIN LEVELS AND NUTRIENT CONTENT OF COMMERCIAL FEEDS IN SELECTED AREAS OF ETHIOPIA

MSc Thesis

By

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Department of Animal Production Studies

MSc Program in Animal Production

June 2022

Bishoftu, Ethiopoa

AFLATOXIN LEVELS AND NUTRIENT CONTENT OF COMMERCIAL FEEDS IN SELECTED AREAS OF ETHIOPIA

A THESIS SUBMITTED TO THE COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ANIMAL PRODUCTION

By

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SIGNATURE PAGE

Title: **AFLATOXIN LEVELS AND NUTRIENT CONTENT OF COMMERCIAL FEEDS IN SELECTED AREAS OF ETHIOPIA**

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And recommend that it be accepted as fulfilling the thesis requirement for the degree of: Master of Science in Animal Production

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I hereby certify that I have read the revised version of this thesis prepared under my direction and recommend that it be accepted as fulfilling the thesis requirement.

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Thesis advisor Signature Date

DEDICATION

The thesis is dedicated to my parents and families for their love, endless support and encouragement.

STATEMENT OF THE AUTHOR

I, the under signed, declare that the thesis, hereby submitted by me for degree of master of science in animal production to the school of graduate studies of Addis Ababa University, College of Veterinary Medicine and Agriculture is my own work and I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate. Materials obtained from other sources are duly acknowledged in the thesis.

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ABBREVIATIONS OR ACRONYMS

DNA	DeoxyriboNucleic Acid
GAP	Good Agricultural Practices
GMP	Good Manufacturing Practices
GHP	Good Hygienic Practice
HCC	Hepatocellular Carcinoma
HPLC	High Performance Liquid Chromatography
IAC	Immunoaffinity Clean-up
KAP	Knowledge, Attitude and Practice
KCl	Potassium Chloride
KH_2PO_4	Potassium Dihydrogen Phosphate
LC	Liquid Chromatography
LD50	Median Lethal Dose
UV	Ultra Violate

ABSTRACT

Contamination of feed with aflatoxin (AF) is a major barrier to long-term food safety and security. Ninety-nine commercial feed samples including poultry and dairy compound feed, and "noug cake" were purposively sampled and analyzed using HPLC and NIR, respectively, to determine the AF levels and the nutrient content. The study areas were selected purposively and clustered into three study locations. AF was discovered in more than a quarter (25%) of the feed sample in each of the study locations. Over all the samples, the contamination of AF levels in Addis Ababa and surroundings was alarming, with high positive samples (>80%), prevalence (>60%), and highest individual AF levels reported; the highest levels of TAF (549 µg/kg) and AFB1 (375 µg/kg) found in "noug cake" with all detected samples prevalent are also alarming. AFB1 was found to be prevalent in compound feed intended for layers in Addis Ababa and surroundings, East Shoa, and Southern Ethiopia at 80, 50, and 16.7%; layer growers at 57, 40 and 20%; lactating dairy cows at 57, 33.3, and 37.5%; broiler finishers at 80 and 50%; broiler starters at 100 and 20%; and for "noug cake" at 37.5, 50, and 33.3%, respectively. On the other hand, in Addis Ababa and surroundings or Southern Ethiopia more than a fifth (>20%) of the samples, as well as more than a quarter (25%) of the samples in each of the three study locations, were unfit for the DM and CP standards, respectively. Except for dairy cow compound feed, the mean DM & CP content in feeds fit the Ethiopian standard in each of the study location. No significant difference (p<0.05) in the mean nutrient content, mean AF level and prevalence of AF in feeds was observed among the study locations, except for the mean CF, mean AFB1 and TAF level and prevalence of AFB1 in broiler starter feed; mean TAF level and mean fiber content in layer grower and *mean TAF level in* layer feed*. These variations may be linked to the type, and proportion of ingredients used in feed formulation. Whereas the highest AF level in oilcake may be associated with limited awareness of AF and proper storage. The highest AF level, prevalence, and positive samples in feeds investigated in this study are animal and public health concerns. It could be decided to implement a feed quality and safety control system in the manufacturing plant, proper regulation, training, and more research on AF in feed ingredients are recommended.*

Key word; Aflatoxin B1, Total Aflatoxin, Nutrient content, commercial feeds

1. INTRODUCTION

Feed is the most important product in the global food industry as it provides the accessibility and safety of animal sources to people all over the world. Food safety is the most pressing issues confronting today's rapidly growing population, as safety has remained a basic human need in recent years and contaminated food consumption poses a clear food security threat globally. As a result, in today's rapidly changing world, food safety has emerged as a major focus of international and national efforts (Gbashi *et al.*, 2019). Safe feed is now a major source of concern in developed countries, particularly for producers and governments, as it is a prerequisite to food safety and human health as well as animal welfare and health (Fumagalli *et al*., 2021).

In the favorable environmental conditions, the most dangerous mycotoxins, Aflatoxins (AFs), are produced by *Aspergillus flavus* and *Aspergillu sparasiticus*. They are widespread and invisible in the environment, wreaking havoc on human food safety and security, particularly in Sub-Saharan Africa, and continue to pose a serious threat to the health of domestic animals and humans. It also has an impact on livestock productivity fed contaminated feeds (Owuor *et al*., 2020; Sipos *et al*., 2021). light, pH, moisture, temperature, water, relative humidity, substrate composition, CO_2, O_2, pesticides, fungicides, plant variety, insects, and spore load, all influence the amount of toxins produced (Ditta et al., 2019; Agriopoulou et al., 2020; Firew et al., 2020). However, moisture, relative humidity, and temperature have a significant impact on mold growth and Aflatoxin(s) production (Saad, 2016; Emana et al., 2017; Demissie, 2018). On the other hand, Aflatoxins, to varying degrees, are toxic to various animal species depending on the dose, duration of exposure, species, breed, diet, and nutritional status (Demissie, 2018). Both monogastric and ruminants animals are diversely sensitive to Aflatoxins due to the presence of ruminal microbiota capable of degrading them (Juan et al., 2019). As a result, monogastric mycotoxicosis is more severe than ruminant mycotoxicosis. Furthermore, susceptibility to mycotoxicoses is more for young and pregnant animals than older animals, with poultry and fish being the most vulnerable (Marta and Bedaso, 2016).

The four most abundant types of toxins in the Aflatoxins group are Aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), and G2 (AFG2), Aflatoxin M1 (AFM1) and M2 (AFM2) are the metabolic products derived from Aflatoxins. However, Aflatoxin B1 is considered the most toxic for mammals due to its mutagenic, hepatotoxic, teratogenic, and immunosuppressive properties (Pereira *et al.*, 2019; Komsky-Elbaz *et al*., 2020). Aflatoxin B1 has been linked to a decrease in productivity and an increase in disease incidence in dairy cattle. Even trace levels of AFB1 reduce growth rate, feed efficiency, hatchability, and disease susceptibility in poultry. Moreover, AFB1 were classified as a group one carcinogen by the International Agency for Research on Cancer (IARC) (Rushing and Selim, 2019). In Ethiopia, about 11–288 HCC cases were attributed to chronic Aflatoxin exposure (Firew et al., 2020).

The presence of Aflatoxin contaminated milk in the market is becoming an alarming problem. A recent study conducted on dairy and feed samples collected from the greater Addis Ababa milkshed indicated that all collected milk and feed samples had detectable levels of Aflatoxin and oil seed cake was found to be the source of AFM1 contamination in 93% of samples of cow's milk (Gizachew *et al.*, 2016). After the discovery of high contamination levels of Aflatoxins in oilseed cakes and compound feeds, there are serious concerns about safety and the nutritional quality of feed throughout the food value chain (Merwe *et al*., 2019).

The AFB1 levels of contamination in feeds obtained from various actors along the value chain were strikingly similar, implying that contamination may occur early in the manufacturing and distribution chain (Gizachew *et al.*, 2016; Merwe *et al*., 2019). On the other hand, maintaining the intended quality requirements of compound feeds is a difficulty for commercial feed producers, regulatory authorities, and livestock producers, resulting in livestock owners' unwillingness to utilize such feeds due to a lack of trust in compound feed nutritional quality (Seyoum *et al*., 2018). Furthermore, the nutritional quality and safety of Ethiopia's feed supplies vary greatly depending on type and proportion of feed ingredient used in ration formulation, processing method, season, handling, storage, transportation, and utilization. This necessitates regular evaluation and laboratory analysis in order to generate current information on nutritional qualities and feed safety, which aids in sensitizing feed producers and the institutions in charge of monitoring and regulating safety and quality of feeds in relation to the standards set for the various groups/species of animals in the country (Demissie, 2020). In addition, previous Ethiopian research efforts had paid little attention to feed quality and safety, particularly Aflatoxins (Rehrahie, 2018). As feed is primarily an important component of the food chain, contamination of feed with aflatoxin (AF) is a major barrier to long-term food safety and security, its quality and safety must be evaluated.

The current study, therefore, was undertaken with the following specific objectives:

1) To determine the levels and prevalence of Aflatoxin B1 and Total Aflatoxin in commercial compound feeds and “noug cake” in the study area;

2) To determine the nutrient content of commercial compound feeds and “noug cake” in the study area;

3) To assess the (KAP) status on Aflatoxin among feed processors and oil producers

2. LITERATURE REVIEW

2.1 Mycotoxins and its Feed Safety Concern

Toxins are substances that, when inhaled, ingested, injected, or absorbed, can cause harm to another organism. They are produced by plants, animals, or microorganisms (Owuor *et al.*, 2020). Climate change, specifically changes in temperature and precipitation patterns, has influenced mold persistence and occurrence patterns, and thus mycotoxins, which have become a hot topic in recent years (Kosicki *et al.*, 2016). Mycotoxins are produced by filamentous fungi of the genera *Aspergillus*, *Alternaria*, *Penicillium*, *and Fusarium* (Pereira *et al.*, 2019; Oliveira & Vasconcelos, 2020). Because they aren't required for the fungus to grow and reproduce, they're classified as secondary metabolites (Smith *et al.*, 2016).

However, not all secondary metabolites are toxic, for example, plant growth regulators and pharmaceutically useful compounds (Udovicki *et al.*, 2018; Ráduly *et al.*, 2020; Yegrem, 2020). Only about 30 of the approximately 450 different types of mycotoxins (Benkerroum, 2020) are actually hazardous, and only a few groups of mycotoxins are considered a safety and economic concern in relation to their prevalence in feeds and known effects on livestock health, namely hepatotoxins (Aflatoxin), nefrotoxins (ocratoxin A), cardiotoxin (moniliformine), and neurotoxins (fumonizine B1) (Kosicki *et al.*, 2016; Marta & Bedaso, 2016).

Mycotoxins can be found in raw materials or feed even when molds are no longer present (Munoz-Solano & Gonzalez-Penas, 2020), and their contamination poses a unique challenge to food safety due to their unavoidable and unpredictable nature (Aboagye-Nuamah *et al.*, 2021). As a result, mycotoxins are the most serious threats to modern feedstuff manufacturing (Peng *et al.*, 2018) and a worldwide safety concern (Munoz-Solano & Gonzalez-Penas, 2020). Climate change, poor agricultural practices, a lack of awareness, and pre- and post-harvest management have all been identified as major contributors to mycotoxins' significant impact in Africa (Firew *et al.*, 2020).

2.2 Aflatoxin History, Property and Production

Aflatoxins are the most important naturally occurring Mycotoxins and have received a lot of attention because they frequently contaminate feed and food commodities in Africa (Gbashi *et al.*, 2019). They are rampant and invisible in the environment, wreaking havoc on food safety and security, particularly in Sub-Saharan Africa (Owuor *et al.*, 2020), and continue to pose a serious threat to the health of both domestic animals and humans (Demissie, 2018; Ismail *et al.*, 2018; Sipos *et al.*, 2021). Also it has an effect on the productivity of livestock fed contaminated feed (Nishimwe *et al.*, 2019). The origin of Aflatoxin, forms of Aflatoxin, its property and production condition are discussed below.

2.2.1 Discovery of Aflatoxins

In the year 2022, it had been 62 years since Aflatoxins were discovered following a "Turkey X disease" outbreak in England in 1960, when over 100,000 turkeys became ill and died (Smith *et al.*, 2016; Oliveira & Vasconcelos, 2020). The turkeys died within a few weeks/days after showing signs of severe poisoning. Many of these turkeys were being fed groundnut meal from Brazil, which had previously been found to be extremely toxic when fed to poultry in feeding trials. A thorough investigation revealed that a mold species known as *Aspergillus flavus* was involved, and that the hepatotoxic metabolites of this mold, which were also detected as components in the toxic groundnut meal, were known as Aflatoxin (Blount, 1961; Pickova *et al.*, 2021).

According to its definition, Aflatoxin encompasses three words: "A" from the genus *Aspergillus*, "fla" from flavis species, and toxin, which means poison (Naveed *et al.*, 2022). As a result, several Aspergillus species, including *A. flavus* and *A. parasiticus*, are the primary producers of Aflatoxins; however, despite their similar geographical ranges, the latter is less common, while the former is the most commonly reported fungus in foods (Yegrem, 2020).

2.2.2 *Conditions for Aflatoxin contamination*

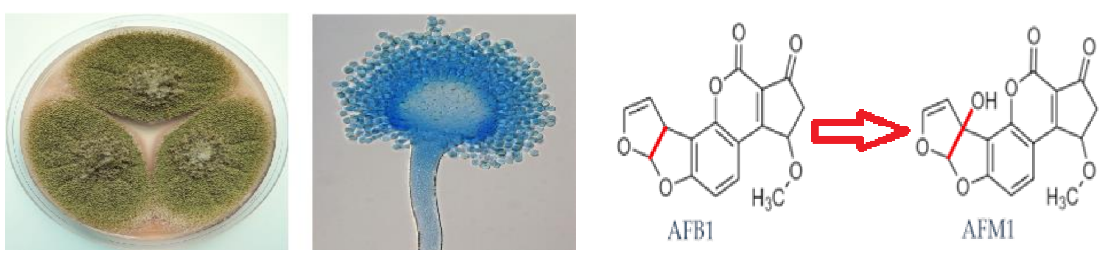
As compared to fungal growth, Aflatoxin production occurs under a more restricted set of conditions (Mannaa & Kim, 2017). Physical factors (pH, light, moisture, temperature, water, relative humidity, and atmospheric gases), chemical factors (substrate composition, CO_2, O_2, pesticides, and fungicides), and biological factors (plant variety, insects, and spore load) all influence the amount of toxins produced (Ditta et al., 2019; Agriopoulou *et al.*, 2020; Firew *et al.*, 2020). However, moisture, relative humidity, and temperature have a significant impact on mold growth and Aflatoxin(s) production (Saad, 2016; Emana *et al.*, 2017; Demissie, 2018). To grow, fungi prefer a pH of 8.4 and a water activity of 0.70 (Agriopoulou *et al.*, 2020 and Firew *et al.*, 2020). Aflatoxin-producing fungi can grow 1.7–9, but 3–7 pH range is ideal. The production of fungal and aflatoxins was promoted at pH > 3 and 6 >, whereas pH of > 1 and 3 > inhibits fungal growth (Eshelli et al., 2015; Kumar et al., 2021). The A. flavus mold can grow at 10 to 43 ° C, and produce Aflatoxins at 15 to 370 ° C temperatures range; and the water activity ranges from 0.78 to 0.84 and above 0.84, respectively (Spanjer, 2018; Agriopoulou *et al.*, 2020; Firew *et al.*, 2020; Yegrem, 2020). Aflatoxin production is higher between 20–30 ^{0}C than at higher or lower temperatures, and A. flavus mold grows best around 30 ^{0}C.

Mold growth generally slows as the moisture and temperature content of the food or feed decrease. *Aspergillus* species infects improperly dried stored food regularly in locations with temperatures ranging from 24 to 35°C and a moisture content greater than 7% (Ditta et al., 2019). *Aspergillus flavus* has relatively high moisture requirements (16–30%) among storage fungi (Agriopoulou *et al.*, 2020; Firew *et al.*, 2020). Aflatoxins can be produced in the field in grain at temperatures ranging from 20 to 40 °C, with humidity levels ranging from 10 to 20% and relative humidity levels ranging from 70–90% (Marta and Bedaso 2016; Agriopoulou *et al.*, 2020). Most feedstuffs with a moisture content above 13% are susceptible to mold growth and the formation of Aflatoxins (Owuor *et al.*, 2020), indicating that Aflatoxins are more common in hot and humid climates. Fungi growth and then Aflatoxin production are both affected by light.

According to Rushing & Selim (2019), in the dark, the production of Aflatoxin is increased, whereas, in the light, it is inhibited. A higher CO_2 level and a lower O_2 level inhibit Aflatoxin production and fungal growth, according to Mahbobinejhad *et al.* (2019), indicating that the availability of O_2 and CO_2 affects Aflatoxin production. Plant insect wounds and competitive weeds both cause plant stress; the latter has been linked to Aflatoxin production, while the former serves as a breeding ground for aflatoxigenic fungi (Kinyungu, 2019). When it comes to fungi varieties, *A. flavus* produces fewer Aflatoxins than *A.parasiticus* (Manjunatha *et al.*, 2018).

2.2.3 Properties of Aflatoxins

Aflatoxins are soluble in acetone, methanol, chloroform, and particularly dimethyl sulfoxide, very slightly soluble in hydrocarbons and water (10–30 g/mL) and insoluble in non-polar solvents. In light and air, they are relatively unstable, particularly in polar solvents or when exposed to oxidizing agents, UV radiation, or solutions with a PH less than 3 or greater than 10 (Vijaya Kumar, 2018; Ditta *et al.*, 2019; Yegrem, 2020). Because they are odorless, tasteless, and colorless (difficult to detect sensorically) posing a significant challenge to food handlers, consumers, and regulators attempting to control or eradicate them (Yegrem, 2020). Aflatoxins are found as colorless to pale yellow crystals or white powder (Pickova *et al.*, 2021).



Picture 1: A.flavus (left); AFB1 and M1 (right)

Source: Yegrem (2020); Pickova *et al.* (2021)

Aflatoxins have high thermal stability in the dry state up to the point of melting (Table 1 shows the melting points of Aflatoxins), preventing them from being completely thermally degraded during human food processing (Ismail *et al.*, 2018). As a result, regular cooking does not remove Aflatoxins, and they are resistant to many common food and feed processing treatments, such as pasteurization, sterilization, and other thermal treatments. Aflatoxins, on the other hand, are removed to some extent when exposed to moisture and high temperatures for an extended period of time, as found in oil seeds, meals, and roasted peanuts, and in aqueous solutions at PH–7 (Owuor *et al.*, 2020). According to Falade, (2019), washed grains may no longer have observable mold growth after being infected with Aflatoxins, but they still contain Aflatoxins, because Aflatoxin is only slightly water soluble.

Table 1: Physicochemical property of Aflatoxins (Vankayalapati et al., 2018)

			Fluorescence			
Aflatoxins	MW (g/mol)	Melting point (^oc)	Under UV Light 360nm	Excitation Wave length (nm)	Spectrum Emission Wavelength (nm)	**Source**
AFB1	312	268-269	Blue	365	425	Rehrahie (2018);
AFB2	314	286-289	Blue	365	425	Vankayalapati et al.(2018):
AFG1	328	244-246	Green	365	450	Benkerroum (2019) and
AFG2	330	237-240	Green-Blue	365	450	(2020)

2.2.4 Types of Aflatoxins

Despite the fact that there are approximately 20 different identified forms of Aflatoxins, only Aflatoxin B1, B2, G1, G2, M2 and M1 are frequently found in foods, and only four of them (B and G) have significant and economic importance, may naturally contaminate animal feeding stuffs, and have been confirmed to be dangerous to humans and livestock

(Smith *et al.*, 2016). When the Aflatoxins were exposed to UV, fluorescent colors of green and blue were produced and represented by "G" and "B" letters respectively, where the minor and major compounds were represented by subscript numbers 2 and 1, respectively (Vijaya Kumar, 2018; Ditta *et al.*, 2019; Owuor *et al.*, 2020; Yegrem, 2020). Aflatoxin B are produced by *A. flavus*, whereas *A. parasiticus* produces Aflatoxin B and G (Ditta et al., 2019). Aflatoxin M2 and M1 are metabolic products of contaminated food or feed that mainly occur in milk (although small amounts of AFM1 have been reported in eggs) as AFB2 and AFB1 metabolites, respectively (Buszewska-Forajta, 2020). Aflatoxin G1, G2, B1 and B2 are thus found in plant-based foods, whereas Aflatoxin M2 and M1are found in animal-based foods.

2.2.5 Aflatoxins B1

AFB1, the most common carcinogenic, teratogenic, and mutagenic toxin among the four Aflatoxins (Buszewska-Forajta, 2020), is the most widely distributed toxin. Animal feed can be contaminated with the fungi *A.flavus* and *A.parasiticus* and produce AFB1 when it is stored at high temperatures and humidity improperly (Zhao *et al.*, 2017). AFB1 is widely distributed in crops with high lipid and starch content and in formulated feed, some of them are used as the primary raw material (e.g., peanuts, beans maize, oily seeds, wheat, and soybeans) (Yang *et al.*, 2020). It has been directly linked to negative health effects, such as liver cancer, and has been classified as a human class I carcinogen (Liu & Wang, 2016). Aflatoxin B1, after ingesting, is quickly absorbed by the gastrointestinal tract, along with other Aflatoxins, and transported to the liver by the circulatory system. Aflatoxins bind irreversibly to DNA and proteins bases in about 1–3 % of those consumed. Aflatoxin B1, G1, B2, and G2 are used in descending order of acute and chronic toxicity potency. Aflatoxin B1 is metabolized to produce more toxic metabolites and its subsequent metabolism determines both chronic and acute toxicity; although it is not toxic in and of itself (Owuor *et al.*, 2020).

2.3 Aflatoxins in feeds

The ingredients and nutritional quality of the feed influence fungi's ability to use their genetic machinery to produce Aflatoxins within such a substrate and thus feed is particularly vulnerable to Aflatoxins contamination (Granados-Chinchilla, 2017). The Aflatoxins contaminations in compound feed and oilseed cake is discussed below.

2.3.1 Aflatoxins in compound feeds

As previously stated, raw materials used in compound feed include “noug cake”, pea hulls, and maize grain, all of which are common sources of Aflatoxins in animal feed (Yegrem, 2020). Even at low concentrations, Aflatoxins present in feed ingredients will contaminate the compounded feed as a whole and posing animals health risk; it is well known that Aflatoxins are not destroyed at high temperature feed manufacturing processes, during the storage and milling (Pereira *et al.*, 2019; Nakavuma *et al.*, 2020). Aflatoxins can be formed before, during, and after feed manufacturing due to improper bulk handling and poor storage conditions, as well as farm feed storage and on-farm feeding systems (Bryden, 2012). As a result, safe ingredients are necessary for the production of safe feed, which is necessary for animal health, the production of safe livestock products for human consumption, and the preservation of the environment. To ensure the security of the agro food chain, feed mills are required to test products and all the raw materials used for identification of possible potential contaminants (Colović *et al.*, 2019).

Aflatoxins contamination of Dairy cow feed in tropical areas such as Sub-Saharan Africa is common and extremely high exceeding European Union (EU), and American Food and Drug Administration (FDA) allowable levels (Kemboi *et al.,* 2020). Aflatoxins levels in Rwanda exceeded EU and FDA limits for dairy and poultry at 108.83µg/kg (dairy farms), 88.64µg/kg (feed vendors), and 94.95µg/kg (feed processors) (Nishimwe *et al.*, 2019). The Aflatoxins contamination of newly manufactured feed and being fed to chickens was 52% and 91 % respectively (Bryden, 2012), indicating feed can be contaminated throughout the feed value chain. Gruber-Dorninger *et al.* (2019) compared the global mycotoxins occurrence in different commodities, including finished feed and feed ingredients, and concluded that finished feed, as a blend of raw materials, had a comparatively high prevalence of all mycotoxins. A study in Japan also indicated the average AFB1 level of contamination in formula feed, 1.6 μg/kg , which was half that of corn (mean 3.9 μg/kg), indicating that contamination of corn contributed to the AFB1 contamination found in formula feed (Nomura *et al.*, 2018).

2.3.2 Aflatoxins in oilseed cake

Oilseed cake (e.g., noug and linseed cake) is primarily used as feed in Ethiopia, and it is highly susceptible to Aflatoxins contamination. Aflatoxins are commonly found in oilseeds, and while the oil may be safe after extraction, the residual seedcake will contain a high concentration of Aflatoxins, which are commonly used as feed ingredients (Stepman, 2018). Concentrated animal feedstuffs harbor the growth of Aflatoxins and can be transferred to animal-sourced food, which is a major source of human exposure (Yegrem, 2020). Agro-industrial by-products, such as wheat bran (100 %), “noug cake” (73 %), pea hulls (37 %), maize grain (12 %), and brewer's dry yeast, are the most common concentrates used every day to feed cattle of all ages in Ethiopia's dairy farms (Gizachew *et al.*, 2016). Concentrated feedstuffs have the highest level of Aflatoxins, according to Marta and Bedaso (2016), with the highest level of Aflatoxin B1 contamination measured at 419 μg/kg in concentrate feeds like “noug cake” and the lowest level of Aflatoxin B1 contamination measured at (7 μg/kg) in silage feed (roughages). The primary source of Aflatoxins contamination in those concentrated feeds, according to (Gizachew et al., 2016), was “noug cake”, with concentrations ranging from 290 to 397 mg/kg AFB1.

2.4 Impacts of Aflatoxins

2.4.1 Health impact of Aflatoxin

Animal diseases and health problems that reduce the production of animal-derived products have a significant impact on global economics and trade. The other important reason to maintain animal health is to protect the health of consumers (Munoz-Solano & Gonzalez-Penas, 2020). Subclinical Aflatoxins exposure in animals usually results in anemia, cancer, poor growth and development, impaired blood clotting, bruising sensitivity, damage to the organs (eg. liver) and, immune responsiveness decreased, increased vulnerability to viral, bacteria, or parasitic infections, reduced feed consumption, lost productivity (drop in milk and egg production), and increased mortality (Gizachew et al., 2016; WHO, 2018; Juan *et al.*, 2019; Benkerroum, 2020). In the literature outbreaks of Aflatoxicosis were primarily reported in poultry, cattle pigs, equine and sheep, even though they occur in any livestock (Peles *et al.*, 2019). Aflatoxins effects in chickens include inferior eggshell quality, decreased egg production, decreased reproductive and productivity efficiency, inferior carcass quality, liver damage and increased disease susceptibility. Reduced weight gain, liver and kidney damage, and decreased milk production were observed in cattle (WHO, 2018). For poultry, the AFM1 median lethal dose (LD50) is 0.3–0.6 mg/kg BW, 2–5 mg/kg BW, and 0.5–1.0 ppm BW for sheep and calves respectively (Jiang *et al.*, 2021). Animal health is therefore a major challenge worldwide and must be protected, especially through adequate nutrition.

Aflatoxins can occur and expose consumers to contamination risks, either directly or indirectly, through food consumption or feed (Agriopoulou *et al.*, 2020). Aflatoxins may be carried over into livestock products, such as meat, eggs, and milk, thereby compromising the safety of human consumers (Demissie, 2018; Gruber-Dorninger *et al.*, 2019). Aflatoxicosis is any disease caused by the consumption of Aflatoxin-contaminated foods and feeds with effects on various organs of the human body that can potentially lead to death. Aflatoxicosis can be acute or chronic (Peles *et al.*, 2019; Agriopoulou *et al.*, 2020). Chronic Aflatoxins exposure is carcinogenic and all organ systems can be affected, particularly the kidneys and liver; it causes liver cancer and has been linked to cancer of other type. Aflatoxins can also cause occupational lung and skin cancer through the respiratory system (inhalation) and the skin (Benkerroum, 2020). Chronic Aflatoxins exposure has been linked to 28.0% of all HCC cases worldwide, with Africa accounting for 40.0%. In central Tanzania (2016) and eastern Kenya (2004), 145 of 385 people with acute aflatoxicosis died (Oliveira and Vasconcelos, 2020). In developing countries 5 billion people and more are exposed to the risk of chronic Aflatoxins from contaminated foods (Liu and Wu, 2016).

2.4.2 Impact of Aflatoxins on livestock production

The dairy industry has major economic, food security, and safety challenges due to Aflatoxins contamination of feed. The direct market costs associated with reduced revenues or lost trade due to the rejection of contaminated animal products, as well as reduced productivity, animal death, particularly more sensitive animal (eg. Calves), and increased treatment and Aflatoxins mitigation costs, all have an economic impact (Kemboi *et al.*, 2020). For several months, a dairy herd exposed to 120 µg/kg Aflatoxins contaminated feed experienced severe health problems as well as a 28% lower in milk production (Nishimwe *et al.*, 2019). All dairy industry participants, including feed manufacturers, dairy farmers, milk processors, and consumers, are affected (Kemboi *et al.*, 2020). The highly nutritious food, Milk, contains both macro-and micronutrients that are required for human development, growth and maintenance. Aflatoxin M1, in milk as well as dairy products, has been a major concern worldwide for the last years, particularly in developing countries. A significant AFM1 level in milk may be resulted when animals are fed highly contaminated feedstuffs (Iqbal *et al.*, 2016). After mammals consume contaminated feeds, AFB1 can be transferred to its milk metabolite, Aflatoxin M1 (Jiang *et al.*, 2021). AFM1 is excreted and found in milk within 12 hours (Demissie, 2018; Sipos *et al.*, 2021), with a rate ranging from 0.3 to 6.2 % between the amount of AFB1 ingested by cows and the amount excreted in milk (Gizachew et al., 2016; Demissie, 2018; Nishimwe *et al.*, 2019; Yegrem, 2020).

Aflatoxins are important in the industry because of the economic losses associated with contaminated feeds, particularly in the poultry industry. Aflatoxin M in poultry accumulates in the liver breast, and gizzard, eggs and other organs (Yang *et al.*, 2020). AFB1 even in Small amounts reduces feed efficiency, growth rate, hatchability, and disease susceptibility in poultry, which are likely the most vulnerable animals to its toxic effects (John & Dhanapal, 2009). Decreased egg production, reduced growth rate and feed efficiency, decreased hatchability and increased susceptibility to disease are all negative effects of Aflatoxins. Poultry fed diets containing an average AFs concentration of 0.95 ppm had lower feed intake, an 11% reduction in daily weight gain, and a 6% reduction in feed conversion. Over a 28-day period, hens fed contaminated feed containing more than 3300 mg/kg AFB1 laid contaminated eggs (Bhat *et al.*, 2010).

2.4.3 Economic impact of Aflatoxins

In livestock production, unsafe feed can result significant economic losses (Dewa & Tikau, 2019), including mortality as well as decreases in productivity, weight gain, feed efficiency, fertility, disease resistance, and the quantity and quality of milk, meat, and egg production . Furthermore, because of the causal relation that exist between Aflatoxins and their impact on health, the high cost of regulatory activities and research aimed at reducing health risks should not be overlooked (Gbashi *et al.*, 2019). Aflatoxin-induced food poisoning, on the other hand, incurs pharmaceutical and health-care costs (Demissie, 2018). Even if Aflatoxins contamination had no economic effects in Ethiopia's domestic market because price differentiation based on Aflatoxins concentration were not existed (Admasu & Tesafa, 2018), Aflatoxins contamination reduces farmer incomes in emerging economies due to contaminated products low market value, high-value markets exclusion and production rejection (Nishimwe *et al.*, 2019).

2.5 Preventing and Mitigating of Aflatoxins

According to Ephrem *et al.* (2015), in Ethiopia, public awareness of molds and Aflatoxins were limited, and many Ethiopians are unaware of the dangers of eating moldy foods. Traders and farmers in Ethiopia are generally unaware of the long-term health risks of Aflatoxins exposure because they only affect food organoleptic qualities, which can be easily corrected through home cooking, drying, or fermenting (Firew *et al.*, 2020). Due to economic considerations and/or a lack of information, it is obvious that smallholders in the animal husbandry industry do not prioritize feed safety. As a result, the first step toward Aflatoxins elimination is for feed handlers to be aware of the contamination of Aflatoxins in feed, feed ingredient and food items, as well as the financial and health hazards they bring (Nishimwe *et al.*, 2019).

Concern for animal feed must extend beyond economic and nutritional value to include food safety, in light of massive economic losses as well as livestock and public health protection (Demissie, 2018;Yegrem, 2020). As a result, technological solutions, effective regulations and standards, and a conducive environment may result in Aflatoxins mitigation and control on the continent (Rehrahie, 2018). Then, regular monitoring of Aflatoxins contamination in feeds should be performed in order to implement proper mitigation strategies that minimize the negative effects of these Aflatoxins and ensure safer derived animal products for consumers (Juan *et al.*, 2019).

Table 1: Pre/post harvest systems for management of Aflatoxins

Aflatoxins						
Pre-harvest				Post-harvest		
Agronomic system	Biological system	Chemical system	Storage management	Biological system	Physical system	Chemical system
Planting harvesting time Crop rotation Tillage Irrigation and fertilization	Fungal antagonists	Fungicides Insecticides	Temperature Humidity O_2,co_2 and N_2	Bacteria Yeasts Moulds	Dryings Separation washing Themal inactivation Irradiation UV and plasma	Bases Oxidizing agents (Ozone) Organic acids

Source: Conte *et al.* (2020)

Furthermore, a proper understanding of the fungi involved and the toxins types they produce is essential for developing mitigation techniques for preventing or lowering Aflatoxins contamination in feeds (Aboagye-Nuamah *et al.*, 2021). Good Manufacturing Practice (GMP), Hazard Analysis and Critical Control Points (HACCP) and Good Agricultural Practice (GAP) should guide each technical process intended for feed/food production and storage to assure food safety and consumer health (Pleadin *et al.*, 2019).

2.6 Quality Feeds in Livestock Production

2.6.1 Importance of quality feed

Regardless of species or production system, feed is the most important variable cost component in animal production (Martin et al., 2018; Dewa & Tikau, 2019), accounting for up to 70% of total animal production costs (Dewa & Tikau, 2019). Feed has also an impact on food, health, productivity, profitability, environmental effects and nutrition security (Makkar, 2016). One strategy for lowering the aforementioned costs is to feed the animals high-quality feed, which increases producer revenue (Kırkpınar & Açıkgöz, 2018). Animals can reach their genetic potential if fed a high-quality ration in sufficient quantities (Martin et al., 2018). Finding a high-quality feed that meets the nutritional needs of livestock based on their age, gender, and physiological stage is the livestock industry's most visible challenges. Even though sufficient nutrients are required for any animal's metabolic function and health, any nutrient deficiency in livestock feed will have an impact on animal growth. (Ogbebor et al., 2021). Poor quality feeds can result in nutritional illnesses, decreased animal production and productivity, poor quality animal products, health hazards to animals and humans, effects on export earnings, and a drop in producer and national income (Kırkpınar & Açıkgöz, 2018).

As a result, one of the goals of any feeding program is to achieve an optimal balance among available feed ingredients so that the total ration nutrient composition meets the animal's or animals' daily nutrient requirements. To pull off such a feat on a daily basis, some information on the nutrient content of feed items is required. Reliable nutritional data is critical for not just balancing rations but also allowing ration balancing programs to determine the most cost-effective ration that meets the animals' demands; the strongest predictor of nutrient availability is laboratory analysis of feeds.

2.6.2 Quality of Compound feeds

Compound feed is a complex industrial mix of blended raw materials primarily derived from cereals, milling byproducts, and oil cakes (Kemboi et al., 2020). Animal protein sources, as well as additives, are also included (Akinmusire et al., 2019; Ogbebor et al., 2021). However, feed processor, in Ethiopia; pay little attention to the quality of thus feed ingredient they use in compound feed (Getachew et al., 2016). Compound feed is designed and manufactured to meet the specific needs of the animal's age and metabolism (Juan et al., 2019). Feed for poultry and cattle is expected to contain specific nutrients such as protein, carbohydrates, fat, minerals, and vitamins in the proper proportions for different stages of growth (Ubiebi, 2017; Ofori et al., 2019). As a result, understanding animal nutritional needs, particularly in terms of protein, fat, and energy, is critical for developing a high-quality compound feed and allowing animals to express their full genetic potential (Martin et al., 2018; Chapter, 2021). As shown in Table 3, the Ethiopian Sstandard Agency established nutrient content (quality) standards for compound feeds aimed at various livestock species and age groups.

In Ethiopia, majority of feed manufacturers are operating below their designated production capacities (short supply of compound feed in Ethiopia) due to a variety of factors including a lack of raw material supply, a lack of commercial orientation among farmers, limited awareness among livestock producers about the use of processed feeds, and a lack of tax exemptions (Seyoum et al., 2018). Moreover, feed manufacturers are one of the most important responsible organizations for ensuring that their feed is fit for its intended use, meets the requirements of the feed products control authority, and does not endanger animals or animal product consumers due to poor safety and quality (Getachew et al., 2016).

2.6.3 Quality of oilseed cakes

The oil processing industry produces a huge amount of oilseed cakes which are high in protein and can be used as valuable livestock feed (Sarkar et al., 2021) and include noug seed cake, cottonseed cake, linseed cake, peanut seed cake, and cakes obtained from other oilseeds (Stepman, 2018). Oilseed cakes contain 20-50 % protein and 0-12 % fat depending on the type of oilseed and method of extraction, but are low in essential amino acids cystine and methionine and have variable and low lysine content. They serve as a protein supplement in concentrate mixtures. Oilseed cakes can also provide significant amounts of energy (2.03 to 3.7 Mcal ME/kg). The protein, fat, and energy content of "noug cake" ranges from 28 - 38 %, 2.1- 12.6% and 2.37 Mcal ME/kg DM respectively. It has high fiber content (34.4% NDF and 8.4% lignin) and a low digestibility of 61.76% when compared to most other oilseed cakes. Cottonseed cake, groundnut cake, and linseed cake have more than 36%, 50%, and about 30% protein content, respectively (Adugna, 2008). The Ethiopian nutrient content standards of oilseed cakes, which are used as feed ingredients for animal feed, are shown in Table 3.

2.7 Ethiopian Feed Standards and Regulation

The confidence of consumer in the safety of the entire food supply chain is critical, and it can only be achieved through strong national standards and regulatory frameworks. National standard bodies, regulatory institutions, and key stakeholders are expected to provide unified leadership for the feed industry in order to contribute to the long-term supply of safe, healthy feed and food of animal origin (VDFACA, 2019).

2.7.1 Feed safety and quality standards in Ethiopia

Various international organizations, including the European Union (EU), CODEX alimentariuos, International Standard Organization (ISO, FAO/WHO, the Food and Drug Administration (FDA), and the International Dairy Federation, can set international standards and maximum limits on Aflatoxins levels to ensure foods and feeds safety and quality (Rehrahie, 2018). As shown in table 4, in the United States, for example, the FDA maximum limits for Aflatoxin B1 in oilseed cakes offered to dairy cattle, and compound feed for breeding cattle and finishing beef cattle were 20 µg/kg , 100 µg/kg , and 300 µg/kg , respectively. Also the European Commission established maximum level for AFB1 in all feedstuffs is 20 µg/kg , 10 µg/kg in complete feeds, and 5 µg/kg in dairy cow feeds (Alvarado et al., 2017). In Ethiopia, the Ethiopian Standard Agency, in collaboration with the Veterinary Drug and Animal Feed Administration and Control Authority (VDFACA), established the nutrient content (table 3) and maximum acceptable/residue Aflatoxins level of feed (Table 2) for each livestock category in 2019. As a result, 22 national feed standards have been updated, and 10 new standards have been developed (VDFACA, 2019). The standards included minimum and/or maximum nutritional requirements as well as maximum permitted contaminant levels for compound feed and feed ingredients. Feed quality standards are also voluntary, whereas feed safety standards are required due to the potentially serious consequences to human health and well-being (VDFACA, 2019).

Taking this into account, the maximum acceptable Aflatoxins level of compound feed for chicken(layer), starter(≤8weeks), layer(>20weeks), chicken(broiler), starter(≤4weeks) was 10µg/kg; compound feed for lactating dairy cows, pregnant dairy cows, dairy heifer, and "noug cake" used as a feed ingredient was 20 µg/kg, while it was 50 µg/kg in fattening cattle compound feed and for linseed cake, rapeseed cake, safflower cake, sunflower cake, and sesame cake as feed ingredients (Table 2) (ESA, 2019). As a result of mycotoxins regulation in human food, compounds not suitable for human consumption may be used in feed preparation in some cases (Munoz-Solano & Gonzalez-Penas, 2020).

Table 2: Aflatoxins limitations in Ethiopian standard (µg/kg)

no	Feed type	AFB1	TAF	Source
1	Compound feed for chicken(layer) (starter(≤8weeks), layer(>20weeks)) , chicken(broiler) (starter(≤4weeks)	10	20	ES6406;2019ES1045;2019ES1041;2019ES1040;2019ES1039;2019ES1038;2019ES1037;2019ES6405;2019ES6403;2019ES1027;2019
2	Compound feed for lactating dairy cows and pregnant, dairy heifer; chicken (layer) grower (8-20weeks), chicken (broiler) finisher (>4weeks), chicken(broiler and layer breeder); Noug cake (meal) as feed ingredient	20	40	
3	Compound feed for fattening cattle; Linseed cake (meal), Rapeseed cake (meal) and Soybean cake (meal) as feed ingredient	50	100	
4	Cotton Seed cake and Groundnut cake (meal) as feed ingredient	100	200	

Feed specification	Feed intended for	Dry matter	Crude Protein	CF/ether % by mass	crude fiber	Total ash	source
compound chicken feed(layer)	starter(≤ 8 weeks)	88	19,min	2,min	7,max		ES1027;ES6403;ES6405;ES6406;ES1037;ES1038; ES1039;ES1040;ES1041;ES1045;2019
	grower(8-20weeks)	88	15,min	2,min	9,max		
	layer (>20weeks)	88	16.5,min	2,min	9,max		
compound chicken feed(broiler)	starter(≤ 4 weeks)	88	20,min	2,min	5,max	8,max	
	finisher(>4weeks)	88	18,min	2,min	6,max	9,max	
compound chicken feed(breeder)	broiler breeder	88	16,min	2.5,min	9,max		
	layer breeder	88	17,min	2,min	9,max		
compound feed for lactating dairy cow		89	17,min	10,max	15,max		
compound feed for dairy heifer		89	16,min	10,max	12,max		
compound feed for fattening cattle		89	14,min	10,max	11,max		
cottonseed cake (meal)	use as animal feed ingredient	90	39,min	5,min	15,max		
groundnut cake (meal)		90	30,max	10,min	20,max	7,max	
linseed cake (meal)		90	24,min	6,min	10,max		
Noug cake (meal)		90	23,min	6,min	20,max	10,ma	
rapeseed cake (meal)		90	25,min	7,min	9,max	10,ma	
soya bean cake (meal)		90	36,min	6,min	9,max		

Table 3: Nutrient content standards of feed in Ethiopia

Table 4: International Aflatoxins limitations

no	Feed type	AFB1
	European Union Aflatoxins maximum limitations (µg/kg)	
1	Complementary feedstuffs for cattle (except dairy); Feed (exceptions below)	50
2	Groundnuts, cottonseed, and products derived from processing thereof; Complete feedstuff for poultry	20
3	Complete dairy feed	5
4	Complete feed for calves ; Other Complementary feedstuffs	10
5	Complementary feedstuff for poultry (except for young animals)	30
	Food and Drug Authority Aflatoxins maximum limitation (µg/kg)	
1	Peanut products for fattening of Beef cattle and Cottonseed meal for Beef cattle and poultry	300
2	products of Peanut for Breeding beef cattle and mature poultry	100
3	All feed and feed ingredient for Dairy animals; feed excluding cottonseed meal for Immature animals	20

Source: Alvarado et al., (2017)

2.7.2 *Feed Safety and Quality Regulations in Ethiopia*

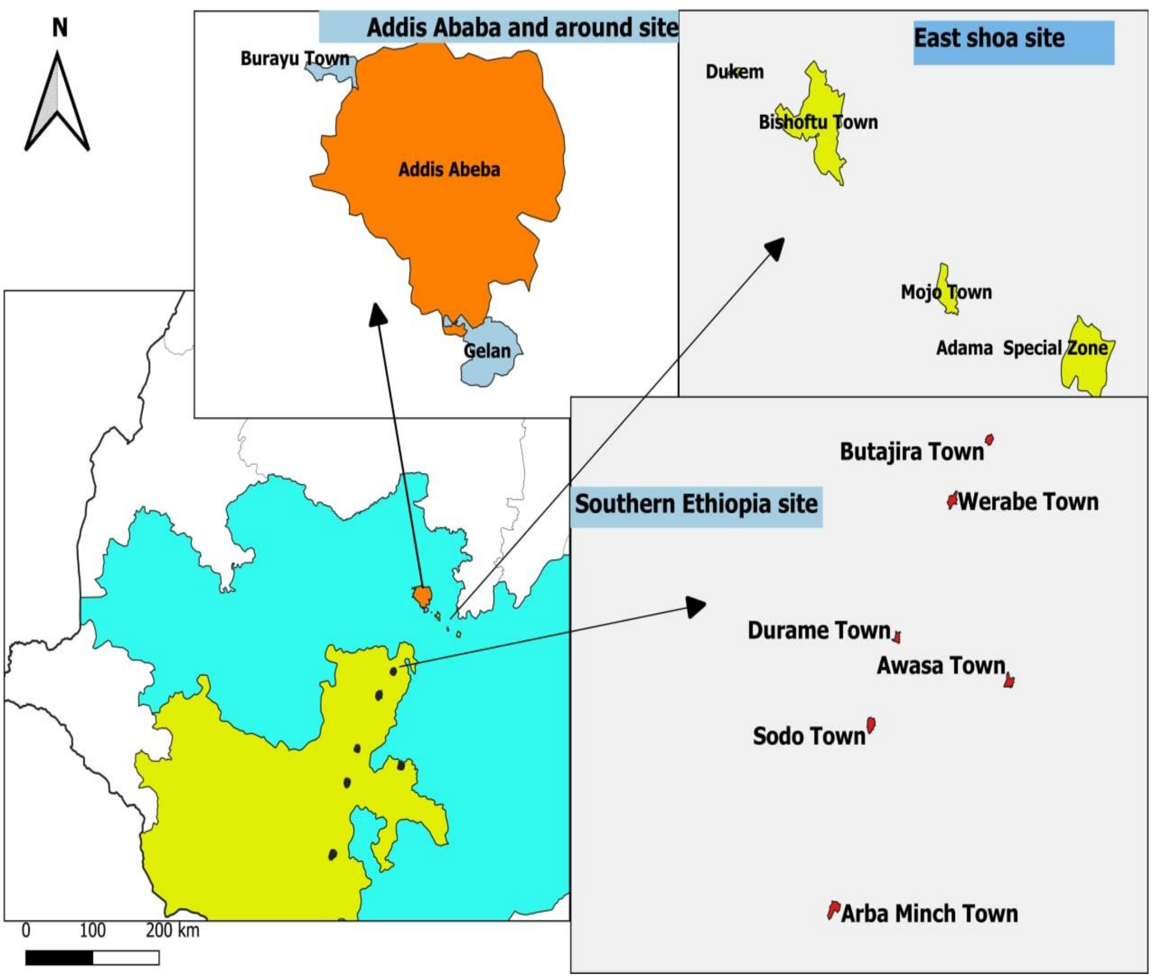
Setting feed safety and quality standards, as previously stated, is not enough of a guarantee; it also necessitates a thorough monitoring and regulation system to reduce potential hazards. Regulatory programs are needed to monitor compliance of the produces with requirements through planned inspections activities and sampling. Current concerns about animal product safety that enter in the food chain must be reflected in an effective regulatory program, while producers and livestock must be protected (VDFACA, 2019). The starting points for ensuring feed safety and quality in Ethiopia are Proclamation No. 728/2011 and Council of Minister Regulation No. 299/2013, where VDFACA was established as the regulatory authority responsible for feed administration and control. However, with Proclamation No. 1263/2021, their rights and obligations are hereby transferred to Ethiopian Agriculture Authority, which is hereby established as an autonomous the Ethiopian federal government organ with its own legal personality.

In 2018, the Authority implemented the guideline prepared for registration and certification of feed/feed ingredient producers, wholesalers, and exporters as the revised Directive number 02/2018. The directive clearly identified issues pertaining to feed safety and quality, as well as appropriate legislative measures. The current numbers for registered and certified feed manufacturers, feed ingredient importers, feed ingredient manufacturers, and feed wholesalers have been updated to 42, 39, 4 and 11 respectively, in accordance with this directive. However, feed safety and quality regulation in Ethiopia is in its early stages and faces several challenges, including a lack of active collaboration among key stakeholders and a weak Ethiopian Animal Feed Industry Association (EAFIA) that can play a key role in feed industry self-regulation (VDFACA, 2019), lack of awareness among major actors in the feed value chain about safety and quality standards, lack of willingness to self-regulate (Getachew *et al.*, 2016), and limited credible laboratories to control the safety and nutrient quality of animal feed through reliable and rapid testing (Seyoum *et al.*, 2018).

3. MATERIALS AND METHODS

3.1 The Study Areas

The study was carried out in Addis Ababa, in the Oromiya special zone towns: "Gelan" and "Bureau", in the East Shoa Zone of the Oromiya region towns: "Dukem," "Bishoftu," "Mojo," and "Adama", in the SNNP region towns: "Sodo," "Arbaminch," "Butajira," "Worabe," and "Durame" and the Sidama region town: "Hawassa". Table 5 and Picture 2 below summarize the study area's climatic characteristics and its map.



Picture 2: Map of the study area

Table 5: Climatic condition of the study area

Study location	Study areas	Temperature (oc)	Relative humidity (%)	latitude (N)	Longitude (E)	Altitude (m.a.s.l)	Location From Addis Ababa (km)
AAS	Addis Ababa	9-23	62	9° 1′ 48″	38° 44′ 24″	2355	-
	Bishoftu	10.9-27	60	8◦45′	38◦59′	1920	47.9 south-east
ES	Modjo	8.5–30.8	57.5	8.36°	39.7°	1777	73 South East
	Adama	18-32	50.5	8° 32′ 24″	39° 16′ 12″	1712	99 South East
SE	Hawassa	23-27	60	7° 3′ 0″	38° 28′ 0″	1708	273south

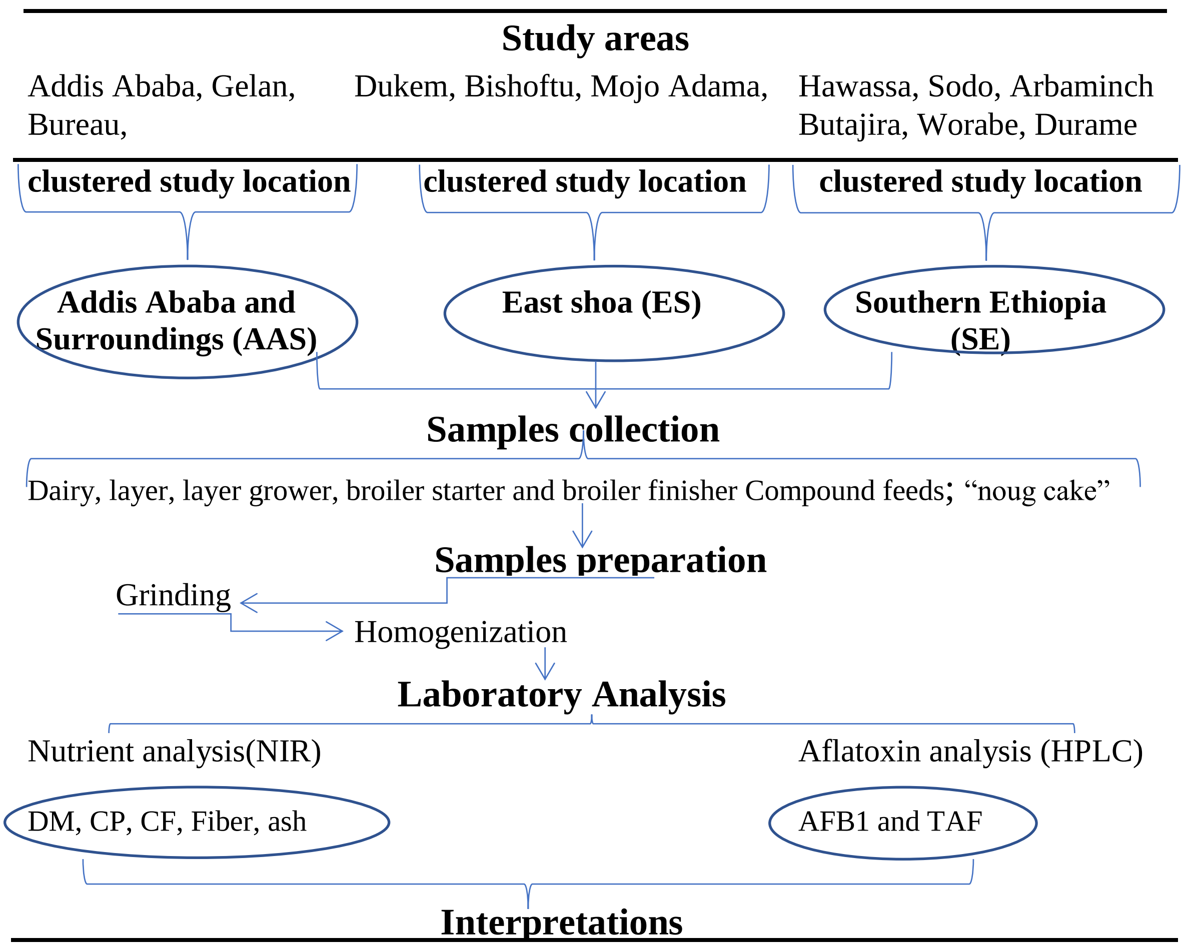
m.a.s.l.= Meters above sea level.
Source: Agricultural offices of the districts

3.2 Study Design and Sampling Method

The current levels of aflatoxins and nutrient content in commercial feeds were investigated during a cross-sectional study from September 2021 to April 2022. Picture 3 shows the study design map that was employed in this study. First off, because they comprised the majority of Ethiopia's compound feed manufacturing plants and/or oilseed cake producers, the study areas stated above were purposefully selected (Adugna, 2008; Seyoum et al., 2018). These study areas were categorized into three study locations based on the administrative demarcation and geographical location, and given the names Addis Ababa and Surroundings (AAS), East Shoa (ES), and Southern Ethiopia (SE). The AAS study location included Addis Ababa and the Oromiya special zone towns "Gelan" and "Bureau"; the East Shoa (ES) study location included the East Shoa Zone of the Oromiya region towns "Dukem," "Bishoftu," "Mojo," and "Adama"; the SE study location also included the SNNP region towns: "Sodo," "Arbaminch," "Butajira," "Worabe," and "Durame"; and the Sidama region town: "Hawassa".

Then, the list of factories in Addis Ababa and Surroundings (AAS), East Shoa (ES), and Southern Ethiopia (SE) study location were identified and the entire nine, ten, and seven compound feed manufacturers, respectively, participated, excluding those that weren't producing compound feed or oilcake during the sampling period. The list of factories was obtained from the Veterinary Drug and Animal Feed Administration and Control

Authority (VDFACA).The targeted commercial feeds, including layer compound feed, layer grower compound feed, lactating dairy cow compound feed, and "noug cake" in each of the study locations; broiler finisher and broiler starter compound feed in AAS and ES study location were sampled. These feeds were selected purposively due to the high contamination level of Aflatoxin ("noug cake") and the risk to livestock and human health (poultry and lactating dairy cow compound feed), as well as based on their availability at the manufacturing plant (broilers compound feed).



Picture 3: Map of study design

The approximate feed sample size was calculated using an expected prevalence of 50%, a defined precision of 10%, and a level of confidence of 95% (Thrusfield, 2005). Accordingly, a total of 99 feed samples were collected in the study area including twenty-two layer growers compound feed (8-20 weeks of age), twenty-one layers compound feed (> 20 weeks of age), nine broiler starters compound feed (4 weeks of age), thirteen broiler finishers compound feed (> 4 weeks of age), twenty-one lactating dairy cows compound feed, and thirteen “noug cake” (Guizotia abyssinica or Niger seed). The variation in the number of samples among the feed type was due to the number of manufactured feed type difference in the study areas.

In addition, to assess the level of Aflatoxin awareness, predisposing practices during feed handling and/or processing, and existing strategies for Aflatoxin contamination (KAP), a semi-structured questionnaire was completed for each factories, and physical observation in each factory was also used as a tool for data collection. Secondary data was gathered through the use of books and other written sources.

Picture 4: Pictures taken during feed samples collection

As shown in Picture 4 above, for each sample, 1 kg of compound feed or oilseed cake was collected and sealed in plastic bags and labeled properly. The samples were transported to the Animal Products, Veterinary Drug, and Feed Quality Assessment Center (APVDFQAC) and kept at room temperature until the analysis was completed. Each sample (1 kg) was made up of at least ten (if the number of bags is between ten and forty) or twenty (if the number of bags is greater than forty) representative subsamples, each pooled from a bulk bag of compound feed/oilseed cake bag that was randomly selected from ten compound feed/ingredient bags. To get representative samples and to limit variability, the randomly selected bags were from compound feed/oilseed cake bags from the same production line (batch). Each subsample was taken by manually probing three places on the 50 kilogram compound feed/ingredient bags (top, center, and bottom). In accordance with Ethiopian feed sampling methods, the samples were well mixed to yield representative samples, and the apparatus used in collecting samples were hand scoops and sack-type spears or triers (from bags) (ESA: 2019).

3.3 Analysis of Feeds

In general, a total of ninety-nine feed samples were analyzed for their nutrient content using a near-infrared (NIR) spectrophotometer and their aflatoxin B1 and Total aflatoxin contamination levels using High Performance Liquid Chromatography (HPLC) in Animal Products, Veterinary Drugs, and Feed Quality Assessment Center (APVDFQAC). The details are presented below.

3.3.1 Feed nutrient content analysis

The dry matter, crude protein, crude fat, crude fiber, and ash contents of the collected “noug cake” and compound feed samples were determined using the near infrared spectrophotometer (NIR) technique with DA7250 instrument (as shown in Picture 5), in the Animal Products, Veterinary Drug, and Feed Quality Assessment Center (APVDFQAC), Addis Ababa. The DA 7250 NIR Analyzer analyzes samples in only 6 seconds, and can determine moisture, protein, fat, ash, starch and many other parameters with excellent accuracy. The procedure starts by opening and preparing the near-spectrophotometer and then placing the sample dish into a sample plate. The feed samples were poured into a sample dish, slightly overfilling it, and the excess feed samples were removed using a ruler before placing the dish on the sample plate. The feed sample type (for example, layer compound feed) was chosen from the screen, and the analysis began after tapping the analysis button. Any information about the sample (for example, manufacturing date) was provided by referring to the specific sample document submitted with the sample, and the analysis was continued. Finally, the analysis result was saved and printed. The analysis report made by a near spectrophotometer shows the contents of the proximate composition in "as is" and thus were converted to dry matter using the formula indicated below in equation 1.

$$\%\ \text{nutrient (DM)} = \frac{\%\ \text{nutrient “as is”}}{\text{Dry matter of sample}}$$

Equation 1: “As is” feed to dry matter feed converting formula
Source**:** (Reiling, 2011)

3.3.2 Feed Aflatoxins Analysis

In general, a total of ninety-nine feeds were analyzed for Aflatoxin B1, B2, G1 and G2 contamination levels. As shown in Picture 5 below, the Homogenization, Extraction, Detection, and Quantification of Aflatoxins by a verified method of reverse-phase High-Performance Liquid Chromatography (HPLC) were done using the method of AOCA (2005:8) and carried out in the Animal Products, Veterinary Drug, and Feed Quality Assessment Center (APVDFQAC), Addis Ababa. Aflatoxins present in feed were extracted from the matrices by a suitable solvent or mixture of solvents and cleaned-up prior to analysis. In the sample preparation, the pretreatment of the sample (protein precipitation, defatting, extraction, and filtration) is an important phase for removing many interferences and for having, in this way, extracts without impurities to allow accuracy and reproducibility in the subsequent instrumental step.

Picture 5: NIR (left side) and HPLC (right side) at APVDFQAC

3.3.2.1 Apparatus and chemicals used

The apparatus used in the homogenizing, extraction, detection, and quantification of Aflatoxin B1 and total Aflatoxin were reverse-phase High Performance Liquid Chromatography (HPLC) systems consisting of an auto sampler with injector, pump, column oven, and fluorescence detector: desktop computer with chromatography software. The HPLC column, AflaCLEAN, and analytical balance were also used for homogenization of the sample and extraction of Aflatoxin. Furthermore, beakers, spatulas, graduated cylinders, blender jars, wrist-action shakers, stopwatches, Filter paper, funnels, sample bottles, clamped lab stands, a centrifuge, sonicator, aluminum foil, disposable syringes Syringe filters (0.45 µm), Falcon tubes, reservoirs and adapters, glass wares, micropipettes, pipettes, pipette fillers, test tubes, and tips, vacuum pump, vials with screw caps, PH meter, magnetic stirrer with bars, stopper with 3 mL adapter AflaCLEAN Other shakers: vortex mixer, measuring flask, disposable gloves, protective masks, are all used. Methanol/water (4/1, v/v), sodium chloride (NaCl), n-Hexane, Phosphate Buffered Saline (PBS), deionized or distilled water, methanol, water/acetonitrile/methanol (60/15/25, v/v), degassed mobile phase and Aflatoxins standards, all of these chemicals are used in the Aflatoxins extraction and detection.

3.3.2.2 Homogenization and extraction of Aflatoxins

Since the distribution of aflatoxins is extremely non-homogeneous, the feed samples were thoroughly ground and mixed to homogenize the samples. The grinder (blender jar) was thoroughly cleaned before grinding each sample to avoid cross-contamination with aflatoxins. To extract the aflatoxin from the corresponding matrix, twenty gram of the feed sample and two gram of NaCl were weighed and added together. Then hundred milliliters of methanol (80% methanol: 20% water, v/v) and fifty milliliters of n-hexane were added and mixed for thirty minutes at 120 revolution per minute (rpm) using a wrist action shaker. The filtered solution through filter paper was centrifuged at 2500 rpm for ten minutes to assist phase separation. The fat portion of the solution separated and remained above the solution in the separator funnel, as shown in Picture 6. The lowest seven milliliters of the solution were then collected and diluted with forty three milliliters of Phosphate Buffer Solution (PBS).The diluted solution was filtered through a syringe filter to remove residual turbidity and thoroughly mixed in a vortex mixer.

3.3.2.3 Cleanup with Immunoaffinity

After extraction from the sample matrix, the Aflatoxin has to be further isolated from any co-extracted matrix constituents. The affinity column contained antibodies raised against Aflatoxin B1, B2, G1, and G2. The detection of aflatoxin is based on antibody-antigen reactions. To clean up the Aflatoxins with immune affinity columns (LCTech AflaCLEAN, Germany), twenty fife milliliters of the dilute extract was passed onto the AflaCLEAN column to isolate and bind the Aflatoxin on an analyte-antibody bond. The Aflatoxin molecules were bound, and the unbound materials were removed by washing the column with ten milliliters of distilled water. A gentle vacuum or overpressure was used in all steps to pass the liquid through the column, and the maximum flow rate was maintained at two milliliters per minute (one drop/sec). All of the samples drained through the column and waited until there were no more samples in the column. However, a complete drying of the column was avoided.

Picture 6: Fat Extraction (top); Elution (below top) at APVDFQAC

3.3.2.4 Elution

After cleanup of the Aflatoxin, an appropriate two milliliters vial was placed below the affinity column. The bound toxins were eluted by using a total of two milliliters of methanol as the elution solvent. The elution process was performed in two steps to ensure complete release of analytes. First, a volume of one milliliters of elution solvent was applied and after that, volume had passed through the column, five minutes were left before the second portion of one milliliters of elution solvent for effective release of toxin from the gel of the column. The remaining solvent solutions were eluted by the application of slight overpressure on the top of the column. Then, the eluate was collected into vials (as shown in Picture 6) and injected into the HPLC for quantitative determination of AFB1, B2, G1 and G2. The AFLATEST®HPLC overview was shown in Figure 1.

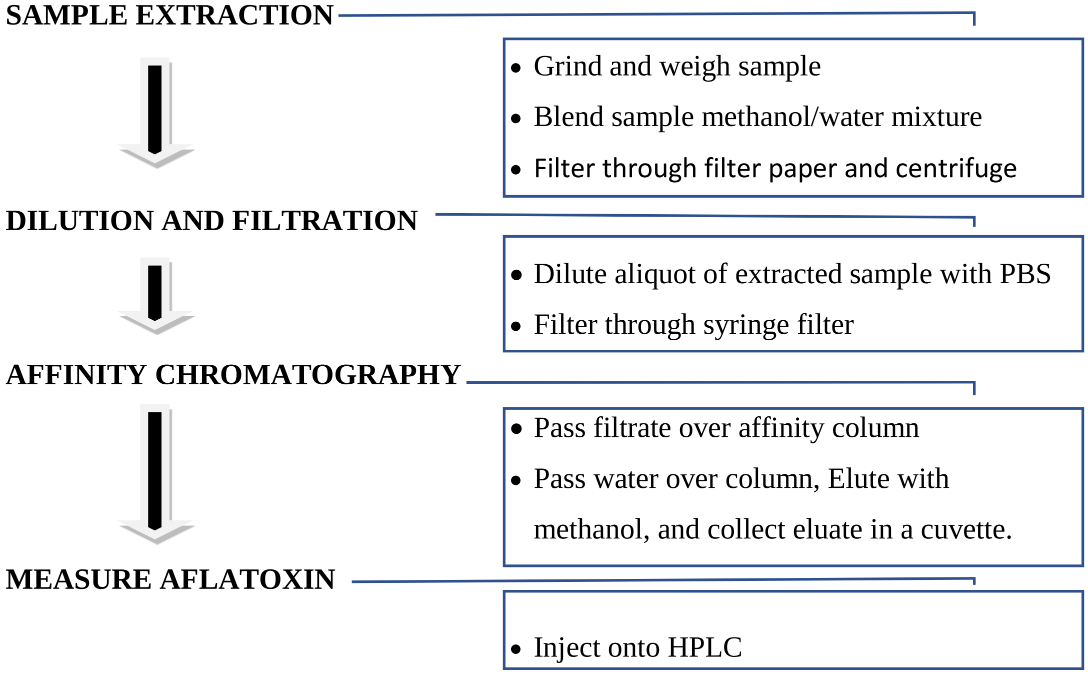


Figure 1: AFLATEST®HPLC overview
Source: (APVDFQAC, 2019)

3.3.2.5 Detection and quantification of Aflatoxins with HPLC

High Performance Liquid Chromatography (HPLC) with fluorescent detection was used to determine the quantity of aflatoxins (B1, B2, G1 and G2) in standards and samples. The HPLC system consisted of a pump and a fluorescence detector. Aflatoxins were separated in HPLC column with a mobile phase of water (methanol: acetonitrile; 60:30:15, v/v/v). Fluorescence detection was at an excitation and an emission wavelength of 365 nm and 440 nm, respectively. The validity of the method was assured and controlled by determining the precision, accuracy, recovery and linearity results as indicated below.

3.3.2.6 Calibration curve and recovery determination

Before injecting the known standard solution of Aflatoxins and the sample into the HPLC, the methods were checked for accuracy (recovery test between 60–120% as required by AOAC (2005:8) and precision using chromatograms of a blank sample and chromatograms of a pure Aflatoxins compound. The calibration curve was established using four calibration points of a mixed Aflatoxin standard of 20 μg/kg concentration with mobile phase (dH2O: ACN: MeOH, 15:25: 65, v/v/v respectively). The standards were run with similar methods to the sample analysis method. Peak areas of the different aflatoxins were plotted against the concentrations, and linear regression analysis was used to calculate the equation and the correlation coefficient of the standard curves. The acceptance criteria for correlation are > 0.99.

3.3.2.7 Accuracy and Precision

The accuracy of the method was determined by analysis of samples containing known aflatoxin amounts and evaluating the recovery against the AOAC guideline. The precision of the method was evaluated through the repeatability of the method by injections of aflatoxin mixed standard as a check after roughly every fife samples per day to evaluate the change in peak area.

3.3.2.8 Calculation results

The concentrations of Aflatoxin at parts per billion (μg/kg) levels in the feed sample were calculated from the quantity of Aflatoxin (g/l) reading from HPLC using the AOAC equations as indicated in equation 2 below:

Aflatoxin, ng/g=A x (T/I) x (1/W)

Where,

A = $\frac{\text{quantity (µg/l) (HPLC reading) x volume eluate injected in to HPLC (µL)}}{1000}$ = __(ng)

T = final test solution eluate volume = ________ (μL)

I = volume eluate injected into HPLC= _______ (μL)

W = mass (g) of commodity taken / volume of Aflatoxin extraction solvent (ml) x volume of the purified extract taken (ml) = ___ (g)

Equation 2: Equation for calculating Aflatoxin concentration

3.4 Statistical Analysis

The statistical model for this study was indicated below in equation 3:

$$Y_i = \mu + L_i + e_i$$ Where,

Y_i = value of dependent variables (levels of AF, value of DM, CP, CF, ash and fiber)

L_i = effect of study location μ = the overall mean, e_i = the error term.

(effect of study location encompasses used feed ingredient: type, contamination level, proportion; storage length and handling; level of knowledge; storage design and storage condition, etc in the study locations).

Equation 3: Equation for statistical model

The data from the PVDFQAC (Animal Products, Veterinary Drugs, and Feed Quality Assessment Center) was entered into an Excel spreadsheet 2010, organized, and then exported to R Statistics software version 4.2.0 for statistical analysis using GLM Procedures. Nutrient content (DM, CP, CF, fiber, and ash) and Aflatoxin (Total Aflatoxin and Aflatoxin B1) were the dependent variables. In order to normalize the variances before analysis, the levels of aflatoxin were log transformed [log10 (x+1)]. The result is reduced by one once the coefficents have been exponentiated. The Shapiro-Wilk and Levene's tests were employed to evaluate the dependent variable's normal distribution and homoscedasticity assumptions.

The effects of study location on the mean value of the dependent variables were investigated using one-way analysis of variance (ANOVA) at $P < 0.05$. A post hoc test was used to compare the means that showed significant differences at $P < 0.05$. While samples with aflatoxins contamination beyond the maximum level of the Ethiopian standard were referred to as prevalent, aflatoxins test results above the limit of detection were referred to as positive. The statistical significance of aflatoxin prevalence among the study locations was investigated using a Fisher's exact test. In order to compare the results of this study to feed recommendations, the Ethiopian feed standards (ESA: 2019) were also used.

4. RESULT

4.1 Commercial feeds Aflatoxin analysis result

4.1.1 Quality control and assurance

Over all, ninety nine feed samples aflatoxin level was analyzed by high performance liquid chromatography (HPLC) using AOAC (2005;8) method. An HPLC system consisted of a pump (Knaur, Germany) and a fluorescence detector (Knauer, Germany). Aflatoxins were separated in HPLC column with a mobile phase of water: methanol: acetonitrile (60:30:15, v/v/v). Fluorescence detection was at an excitation wavelength of 365 nm an emission wavelength of 440 nm. The precision, accuracy, recovery and linearity results obtained before and during feed analysis, as indicated below validated the analysis method.

The order of the individual aflatoxin's elution was AFG2, AFG1, AGB2, and AFB1. Aflatoxin retention times with maximum 2 milliliter per minutes flow rate were 7-7.5 minuts for AFG2, 8.5-9 minutes for AFG1, 9.9-10.5 minutes for AFB2, and 12-12.5 minutes for AFB1. Total run time was 25 minutes. The calibration curve was established using four calibration points of a mixed Aflatoxin standard of 20 µg/kg concentration with mobile phase (dH2O: ACN: MeOH, 15:25: 65, v/v/v respectively). The standards were run with similar methods to the sample analysis method. Peak areas of the different aflatoxins were plotted against the concentrations, and linear regression analysis was used to calculate the equation and the correlation coefficient of the standard curves and all correlation curves were ≥ 0.9976, which is on the acceptance criteria of >0.99.

Before injecting the known standard solution of Aflatoxins and the sample into the HPLC, the methods were checked for accuracy and precision using chromatograms of a blank sample and chromatograms of a pure Aflatoxins compound (Figure 2). By spiking a mixed 20 µg/kg Aflatoxin standard solution to the initial weight of an Aflatoxin-free material, the calculated recovery ranged from 84% to 98% for AFB1, AFB2, AFG1 and AFG2, in descending order, which is within the acceptable range (60-120%), demonstrating the method's accuracy. Also, the change in peak area was assessed by injecting a mid-ranged calibration standard as a check following roughly every fife samples per day. The area did not deviate more than 10% from the calibration curve's standard peak area.

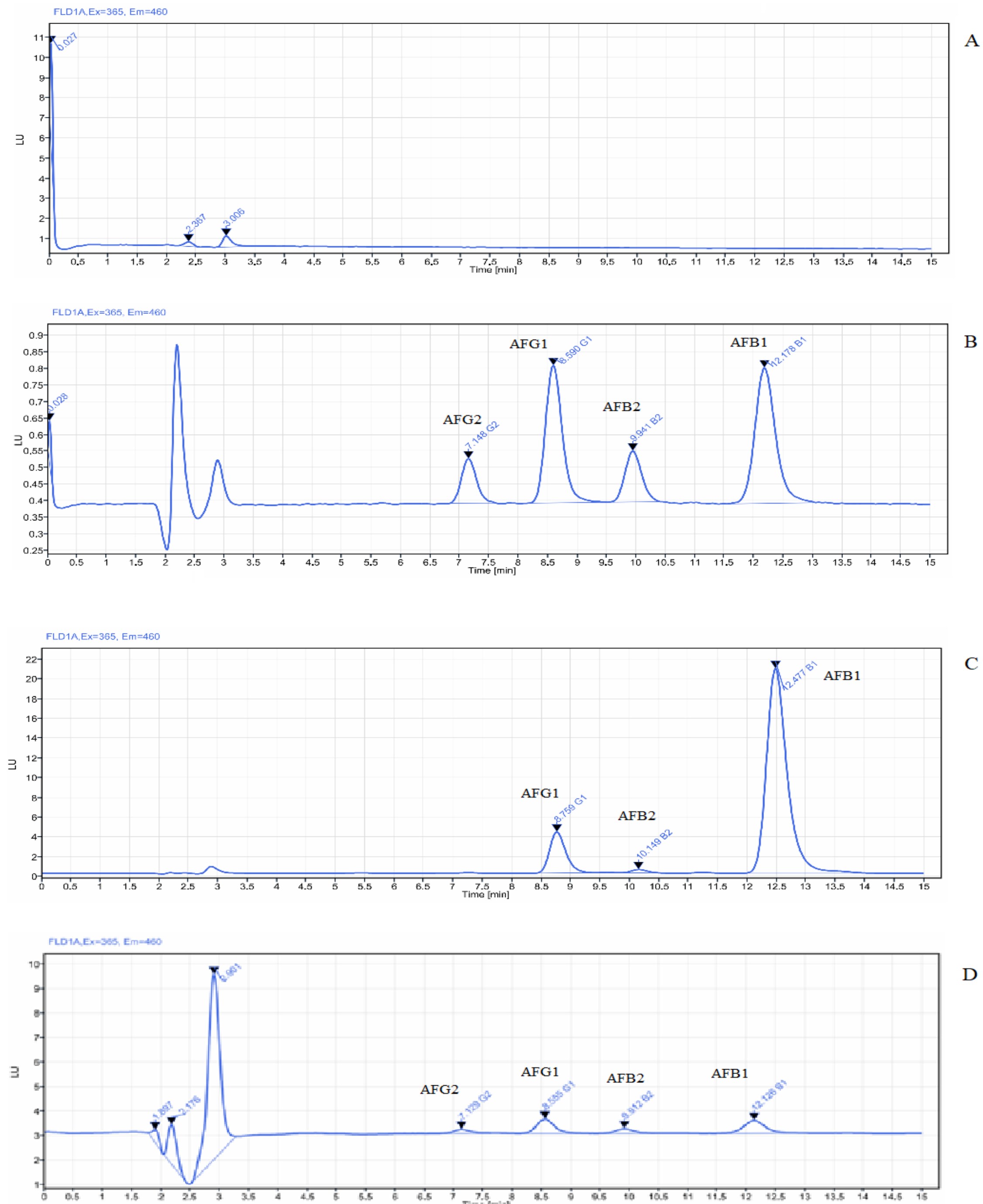


Figure 2: A=Fluorescence detector (FLD) chromatograms of blank,B= standard Aflatoxins (AFs) mixture and C & D = a feed sample contaminated with AFs

4.1.2 Aflatoxins in Feeds in the study areas

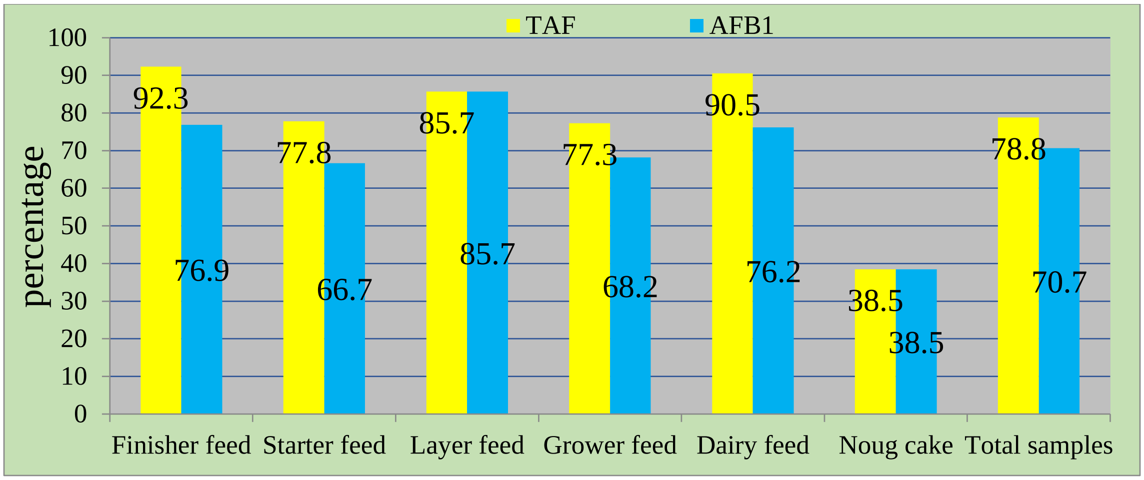
A total of ninety-nine feed samples were analyzed for TAF and AFB1 quantification. The results of the study's ANOVA and Fisher's exact test are shown in the appendices, as well as in Table 6-9 and Figure 3-12 below. From Table 6 below, TAF and AFB1 contamination in the study area were, respectively: in lactating dairy cow compound feed: positive samples (90.5 and 76.2%), prevalence (47.6 and 42.9%), mean (21.45 and 10.19 µg/kg) and maximum level (426 and 316 µg/kg); in "noug cake": positive samples and prevalence for both TAF and AFB1 38.5%, mean (6.67 and 5.01µg/kg) and maximum level (549 and 375µg/kg); in broiler finisher compound feed: positive samples (92.3 and 76.9%), prevalence(61.5%) for both TAF and AFB1, mean (28.88 and 14.96 µg/kg) and maximum level (393 and 190 µg/kg); in broiler starter compound feed: positive samples (77.8 and 66.7%), prevalence (55.6% for both TAF and AFB1), mean (21.36 and 11.87 µg/kg) and maximum level (269 and 128 µg/kg); in layer compound feed: positive samples (85.7%), prevalence (47.6%) for both TAF and AFB1, mean (16.07 and 8.27 µg/kg) and maximum level (389 and 174µg/kg) and in layer grower compound feed: positive samples (77.3 and 68.2%), prevalence(40.9%) for both TAF and AFB1, mean (12.43 and 6.83µg/kg) and maximum level (233 and 116 µg/kg); in total samples: positive samples (78.8 and 70%), prevalence (47.5 and 46.5%), mean (16.05 and 8.05 µg/kg) and maximum level (549 and 375 µg/kg).

Layer compound feed had the highest AFB1 positive samples (85.7%), followed by broiler finisher compound feed (76.9%), and lactating dairy cow compound feed (76.2%). Broiler finisher compound feed had the highest TAF positive samples (92.3%) followed by lactating dairy cow compound feed (90.5%). "noug cake" had the least positive samples (38.5%) of TAF or AFB1 (Figure 3). Broiler finisher compound feed had the highest TAF and AFB1 prevalence (for both 61.5%) followed by broiler starter compound feed (for both 55.6%) and layer compound feed (for both 47.6%), and lactating dairy cow compound feed (TAF=47.6 and AFB1=42.9%); "noug cake" had the least prevalence (38.5%) of TAF or AFB1 (Figure 5). With the exception of broiler starter feed, the mean AF concentrations in each feed type were within Ethiopian allowed limits (Figure 6). The highest individual level of TAF and AFB1 contamination was found in “noug cake” (549 and 375 µg/kg, respectively), followed by lactating dairy cow compound feed (426 and 326 µg/kg, respectively) (Figure 7), with the highest mean TAF and AFB1 contamination found in broiler finisher compound feed (28.8 and 14.96 µg/kg, respectively), broiler starter compound feed (21.36 and 11.87 µg/kg, respectively), and lactating dairy cow compound feed (21.45 and 10.19 µg/kg, respectively), in that order (Table 6). As shown in Table 7 and Figure 4, over all the sample 46.5, 8, 5 and 19% of the samples for TAF and 29, 16, 8 and 17% of the samples for AFB1 contamination lies in more than 40 µg/kg, >20 and <40 µg/kg, >10 and <20 µg/kg and >0and <10 µg/kg, respectively.

Table 6: Aflatoxin contamination in feeds

			Samples (%)							
Feed type	AFs	N	+ve	+ve %	Mean	Max	Median	SD	ST	prevalence
Total samples	TAF	99	78	78.8	16.05	549	16.05	-	-	47.5
	AFB1	99	70	70.7	8.05	375	16	-	-	47.5
Broiler Finisher feed	TAF	13	12	92.3	28.88	393	55.3	8.4	40	61.5
	AFB1	13	10	76.9	14.96	190	30	8.1	20	61.5
Broiler starter feed	TAF	9	7	77.8	21.36	268.7	63.4	9.1	20	55.6
	AFB1	9	6	66.7	11.87	128	28.4	6.9	10	55.6
Layer feed	TAF	21	18	85.7	16.07	389	15	5.9	20	47.6
	AFB1	21	18	85.7	8.27	173.9	7	4.5	10	47.6
Layer grower	TAF	22	17	77.3	12.43	233	25.1	7.4	40	40.9
	AFB1	22	15	68.2	6.83	116	12.56	5.5	20	40.9
Dairy cow feed	TAF	21	19	90.5	21.45	426	38.74	5.6	40	47.6
	AFB1	21	16	76.2	10.19	316	19	5.4	20	42.9
“noug cake”	TAF	13	5	38.5	6.67	549	0	14.6	40	38.5
	AFB1	13	5	38.5	5.01	375	0	10.4	20	38.5

AFs=Aflatoxins, TAF=Total Aflatoxins, AFB1= Aflatoxin B1, N=number of samples, +ve=number of positive samples, +ve (%)=percentage of positive samples, SD=standard devation, ST= Ethiopian feed standards, total samples=over all feeds samples analysis result, - = not available.



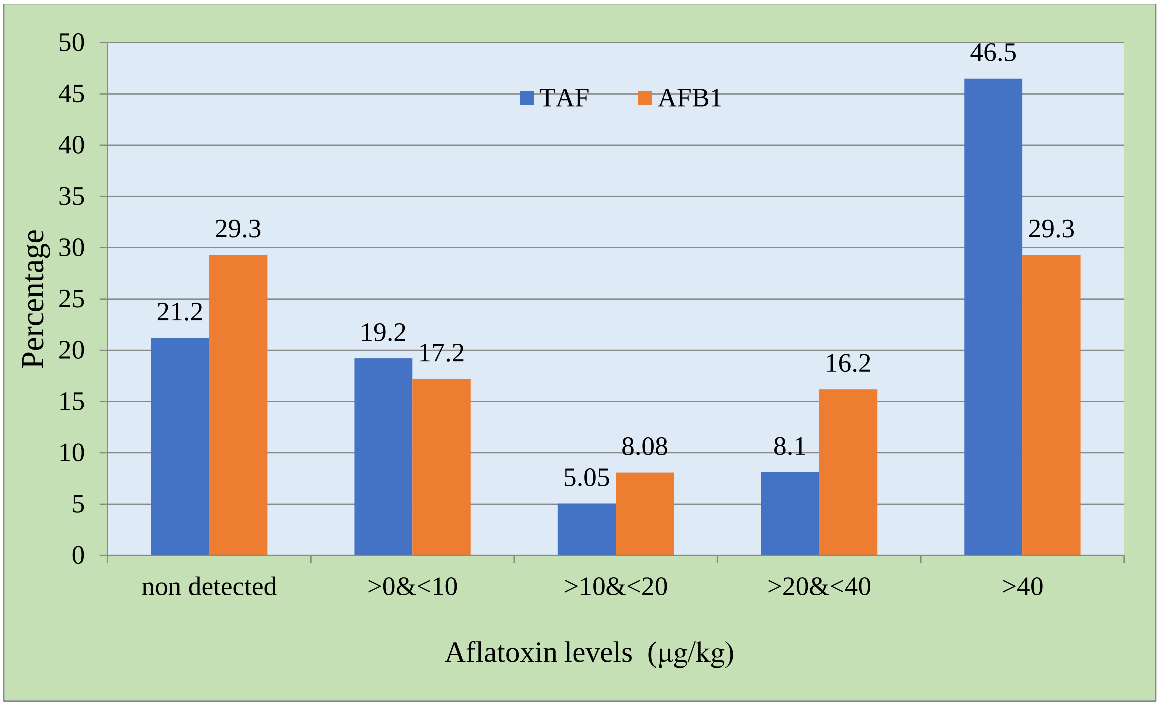
TAF=Total aflatoxin, AFB1=Aflatoxin B1, total samples= sum of all feed samples

Figure 3: Aflatoxin positive samples in the study area (%)

Table 7: Aflatoxins positive samples with various concentrations

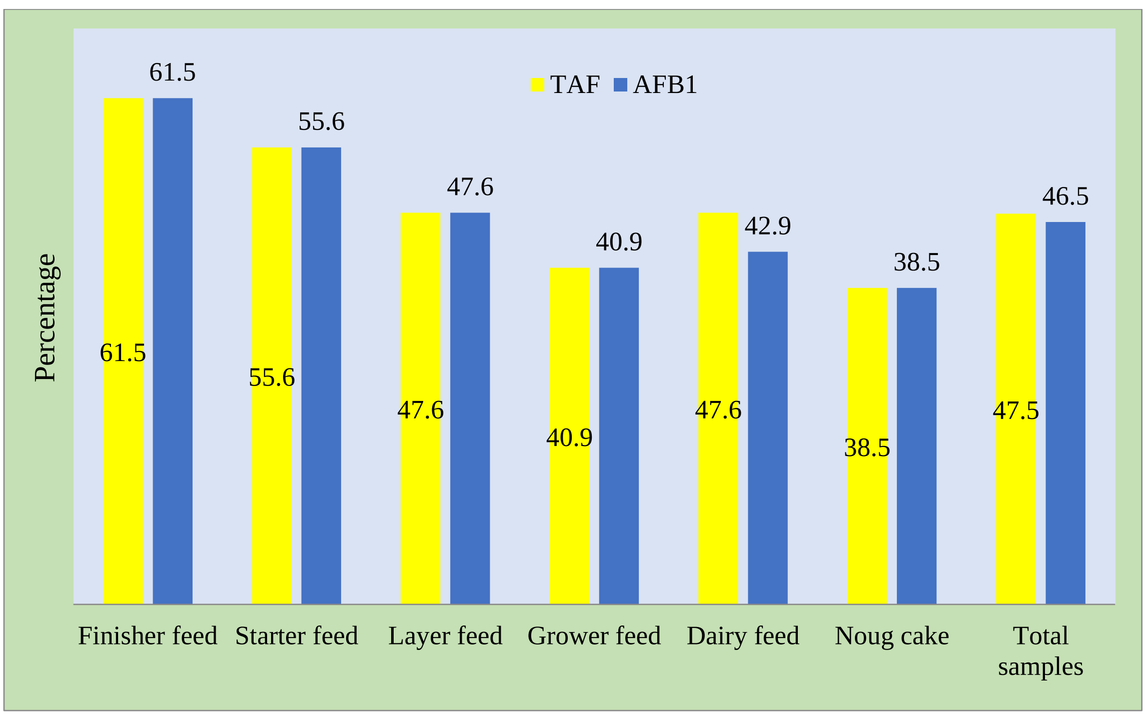
			Proportion of samples with different Aflatoxin level (µg/kg)				
			Proportion of individual positive samples (%)				
Feed type	AFs	N	ND	>0 &<10	>10&<20	>20&<40	>40
Total samples	TAF	99	21.2	19.2	5.05	8.1	46.5
	AFB1	99	29.3	17.2	8.08	16.2	29.3
Poultry samples	TAF	65	16.9	21.5	7.69	6.2	47.7
	AFB1	65	24.6	21.5	6.15	18.5	29.2
Broiler finisher feed	TAF	13	7.7	30.8	0.00	0.0	61.5
	AFB1	13	23.1	15.4	0.00	23.1	38.5
Broiler starter feed	TAF	9	22.2	11.1	11.11	0.0	55.6
	AFB1	9	33.3	11.1	0.00	22.2	33.3
Layer feed	TAF	21	14.3	23.8	14.29	4.8	42.9
	AFB1	21	14.3	38.1	4.76	19.0	23.8
Layer grower feed	TAF	22	22.7	18.2	4.55	13.6	40.9
	AFB1	22	31.8	13.6	13.64	13.6	27.3
Dairy cow feed	TAF	21	9.5	23.8	0.00	19.0	47.6
	AFB1	21	23.8	14.3	19.05	14.3	28.6
"noug cake"	TAF	13	61.5	0.0	0.00	0.0	38.5
	AFB1	13	61.5	0.0	0.00	7.7	30.8

N=number of samples, ND=Non detected sample, total samples= sum of all feed samples, poultry samples= sum of all poultry feed samples



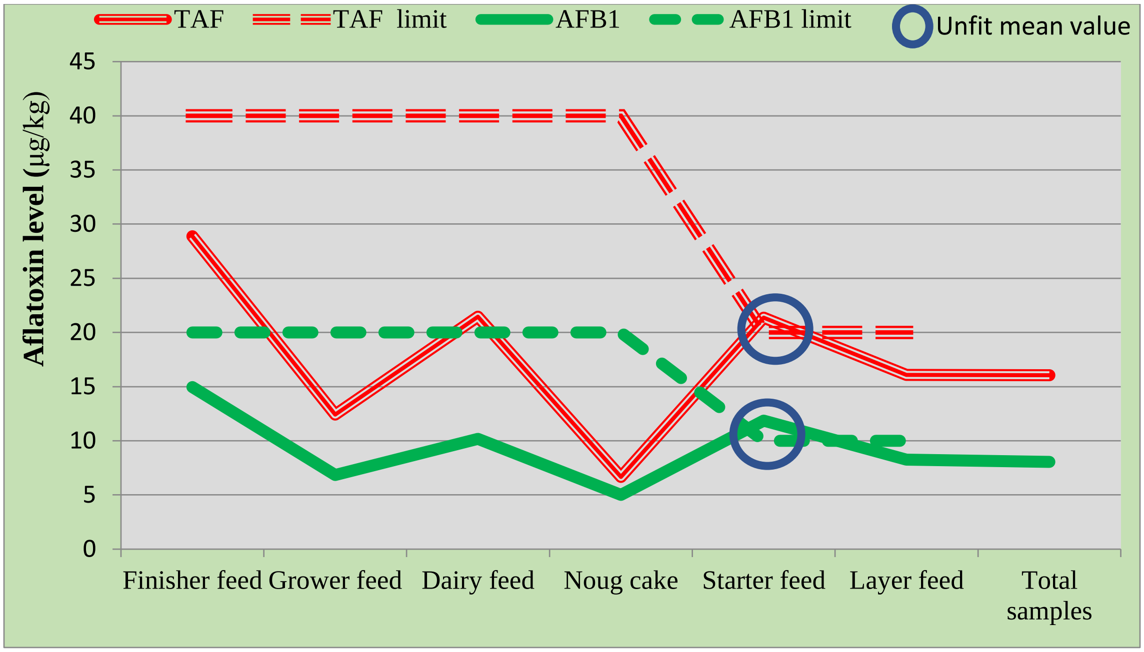
TAF=Total aflatoxin, AFB1=Aflatoxin B1

Figure 4: Aflatoxins positives in total samples with various concentrations



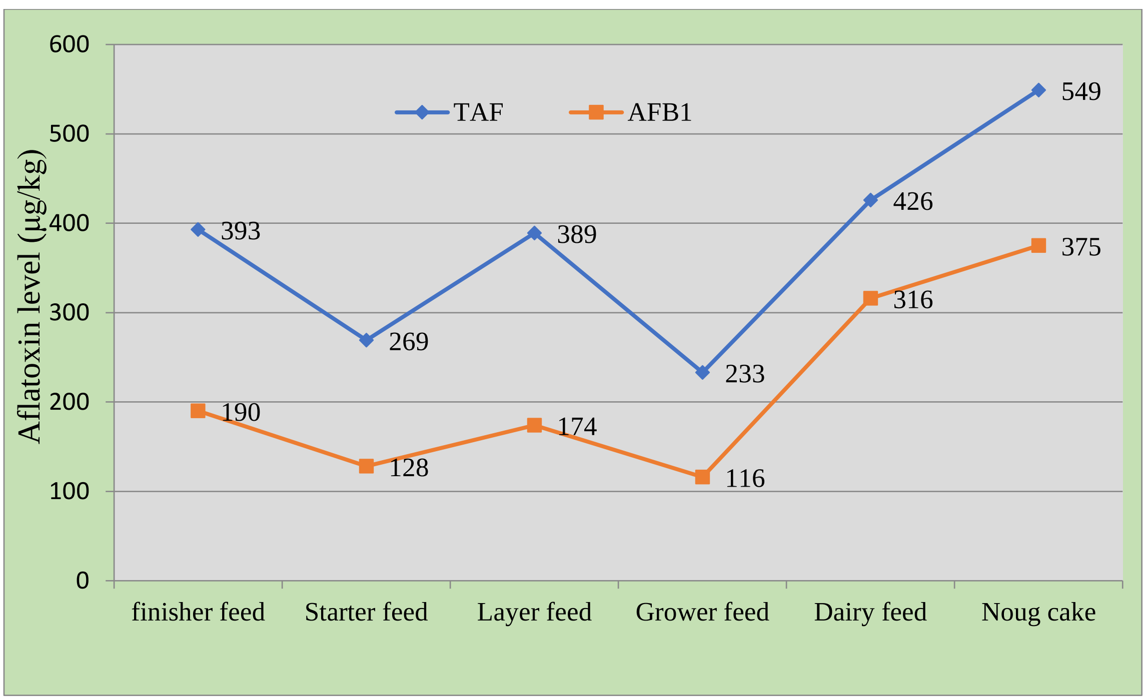
TAF=Total aflatoxin, AFB1=Aflatoxin B1, total samples= sum of all feed samples

Figure 5: Prevalence of Aflatoxin in the study area (%)



TAF=Total aflatoxin, AFB1=Aflatoxin B1, limit= Ethiopian standard maximum limit

Figure 6: Aflatoxin mean value vs. Ethiopian standard in the study area (µg/kg)



TAF=Total aflatoxin, AFB1=Aflatoxin B1

Figure 7: Maximum Aflatoxin level in individual sample (µg/kg)

4.1.3 Aflatoxins in Feeds at the study locations

As previously stated, TAF and AFB1 levels in compound feeds of lactating dairy cows, layers, layer growers, broiler finishers, broiler starters, and in “noug cake” were determined. In the study locations of Addis Ababa and surroundings (AAS), East Shoa (ES), and Southern Ethiopia (SE), the number of samples examined for Aflatoxin level were: lactating dairy cow compound feed (7, 8 and 6), layer grower compound feed (7, 5 and 10), layer compound feed (5, 6 and 10), broiler starter compound feed (4 and 5), and broiler finisher compound feed (5 and 8), respectively. Due to a lack of availability, broiler compound feed was not collected from SE. The analysis results for each type of feed in the study area were presented in the previous section. Furthermore, Aflatoxin levels, positive samples and prevalence in each feed type at the study locations are presented in Tables 8 and 9 and Figures 8-12 below.

4.1.3.1 Aflatoxins in dairy compound feeds

In lactating dairy cow compound feed, the recorded TAF and AFB1 positive samples was 87.5% and 75% in SE, 83.3% and 50% in ES, respectively, and 100% in AAS. Of the lactating dairy cow compound feed samples collected from AAS, 42.9% and 57.1% exceeded the Ethiopian Standard recommended level of 40 µg/kg TAF and 20 µg/kg AFB1, respectively, whilst 50% and 33.3% of the samples from ES and 50% and 37.5% from SE, respectively, exceeded the limits (Table 8).

The total aflatoxin (TAF) level of lactating dairy cow compound feed collected from AAS feed factories ranged between 1.4 µg/kg and 426µg/kg with a mean value of 30.4µg/kg, while it ranged from 0 to 125 µg/kg with a mean value of 17.1 µg/kg in ES, and from 0 to 182 µg/kg with a mean value of 18.7 µg/kg in SE. On the other hand, the AFB1 content of lactating dairy cow compound feed collected from AAS feed factories ranged between 1 µg/kg and 316 µg/kg with a mean value of 17.6 µg/kg, while it ranged from 0 to 60 µg/kg with a mean value of 5.5 µg/kg in ES, and it ranged from 0 to 80 µg/kg with a mean value of 9.8 µg/kg in SE (Table 9). The mean TAF and the mean AFB1 value of lactating dairy cow compound feed recorded in each study location were below the Ethiopian Standard recommended limits of 40 µg/kg TAF and 20 µg/kg AFB1. In this study, the prevalence and mean Aflatoxin levels in lactating dairy cow compound feed were not statistically different among the sample locations at $p<0.05$.

4.1.3.2 Aflatoxins in layer compound feeds

Aflatoxin positive samples in layer compound feed were 100% in AAS, 66.7% in SE, and 90% in ES for both TAF and AFB1. Of the layer compound feed samples collected from AAS, 80% exceeded the Ethiopian Standard recommended level of 20 µg/kg TAF and 10 µg/kg AFB1, whilst 50% of the samples from ES and 16.7% from SE exceeded the limits. The TAF content of layer compound feed collected from AAS feed factories ranged between 11.3 µg/kg and 139 µg/kg with a mean value of 52.6 µg/kg, while it ranged from 0–389 µg/kg with a mean value of 23.5 µg/kg in ES and it ranged from 0–163 µg/kg with a mean value of 2.6 µg/kg in SE (Table 8).

On the other hand, the AFB1 content of layer compound feed collected from AAS feed factories ranged between 4.1µg/kg and 63.7 µg/kg with a mean value of 24.1µg/kg, while it ranged from 0-174 µg/kg with a mean value of 10.5 µg/kg in ES and it ranged from 0-74 µg/kg with a mean value of 1.8 µg/kg in SE. The mean TAF and the mean AFB1 value of layer compound feed recorded in AAS and ES were exceeded the Ethiopian Standard recommended limits of 20 µg/kg TAF and 10 µg/kg AFB1, whereas, in SE below the limits was registered (Table 9). Statistically different mean TAF levels in layer compound feed was observed in study location of AAS and SE at $p<0.05$. However, no significant difference was observed in mean AFB1 and prevalence of aflatoxin among the study locations.

4.1.3.3 Aflatoxins in layer grower compound feeds

In this study, TAF and AFB1 positive samples in layer grower compound feed was 90 and 70% in ES respectively, 100% in AAS and 20% in SE. Of the layer grower compound feed samples collected from AAS, 57.1% exceeded the Ethiopian standard recommended limit of 40 µg/kg TAF and 20 µg/kg AFB1, whilst 40% of the samples from ES and 20% from SE exceeded the limits. The TAF content of layer grower compound feed collected from AAS feed factories ranged between 14.6 µg/kg and 233 µg/kg with a mean value of 63.6µg/kg, While it ranged from 0-125 µg/kg with a mean value of 9.3 µg/kg in ES and it ranged from 0-102 µg/kg with a mean value of 1.5 µg/kg in SE (Table 8). On the other hand, the AFB1 content of layer grower compound feed collected from AAS feed factories ranged between 0.8 µg/kg and 116 µg/kg with a mean value of 25µg/kg, while it ranged from 0-55 µg/kg with a mean value of 5.5 µg/kg in ES and it ranged from 0-46 µg/kg with a mean value of 1.2 µg/kg in SE. The mean TAF and the mean AFB1 value of layer grower compound feed recorded in AAS were exceeded the Ethiopian Standard recommended limits of 40 µg/kg TAF and 20 µg/kg AFB1, whereas, in ES and SE below the limits was registered (Table 9). Statistically different mean Aflatoxin levels in layer grower compound feed was observed in study location of AAS and SE.

4.1.3.4 Aflatoxins in Broiler finisher compound feeds

In broiler finisher compound feed the recorded TAF and AFB1 positive samples were 100% and 88% in AAS, and 80% and 75% in ES, respectively. Of the broiler finisher compound feed samples collected from AAS, 80% exceeded the Ethiopian Standard recommended level of 40 µg/kg TAF and 20 µg/kg AFB1, whilst 50% of the samples from ES exceeded the limits (Table 8). The TAF content in broiler finisher compound feed collected from AAS feed factories ranged between 8 µg/kg and 393 µg/kg with a mean value of 130 µg/kg and the AFB1 content ranged between 0 and 190 µg/kg with a mean value of 52.6 µg/kg, whereas in ES the TAF content ranged from 0–218.3 µg/kg with a mean value of 10.9 µg/kg and the AFB1 content ranged 0-109.4 µg/kg with a mean value of 6.5 µg/kg. The mean TAF and the mean AFB1 value of broiler finisher compound feed recorded in AAS were exceeded the Ethiopian Standard recommended limits of 40 µg/kg TAF and 20 µg/kg AFB1, whereas in ES, below the limits was registered (Table 9). Statistically the mean Aflatoxin levels and prevalence of aflatoxin in broiler finisher compound feed were not different in the study location of AAS and ES at $p<0.05$.

4.1.3.5 Aflatoxins in Broiler starter compound feeds

Positive samples for TAF and AFB1 in broiler starter compound feed were for both, 100% in AAS, and 60% and 40% in ES, respectively. Of the broiler starter compound feed samples collected from AAS, 100% exceeded the Ethiopian Standard recommended level of 20 µg/kg TAF and 10 µg/kg AFB1, whilst 20% of the samples exceeded the limits (Table 8). The TAF content of broiler starter compound feed collected from AAS feed factories ranged between 75.8 µg/kg and 268.7 µg/kg with a mean value of 157.2 µg/kg and AFB1 content ranged from 35–128.4 µg/kg with a mean value of 75.9 µg/kg, whereas in ES, TAF content ranged from 0–63.4 µg/kg with a mean value of 3.7 µg/kg and AFB1 content ranged between 0 µg/kg and 28.4 µg/kg with a mean value of 2.1 µg/kg . The mean TAF and the mean AFB1 value of broiler starter compound feed recorded in AAS were exceeded the Ethiopian Standard recommended limits of 20 µg/kg TAF and 10 µg/kg AFB1, whereas in ES below the limit was registered (Table 9). Statistically different mean Aflatoxin levels and prevalence of aflatoxin in broiler starter compound feed was observed among the study locations at $p<0.05$.

4.1.3.6 Aflatoxins in "noug cake"

Positive samples and prevalence of AF (above maximum limit) in "noug cake" was 37.5% in AAS, 33.3% in SE, and 50% in ES for both TAF and AFB1 (Table 8). The TAF content of "noug cake" collected from AAS feed/oil factories ranged between 0 and 549.1 µg/kg with a mean value of 6.8 µg/kg, whereas it ranged from 0–44 µg/kg with a mean value of 5.7 µg/kg in ES and it ranged from 0–512 µg/kg with a mean value of 7 µg/kg in SE. In addition, the AFB1 content of "noug cake" collected from AAS feed/oil factories ranged between 0 and 227 µg/kg , with a mean value of 4.7 µg/kg , whereas it ranged between 0 and 31 µg/kg with a mean value of 4.7 µg/kg in ES and it ranged between 0 and 375 µg/kg with a mean value of 6.2 µg/kg SE. The mean TAF and the mean AFB1 value of "noug cake" collected in either of the study location was below the Ethiopian Standard recommended limits of 40 µg/kg TAF and 20 µg/kg AFB1 (Table 9). Statistically the mean aflatoxin levels and prevalence of aflatoxin in "noug cake" was not different along the study location at $p<0.05$.

4.1.3.7 *Aflatoxins in total feed samples*

Overall 99 feed samples analyzed, AAS had the highest AF positive samples (TAF=86.1% and AFB1=83.3%), followed by ES (TAF=82.9% and AFB1=68.3%), while SE had the lowest (TAF=59.1 and AFB1=54.5%). Except for "noug cake" (TAF and AFB1=37.5%) and broiler finisher compound feed (AFB1=80%), all of the AAS feed samples were positive for TAFs and AFB1 (Figure 8 and 9). The TAF and AFB1 levels were found to be higher than the Ethiopian Standard recommended limits in 61.5 and 63.91% of AAS feed samples, 31.8 and 27.3% of SE feed samples, and 43.9 and 41.5% of ES feed samples, respectively. AAS had the highest prevalence of TAF and AFB1 in each feed type studied, with the exception of lactating dairy cow compound feed and "noug cake" for TAF and "noug cake," for AFB1 (Figure 10 and 11). The maximum individual AF level was recorded in AAS than the ES and SE for each feed type except TAF and AFB1 in layer compound feed and AFB1 In “noug cake” (Figure 12).

Table 8: Aflatoxins in feeds at the study locations

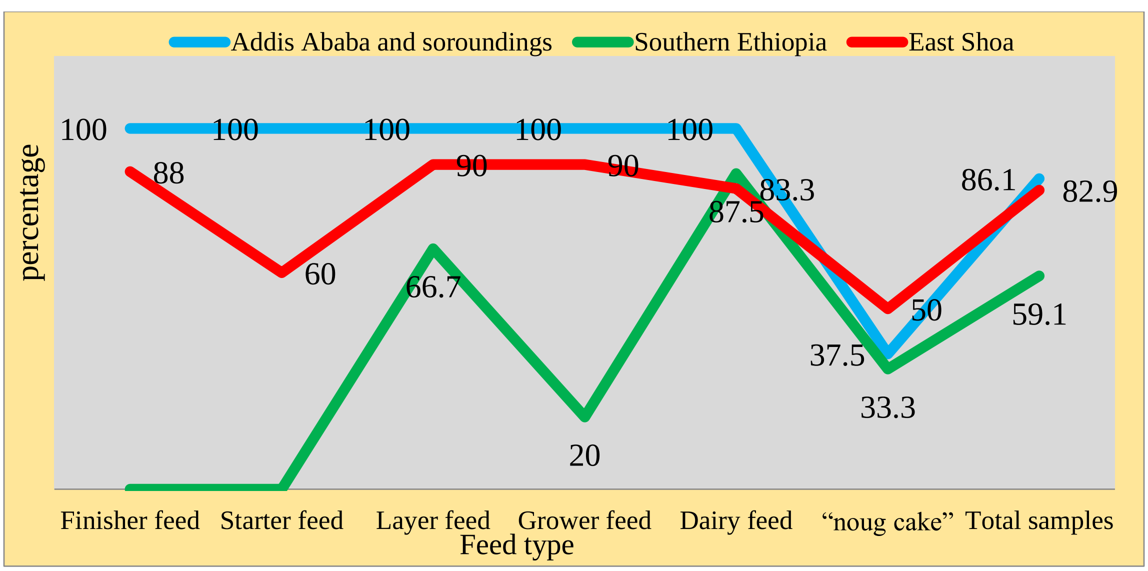
Feed type	Addis Ababa & surroundings				Southern Ethiopia				East Shoa			
	Positive(%)		prevalence(%)		positive(%)		prevalence(%)		positive(%)		prevalence(%)	
	TAF	AFB1	TAF	AFB1	TAF	AFB1	TAF	AFB1	TAF	AFB1	TAF	AFB1
Finisher feed	100	80	80	80	na	na	na	na	88	75.0	50	50
Starter feed	100	100	100	100	na	na	na	na	60	40.0	20	20
Layer feed	100	100	80	80	66.7	66.7	16.7	16.7	90	90.0	50	50
Grower feed	100	100	57.1	57.1	20.0	20.0	20	20	90	70.0	40	40
Dairy feed	100	100	42.9	57.1	87.5	75.0	50	37.5	83.3	50.0	50	33.3
“noug cake”	37.5	37.5	37.5	37.5	33.3	33.3	33.3	33.3	50.0	50.0	50	50
Total samples	86.1	83.3	61.5	63.9	59.1	54.5	31.8	27.3	82.9	68.3	43.9	41.5

na = not available (In Southern Ethiopia broiler feeds not found), TAF= total aflatoxn, AFB1=aflatoxin B1 positive(%)=proportion of AF detected (above 0) samples, prevalence(%)=proportion of samples contained AF level above the maximum limit, total sample= sum of all feed samples.

Table 9: Aflatoxin levels in feeds along the study locations (µg/kg)

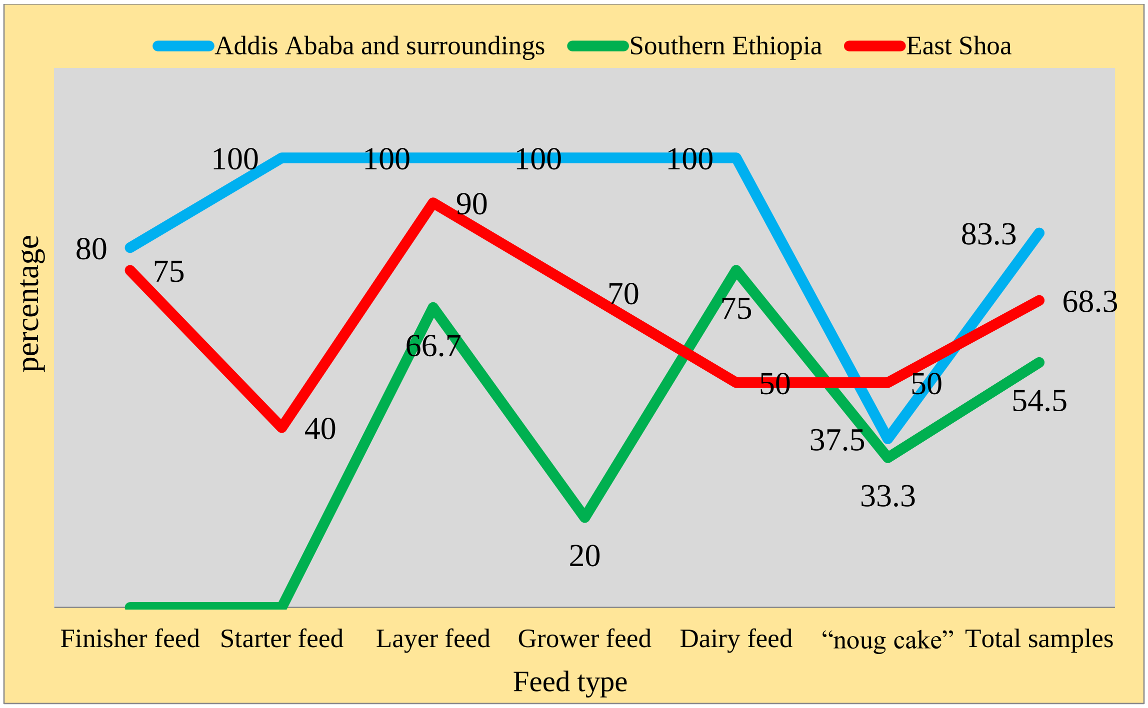
Feed type		Total Aflatoxin(µg/kg)			Aflatoxin B1(µg/kg)		
		AAS	SE	ES	AAS	SE	ES
Dairy cow feed	Mean	30.4	18.7	17.1	17.6	9.8	5.5
	Range	1.4-426	0-182	0-125	1-316	0-80	0-60
Layer feed	Mean	52.6[a]	2.6[b]	23.5[ab]	24.1	1.8	10.5
	Range	11.3-139	0-163	0-389	4.1-63.7	0-74	0-174
Grower feed	Mean	63.6[a]	1.5[b]	9.3[ab]	25	1.2	5.5
	Range	14.6-233	0-102	0-125	0.8-116	0-46	0-55
Finisher feed	Mean	130	na	10.9	52.6	na	6.5
	Range	8.0-393	na	0-218.3	0-190	na	0-109.4
Starter feed	Mean	157.2[a]	na	3.7[b]	75.9[a]	na	2.1[b]
	Range	75.8-268.7	na	0-63.4	35-128	na	0-28.4
"noug cake"	Mean	6.8	7.0	5.7	4.7	6.2	4.7
	Range	0-549.1	0-512	0-44	0-227	0-375	0-31

The results are on a dry matter basis, [ab] = means of total aflatoxin or aflatoxin B1 in the raw with different superscript are significantly different at ($p<0.05$), AAS denotes Addis Ababa and surroundings, while ES denotes the East Shoa, and SE denotes the Southern Ethiopia, na = not available (In Southern Ethiopia broiler feeds samples were not found), ns = no standard limitation in Ethiopian standards list. The Ethiopian standard maximum limits of total aflatoxin and aflatoxin B1 are 40 and 20 µg/kg, respectively, in "noug cake", broiler finisher feed, layer grower feed, and lactating dairy cow feed, and 20 and 10 µg/kg in layer feed and layer starter feed.



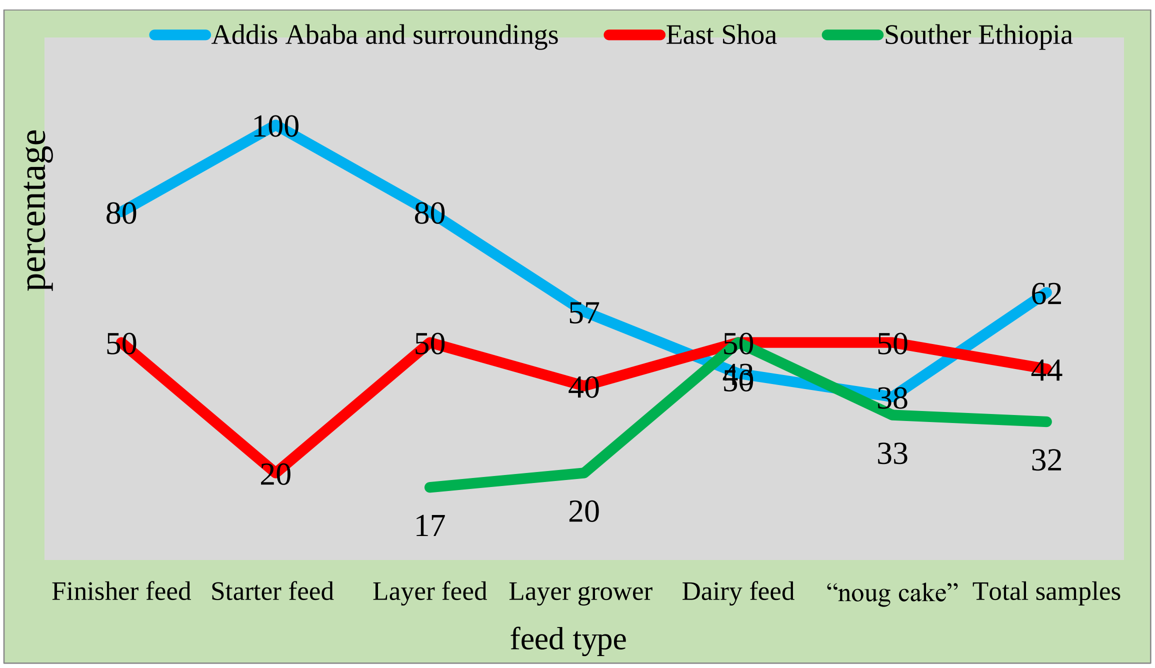
No broilers feed samples was taken from southern Ethiopia, total sample= sum of all feed type samples

Figure 8: TAF positive samples among the study locations (%)



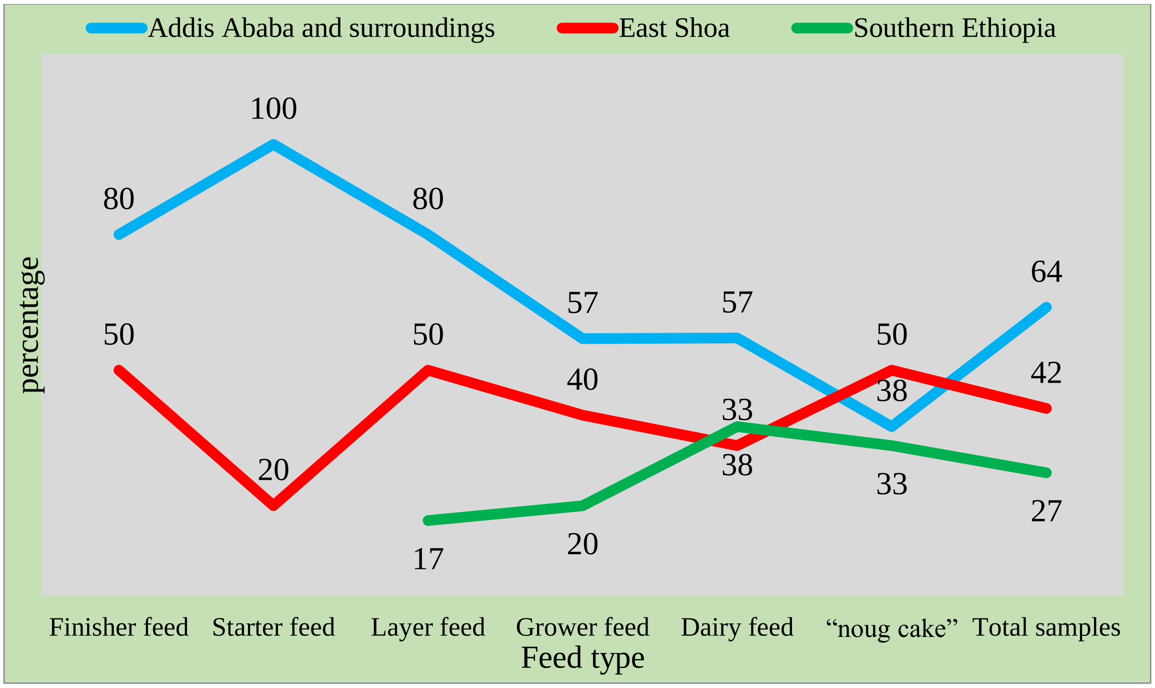
No broilers feed samples was taken from southern Ethiopia, total sample= sum of all feed type samples

Figure 9: AFB1 positive samples among the study locations (%)



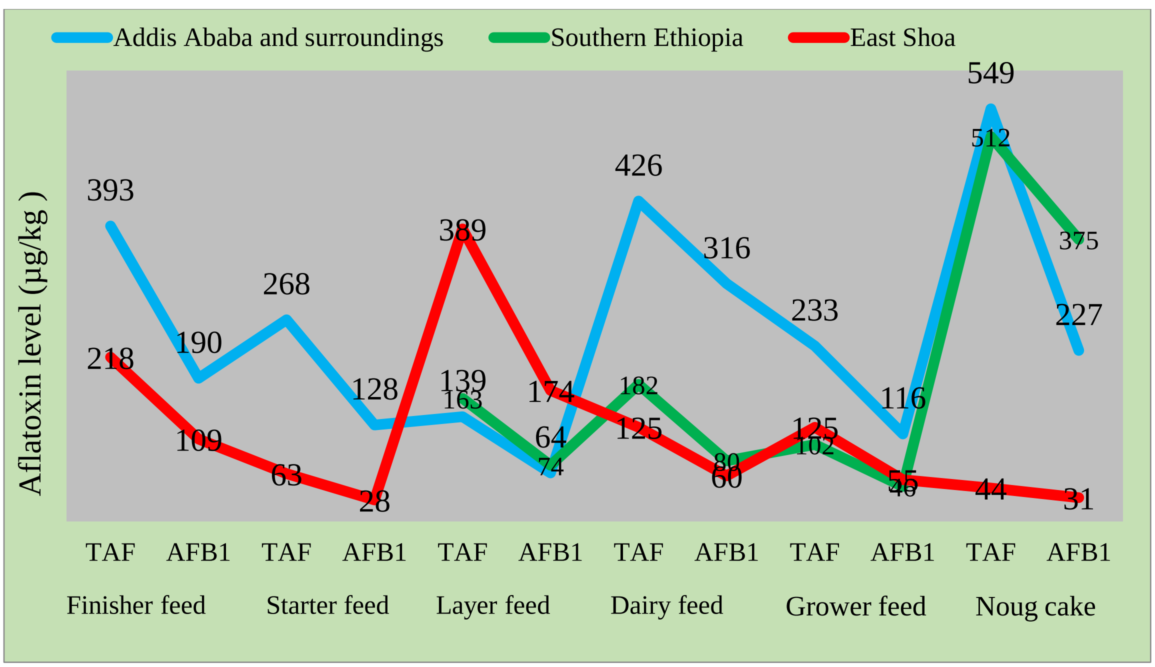
No broilers feed samples was taken from southern Ethiopia, total sample= sum of all feed type samples

Figure 10: Prevalence of TAF among the study locations (%)



No broilers feed samples was taken from southern Ethiopia, total sample= sum of all feed type samples

Figure 11: Prevalence of AFB1 among the study locations (%)



No broilers feed samples was taken from southern Ethiopia, TAF=total aflatoxin, AFB1=aflatoxinB1

Figure 12: Maximum aflatoxin levels in feed among the study locations

4.2 Commercial Feeds Nutrient Content Analysis Result

A total of ninety-nine feed samples were analyzed for the dry matter (DM), crude protein (CP), crude fat (CF), fiber and ash content using NIR. The ANOVA test results are presented in the appendices, and Tables 10-13 and in Figures 13and 14 below.

4.2.1 Individual sample nutrient content

4.2.1.1 Individual samples nutrient content in the study area

As shown in Table 10, when individual sample content was taken into account, over all the feed samples (n=99): 6.6 % (n = 6/99), 25.3 % (n = 25/99), 9.1 % (n = 9/99), and 14.1 % (n = 14/99) of the feed samples evaluated were unfit for the DM, CP, CF, and fiber requirements of Ethiopian standards, respectively. Except for broiler starter compound feed and "noug cake" (44.4 % and 15.4 % unfit), none of the samples in each feed were unfit for the Ethiopian standard DM requirements.

Except for layer grower compound feed, 38.5 % of broiler finisher compound feed, 11.1 % of broiler starter compound feed, 42.9 % of layer compound feed, 42.9 % of lactating dairy cow compound feed, and 7.7% of "noug cake" samples were unfit for the CP requirement in the Ethiopian standard. Except for 23.3 % of the broiler finisher compound feed sample, 11.1 % of the broiler starter compound feed sample, 4.5 % of the layer grower compound feed sample, and 30.8 % of the "noug cake", none of the layer or lactating dairy cow compound feed samples had a CF concentration that was unfit for the Ethiopian standard.

With the exception of 53.8 %, 15.4 %, and 55.6 % of the "noug cake", Broiler finisher compound feed, and Broiler starter compound feed samples, respectively, none of the layer, layer grower, or lactating dairy cow compound feed samples was unfit for the fiber content of the Ethiopian standard.

Table 10: unfit individual samples in nutrient content in the study area (%)

Feed type	N	DM		CP		CF		Crude fiber		Ash	
		n	%	n	%	n	%	n	%	n	%
Total samples	99	6	6.1	25	25.3	9	9.1	14	14.1	-	-
Poultry feed	65	4	6.2	15	23.1	5	7.7	7	10.8	-	-
Finisher feed	13	13	100	5	38.5	3	23.1	2	15.4	12	92.3
Starter feed	9	4	44.4	1	11.1	1	11.1	5	55.6	9	100
Layer feed	21	21	100	9	42.9	21	100	21	100	ns	-
Grower feed	22	22	100	22	100	1	4.5	22	100	ns	-
Dairy cow feed	21	21	100	9	42.9	21	100	21	100	ns	-
"noug cake"	13	2	15.4	1	7.7	4	30.8	7	53.8	0	-

N=sample number, n=number of unfit samples, ns= no standard in Ethiopia, - =not calculated, DM=dry matter, CP=crude protein, CF=crude fat, Total sample= sum of all feed samples, poultry feed= sum of all poultry feed.

4.2.1.2 Individual samples nutrient content in the study locations

As previously stated, the nutrient content of lactating dairy cows, layers, layer growers, broiler finishers, broiler starters, and "noug cake" was determined using a near-infrared spectrophotometer. In the study locations of Addis Ababa and surroundings (AAS), East Shoa (ES), and Southern Ethiopia (SE), the number of samples examined for nutrient content were: lactating dairy cow compound feed (7, 8 and 6), layer grower compound feed (7, 5 and 10), layer compound feed (5, 6 and 10), broiler starter compound feed (4 and 5), and broiler finisher compound feed (5 and 8), respectively. Due to a lack of availability, broiler compound feed was not collected from SE.

The nutrient content of feed among the study locations was compared and presented below. Considering the individual sample content, as shown in Table 11, the dry matter content of 22.2, 4.9, and 22.7% of AAS, ES, and SE samples, respectively, was unfit the Ethiopian standard recommended limit, as was the crude protein content of 25, 26.8 and 36.4% of AAS, ES, and SE samples, respectively. Among broilers, 50% of broiler starter compound feed in AAS; 20% of broiler starter compound feed in ES; 40 and 37.5 % of samples of broiler finisher compound feed collected from AAS and ES, respectively were unfit for the DM, CF and CP limit of the Ethiopian standard, as did the ash content of 80 and 100 % of broiler finisher compound feed and 100 and 100 % of broiler starter compound feed and the fiber content of 20 and 13 % of broiler finisher compound feed and 75 and 40 % of broiler starter compound feed.

With the exception of the crude protein content in layer compound feed, all samples of layer and layer grower compound feed collected in either of the study locations were found fit for the dry matter, crude protein, crude fat, and crude fiber content. In the lactating dairy cow compound feed, 71, 33, and 50% of samples for DM; 71, 100, and 63% of samples for CP and 0, 17 and 13% of samples for fiber in AAS, ES and SE, respectively, were unfit based on the Ethiopian standard. In “noug cake”, 13, 13, 38 and 38% in AAS were found unfit for the DM, CP, CF and fiber limit of the Ethiopian standard, as did in 50% of CF or fiber in ES and 33% of DM and 67% of fiber in SE.

Table 11: Unfit individual samples in nutrient content at the study locations (%)

Feed type	Finisher feed		Starter feed		Dairy feed			Layer feed			“noug cake”			Total sample		
	AAS	ES	AAS	ES	AAS	ES	SE	AAS	ES	SE	AAS	ES	SE	AAS	ES	SE
DM	F	F	50	F	71	33	50	F	F	F	13	F	33	22.2	4.9	22.7
CP	40	38	F	20	71	100	63	20	10	50	13	F	F	25	26.8	36.4
CF	F	25	F	20	F	F	F	F	F	F	38	50	F	8.3	9.8	F
Fiber	20	13	75	40	0	17	13	F	F	F	38	50	67	19.4	12.2	13.6
ash	80	100	100	100	ns	ns	ns	ns	ns	ns	ns	ns	ns	22.2	31.7	F

No samples of grower feed were found unfit for DM, CP, CF and fiber, na = not available (In Southern Ethiopia broiler feed type were not found), ns = no limitation in Ethiopian standards list, F= all samples are fit the Ethiopian standard limit, AAS = Addis Ababa and surroundings, ES=East Shoa, SE= Southern Ethiopia, DM=dry matter, CP=Crude protein, CF=Crude fat, total sample= sum of all feed type samples.

4.2.2 Mean nutrient content in feeds

4.2.2.1 Mean nutrient content of feeds in the study area

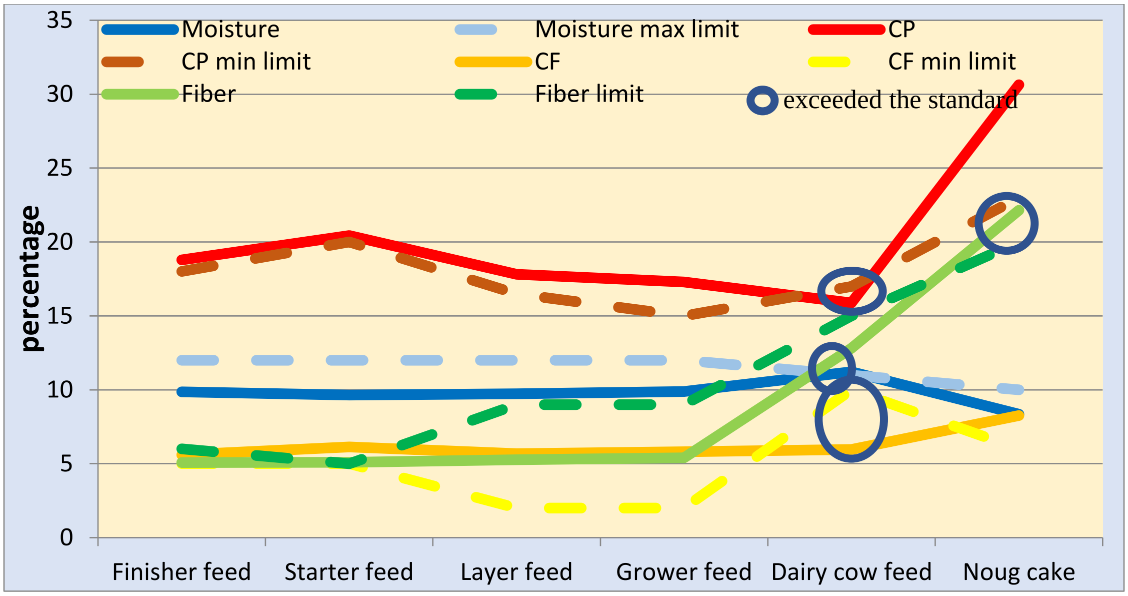
As shown in Table 12 below, in the study area as a whole,, the mean content of broiler finisher compound feed, broiler starter compound feed, layer compound feed, layer grower compound feed, lactating dairy cow compound feed, and “noug cake” were DM (90.14, 90.35, 90.27, 90.11, 88.78, and 91.65%), CP (18.78, 20.43, 17.82, 17.3, 15.88, and 30.64%), CF (5.6, 6.13, 5.69, 5.82, 5.97%, and 8.26%), fiber (5.08, 5.10, 5.28, 5.4, 12.81, and 22.15%), and ash (11.27, 12.21, 11.89, 10.92, 9.31, and 8.52%), respectively.

As shown in Figure 13 below, the mean DM content in each feed type was found to be higher than the Ethiopian standard limit (within the limit) except in lactating dairy cow compound feed. With the exception of lactating dairy cow compound feed, none of the feed mean CP content did not meet the Ethiopian standard requirement. In addition to this, all of the feeds' mean CF and fiber content met the Ethiopian standard requirement, except fiber content in “noug cake”.

Table 12: Mean nutrient contents in feeds in the study area (%) (Mean±sd)

Feed type	DM	CP	CF	Fiber	Ash
Finisher feed	90.14±0.63	18.78±1.80	5.60±0.98	5.08±1.10	11.27±1.61
Starter feed	90.35±0.68	20.43±2.13	6.13±1.09	5.10±0.92	12.21±1.02
Layer feed	90.27±0.56	17.82±1.90	5.69±0.74	5.28±0.62	11.89±1.13
Grower feed	90.11±0.61	17.30±2.25	5.82±0.91	5.40±0.77	10.92±1.66
Dairy feed	88.78±0.65	15.88±1.64	5.97±1.41	12.81±1.97	9.31±1.64
“noug cake”	91.65±1.40	30.64±4.50	8.26±3.36	22.15±5.04	8.52±5.62

Results are on dry matter basis, DM=dry matter, CP=crude protein, CF=crude fat



CP=crude protein, CF=crude fat, limit= the Ethiopian standard limitation

Figure 13: Mean nutrient content of feed in the study area Vs the Ethiopian standard

4.2.2.2 Mean nutrient content of feed at the study locations

As shown in Table 13, the mean nutrient content in feeds at the study locations of AAS, SE and ES was, respectively, layer compound feed: DM (90.5, 90.4, and 90.1%), CP (18.5, 17.9, and 17.4%), CF (5.5, 5.8 and 5.7%), fiber (5.3, 5.7, and 5.0%), and ash (11.6, 11.6, and 12.2%); layer grower: DM (90.4, 90.3, and 89.8%), CP (17.3, 16.9, and 17.5%), CF (5.5, 5.8, and 5.9%), fiber (5.5, 5.8, and 5.1%), and ash (11.5, 11.3, and 10.3%); lactating dairy cow compound feed: DM (88.6, 88.8, and 89%), CP (15.3, 16.4 and 15.9%), CF (6.3, 6.1, and 5.3%), fiber (12, 13.1, and 13.3%), and ash (8.2, 9.9, and 9.8%); and "noug cake": DM (91.7, 91.3, and 92%), CP (29.8, 29.6, and 35.5%), CF (8.3, 10 and 5.3%), fiber (23.2, 19.58 and 21.9%), and ash (7.9, 12.1 and 5.6%). The mean nutrient content in broiler compound feed was: broiler finisher compound feed: DM (90.4 and 90%), CP (19.2 and 18.5%), CF (6 and 5.3%), fiber (5.4 and 4.9%), ash (11.3 and 11.3%) and in broiler starter compound feed: DM (90.3 and 90.4%), CP (20.2 and 20.6%), CF (6.9 and 5.5%), fiber (5.7 and 4.6 %), ash (12.2 and 12.2%) in AAS and SE, respectively.

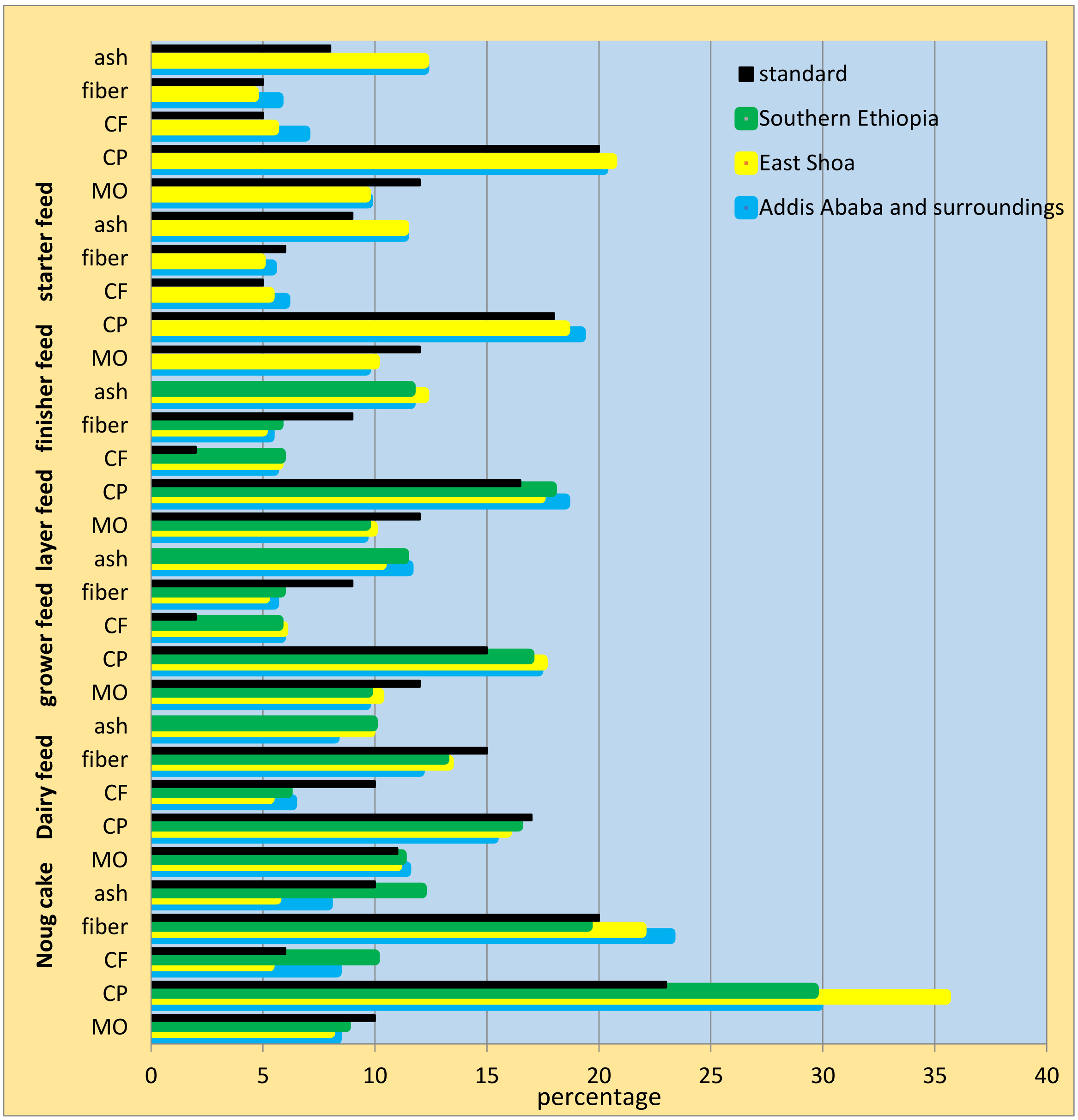
As shown in Figure 13, the mean DM, CF, and fiber content of lactating dairy cow compound feed in each study location met the Ethiopian Standard. However, the mean CP content of lactating dairy cow compound feed samples collected in AAS (15.3%), ES (15.9%), and SE (16.4%) did not meet the Ethiopian standard (17%). In "noug cake", the mean CF content of in ES (5.3%), the mean fiber content in AAS (23.2%) and ES (21.9%), and the mean ash content in SE (12.1%) were unfit for the Ethiopian standard limit (min 6%, max 20% and max 10%, respectively). The ash content of broiler starter and broiler finisher compound feed in each of study locations were unfit based on Ethiopian standard requirements (max 8% and 9%, respectively).

Table 13: Mean nutrient content of feeds at the study locations (%)

Nutrient	Site	*Noug cake*	Dairy Cow	Layer grower	layer	Broiler finisher	Broiler start
Dry matter	AAS	91.7	88.6	90.4	90.5	90.4	90.3
	ES	92.0	89.0	89.8	90.1	90	90.4
	SE	91.3	88.8	90.3	90.4	na	na
	St(min)	90	89	88	88	88	8
Crude protein	AAS	29.8	15.3	17.3	18.5	19.2	20.2
	ES	35.5	15.9	17.5	17.4	18.5	20.6
	SE	29.6	16.4	16.9	17.9	na	na
	St(min)	23	17	15	16.5	18	20
Crude fat	AAS	8.3	6.3	5.8	5.5	6	6.9[a]
	ES	5.3	5.3	5.9	5.7	5.3	5.5[b]
	SE	10	6.1	5.7	5.8	na	na
	St(min)	6	max10	2	2	5	5
Crude fiber	AAS	23.2	12	5.5 [ab]	5.3	5.4	5.7
	ES	21.9	13.3	5.1 [b]	5.0	4.9	4.6
	SE	19.5	13.1	5.8 [a]	5.7	na	na
	St(max)	20	15	9	9	6	5
Total Ash	AAS	7.9	8.2	11.5	11.6	11.3	12.2
	ES	5.6	9.8	10.3	12.2	11.3	12.2
	SE	12.1	9.9	11.3	11.6	na	na
	St(max)	10				9	8

The results are on a dry matter basis., ab = means in the column for each nutrient with different superscript are significantly different at ($p<0.05$), AAS denotes Addis Ababa and surroundings, while ES denotes the East Shoa, and SE denotes Southern Ethiopia, na = not available (In southern Ethiopia, broiler feed samples were not found), ns = there are no standard limitations in the Ethiopian standards list, St = Ethiopian standard requirements, min = minimum, max = maximum.

Based on the ANOVA result, P<0.05, statistically there was no significant difference in mean DM, CP, CF, fiber and ash content of each feed (layer compound feed, layer grower compound feed, broiler starter compound feed, broiler finisher compound feed, lactating dairy cow compound feed and "noug cake") along the study location, with the exception of the fat content of broiler starter compound feed sampled from AAS and ES, and the fiber content of layer grower compound feed in the study locations of ES and SE.



MO=moisture, CP=crude protein, CF=Crude fat

Figure 14: Mean nutrient content of feeds at the study locations Vs Ethiopian standard

4.3 KAP Survey results

Socio-demographic characteristics of feed or oil producers have been identified in Addis Ababa and surroundings (AAS), East Shoa (ES) and Southern Ethiopia (SE) study locations. As shown in Figure 15, a total of 40 respondents (twenty-six feed processors and fourteen oilseed producers) participated in this interview. As shown in Tables 14 from the summary, the majority of compound feed and oil producers who participated in the study were over the age of 30 (85%) and were generally married (62.5%). They were also all literate. All compound feed producers in AAS had at least a bachelor's degree, compared to 90% in ES and 43% in SE. Males made up 83 % of the participants, while females made up 17 %. The majority of those who responded to the survey (75%) are employees of the factory.

Table 14; Socio-demographic characteristics of participants (%)

Location	M	F	<30	30-39	40-49	Single	Married	Households	Employee	Elementary	High school	Degree
SE	71.4	28.6	14.3	42.9	42.9	28.6	71.4	28.6	71.4	0	57	43
ES	87.5	12.5	18.8	37.5	37.5	50	50	37.5	62.5	13	31	56
AAS	82.4	17.6	11.8	64.7	29.4	29.4	70.6	11.8	88.2	6	29	65
Sum	82.5	17.5	15	50	35	37.5	62.5	25	75	8	35	58

AAS=Addis Ababa and surroundings, ES=East shoa , SE=Southern Ethiopia, M=male, F=Female

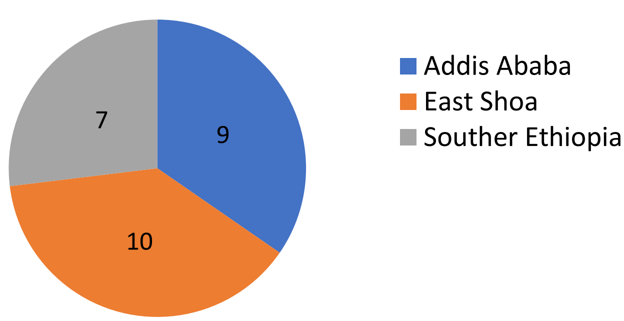


Figure 15: Number of participants (factories) in the study area

Based on purposive sampling approaches, a semi-structured questionnaire result shows KAP (knowledge, attitude, and practice) assessments related to Aflatoxin formation among compound feed and oil factories. According to the findings of the KAP survey, as presented in Tables 15 below, oil producers' awareness of AF, its production, and its impact on animals and humans' health, and livestock productivity is quite poor. Only about 21% of oil producer respondents were aware of aflatoxins' formation, while the remaining 79% was not. Aflatoxin, its causes, and effects, on the other hand, were well understood by 92% of compound feed manufacturing plant respondents.

According to the participants, 54 % of feed producers and 93 % of oil producers never store feed or oil seed cake for more than one month. However, feed ingredients in a compound feed manufacturing plant may be stored for more than a month. All of the respondents stated that they would not purchase contaminated material for use as feed ingredients or for processing into oil.

Moreover, ingredients were physically inspected before being accepted from the supplier by the majority of both compound feed and oil producers (92%), and based on contaminants found in the ingredients, unloading was not permitted. During an interview of four compound feed manufacturing plants, I observed that infected maize and cake were all sensorically checked before being unloaded, and none were accepted. According to the respondent's response and physical observation, only two compound feed producers (8 %) conduct Aflatoxin testing of their finished products and raw materials in their own laboratory.

On the other hand, all compound feed manufacturers and oil producers have feed or feed ingredient storage, but the design of some of them (12 and 14%, respectively) has been called into question. Despite the fact that all of the companies had pallets in their storage, few of the compound feed and feed ingredients were stored on bare ground, according to my observations. Prior to and after storage, all responders reported all cleaning and visual examination of the feed storage.

Table 15; Summary of Aflatoxin KAP result (%)

No	Questions	Compound feed factories		Edible oil factories	
		yes	no	yes	no
1	Being aware of aflatoxins (AFs): the factors that lead to their production; their contamination of feeds; their impacts on human and animal health and livestock productivity; their occurrence in livestock products; and treatments for AFs-contaminated ingredients.	92	8	21	79
2	Being aware of the maximum Aflatoxin limitations in commercial feeds established by the Ethiopian Standard Agency: compound feeds and oilseed cakes.	100	100	43	57
3	Being informed of the feed regulatory body's (VDFACA) and the safety and quality guidelines for feed.	100	100	43	57
4	Implementing selection criteria to minimize AF contamination in ingredients entering into final feed products.	92	8	93	7
5	Holding feed ingredients in storage for less than a month.	54	46	93	7
6	Adequate drying of feed ingredients before storage.	96	4	57	43
7	Preventing mold formation on feed ingredients.	85	15	86	14
8	Solution to reduce the effects of Aflatoxin.	62	38	0	0
9	Using contaminated feed ingredients in compound feed.	0	100	0	100
10	The storage design minimizes the growth of mold.	88	12	86	14
11	Inspecting the feed ingredient storage.	100	0	100	0
12	Feed (bag) storage using palettes.	100	0	100	0
13	Checking the levels of Aflatoxin in feed and feed ingredients before being used.	8	92	0	100

5. DISCUSSION

5.1 Aflatoxins in feeds

Aflatoxins are toxic fungal metabolites that naturally contaminate poultry feed. A. flavus and A. parasiticus are the major species that produce these toxic secondary metabolites. High temperatures and humidity are optimal for mold growth and toxin production. As presented in the results section, aflatoxins were found to be widely distributed in compound feed and oilseed cakes throughout the study area. On the other hand, different studies have reported varying frequencies and levels of aflatoxins in feed globally. However, the current findings (positive samples, 78.8% in all sample and 38.8% in "noug cake") were consistent with Rossi et al. (2012), who used HPLC to investigate Aflatoxin contamination of broiler and laying hen feeds in Brazil and found that 88.2% and 89.7% of samples collected were positive for AFB1, respectively. In Nigeria, Akinmusire et al. (2019) discovered Aflatoxin B1 contamination in 83% of the analyzed feed samples (range, 0.5–760 μg/kg ; mean, 74 μg/kg). In India, Kotinagu et al. (2015) investigated Aflatoxin in feed ingredients and discovered 35.2 % were contaminated with Aflatoxin B1. Nishimwe et al. (2019) found Aflatoxin levels in Rwanda exceeded EU and FDA limits for dairy and poultry at 94.95 µg/kg.

On the other hand, higher AFB1 contamination levels, to a maximum of 9661 μg/kg, were reported in Kenya by Senerwa et al. (2016), with 46% of the feed samples failing to fulfill the FDA AFB1 level recommended for feeds. This report was found in contrast with the current findings, a maximum of 316 μg/kg of AFB1 and 74% of samples was unfit for the Ethiopian standard. However, in Kenya (Kang'Ethe and Lang'A, 2009), similar results were reported that a total of 830 animal feed samples were analyzed for Aflatoxin B1 using a competitive enzyme immunoassay and 86% of the feed samples were positive for Aflatoxin B1, 67% of these exceeded the regulatory limits.

In Ethiopia, different AFs levels in feed were reported. The current findings in total samples (70.8% AFB1 positive samples with a maximum of 316 μg/kg) were found in contradiction with earlier findings by Rehrahie (2018) that the overall sample ranged from 0-20 μg/kg and half of the feed samples were free from Aflatoxin contamination. This variation may have resulted from the feed type included in his study, since low Aflatoxin susceptible feeds like wheat bran were included.

The current findings of AFs in Addis Abeba and surroundings (AAS) study location, 100% positive samples, mean 17 μg/kg, and maximum TAF=426 μg/kg and AFB1=316 μg/kg contamination in dairy cow feed were found to be in good agreement with what Gizachew et al., (2016), Fikadu et al., (2021), and Mengesha et al., (2019) reported from AAS. Gizachew et al. (2016) studied in the Great Addis Ababa milk shed area in dairy cow feed collected from the feed manufacturer and reported a maximum of 412 μg/kg AFB1 contamination level. Fikadu et al., (2021) reported that all feed samples were detected with a mean AFB1 content of 21.9±1. 9, and TAF content in dairy cattle feed was 557.12 μg/kg, and AFB1 content was 370.51 μg/kg (Mengesha et al., 2019). Because AFB1 can be transferred to its milk metabolite, Aflatoxin M1, when mammals consume contaminated feeds, the maximum Aflatoxin value and prevalence measured in dairy cow feeds may lead to poor health effects in both humans and animals.

On the other hand, the mean AF concentrations in the majority of poultry feeds at the AAS study location exceeded the maximum limit of the Ethiopian standards, but among the total samples, only the broiler starter was found to exceed the limit. Also, broiler starter feed are high in crude protein, as indicated in the standard (20%) and as confirmed in this study (20.43%). To overcome this, more concentrates, including “noug cake”, may incorporated into the formulation, and this may result in a high mean AF level.

These levels may negatively impact on the performance of broilers and may end up in broiler meat and human food, with disastrous consequences for human health. The Aflatoxin B1 level and prevalence in layer and layer grower feed may also adversely influences egg quality by decreasing shell thickness, egg weight, and egg energy deposition and leads to reduced egg production, poor egg quality, and increased mortality of challenged hens. Besides, the presence of Aflatoxin in laying hen feed may result in an Aflatoxin residue in the eggs.

In this study, AAS had the most Aflatoxin contaminated feed samples, followed by feeds from the ES and SE. This confirms previous findings by (Gizachew et al. (2016) that AFB1 in dairy cow feed samples in the Greater Addis Ababa milk shed ranged between 9 µg/kg and 412 µg/kg; Mengesha et al. (2019) in dairy cattle feed collected from Addis Ababa and surroundings feed factories reported TAF and AFB1 maximum content of 557.12 µg/kg and 370.51 µg/kg with a mean value of 313.03 µg/kg and 192.80 µg/kg respectively.

Although the findings of this study in the study locations of ES and SE were in agreement with what Rehrahie et al. (2018) reported in Bishoftu and Hawassa, It was found to have an Aflatoxin contamination level of 9.76 µg/kg in Bishoftu and was higher than AFB1 in Hawassa (3.8 µg/kg), thus the lowest AFB1 content of feeds observed in SE. According to Rehrahie et al. (2018), concentrate feeds belonging to feed manufacturers in SE were mainly (80%) wheat bran with some mixture of linseed cake and other home-produced grain by products from barley and pulses, which are relatively less susceptible to Aflatoxin contamination. The author also revealed that oil seed cake based feed contained significantly ($P<0.05$) higher AFB1 (13.09µg/kg) than those without oilseed cake.

As a result of this, the selection of ingredients used in formulating dairy and poultry feeds should be done based on a risk minimization strategy. In formulating this feed, high-susceptible ingredients should not be incorporated or be incorporated with no impact on animal and human health, as well as on the productivity of the poultry and dairy cows.

In this study, the TAF and AFB1 contamination in “noug cake” were registered with a maximum TAF of 549 µg/Kg and a maximum of AFB1 of 375 µg/Kg. This high level of Aflatoxin contamination in “noug cakes” could be attributed to their high fat content when compared to other samples, a lack of knowledge about Aflatoxin contamination and proper storage, as well as a lack of suitable storage. As a result, feed processors should take precautions while utilizing “noug cake” as a feed ingredient to reduce Aflatoxin contamination in finished products.

This study confirmed an earlier study by Gizachew et al. (2016) in Ethiopia, which found that "noug cake" was highly contaminated with AFB1 with a range of 290–397 μg/Kg; Mengesha et al. (2019) found an AFB1 maximum of 438.86 μg/Kg in "noug cake" in Addis Ababa and surroundings ; and Mulugeta (2017) reported AFB1 in "noug cake" ranged from 149 to 887 μg/kg and was also the maximum as found in this study.

In this study, most Aflatoxin contaminated feed samples was recorded in AAS, followed by feeds from the ES and SE. On the other hand, statistically, significant contamination of TAF along the study location was observed in broiler starter, layer grower and layer feed, and significant contamination of AFB1 and prevalence was observed in broiler starter. The variation in Aflatoxin levels among the study locations may be associated with the storage conditions (in the southern region, feed ingredients were not stored for more than a month (Rehrahie et al., 2018)) and also the type and proportion of ingredients used in compound feed formulation (e.g., high proportion of susceptible ingredients like oil seed cakes) (Demissie, 2020). As a result, safe ingredients are necessary for the production of safe feed, which is necessary for animal health, the production of safe livestock products for human consumption, and the preservation of the environment.

5.2 Feeds Nutrient Content

Animals can reach their genetic potential if fed a high-quality ration in sufficient quantities (Martin et al., 2018). Finding a high-quality feed that meets the nutrient needs of livestock based on their age, gender, and physiological stage is the livestock industry's most visible challenge. Even though sufficient nutrients are required for any animal's metabolic function and health, any nutrient deficiency in livestock feed will have an impact on animal growth (Ogbebor et al., 2021).

According to Vakili et al. (2015), the major nutrient values of poultry and dairy cow feeds that are taken into account when formulating diets are crude protein, crude fat, moisture, crude fiber, and ash in the appropriate proportions for different stages of growth. The study found the nutrient content of poultry feed, dairy cow feed, and oilseed cakes manufactured and collected in the study area at the time of sampling.

Accordingly, one fifth of the samples collected in Addis Ababa and surroundings (AAS) or Southern Ethiopia (SE) failed to meet the dry matter requirements of the Ethiopian standards. Feed samples with unfit nutrient content may also be caused by the presence of molds in the feed that produce aflatoxins. In the literature, it is reported that when conditions are optimal for molds to colonize feed and feed ingredients in the field or storage, their first effect is the utilization of nutrients for their metabolism and propagation, which results in a decreased nutrient value of feeds. The crude protein and crude fat values of moldy feed and feed ingredients were decreased by 6% and 63%, respectively, and dietary fats were affected more extensively than proteins; they decreased by 37% to 40% after 25 days of storage or 52% to 57% after 50 days of storage.

In the current study, the majorities of feed samples were contaminated with AF and this may have resulted, as one factor, in the nutrient content of feed being unfit. Thus, preventing mould growth and the formation of Aflatoxin in feed and feed ingredients is critical, not only to save the health of animals and humans but also to safeguard the nutrient content of feed or feed ingredients for livestock productivity.

To prevent the production of Aflatoxin, the dry matter (DM) in feed is a key to safe storage and as a quality indicator. Aspergillus species frequently infect improperly dried and stored feed with temperatures ranging from 24 to 35 °C and a dry matter content of less than 7%, exposing the feed to aflatoxins. Thus, some feed samples observed with a lower dry matter content in this study are susceptible to mold growth and the formation of aflatoxins and were found to have high Aflatoxin contamination as registered in this study. Thus, proper drying of feed and feed ingredients should be practiced along the feed value chain actors.

The nutrient content of poultry feed has been reported by various researchers with various composition levels in the literature. Some of the findings are in agreement with the current findings. Vakili et al. (2015) discovered DM 90.45% and CP 23.59% for starters, and CP 19.64% and ash 3.79% for finishers; Mokubedi et al. (2019) conducted research in South Africa and reported CP at 18% for broiler starters; Dewa and Tikau (2019) conducted research in Nigeria and found CP 20.16%, CF 7.5%, and ash 11.53% for starter feed, fat 6.04% for grower feed, and fat 6.04% for finisher feed.

The results of this research were also similar to Souliana et al. (2020) findings: DM% 89.1%, CP 21.23%, CF 4.98%, fiber 7.11%, and ash 15.34%-21.57% for broiler feed and ash 15.67% for layer feed. Ofori et al. (2019) reported consistent results with the current findings; DM and CP for starter were 89.52% and 18.50%; grower 89.6% and 20.97%; layer 89.3% and 16.76%; and broiler finisher 89.6%; as well as fat content of 2.4737%, 3.657%, 2.7095%, and 2.7%; and ash content of 6.7%, 8.0%, 17.07%, and 14.84% for starter, grower, layer, and broiler were reported, respectively.

In Ethiopia, a study done by Demissie (2020) in Oromiya, Amhara, Tigray, Addis Ababa, and southern regions reported fat of 4.91% and fiber of 7.34% for layer feed; Feyissa et al. (2015) studied in Ethiopia with a DM of 90.9%, CP of 19.08%, and ash of 9.25% in dairy cow feed and both findings were in agreement with the current results except CP content in dairy feed. The current manufactured dairy cow feed was in shortage of CP in each of the study locations. Feeding dairy cows with low-protein diets can result in production losses. These low protein content samples, registered in some of the feed, might have resulted from using too much of the low protein raw materials like wheat bran and middlings. Thus, the feeds need to be improved with the addition of an adequate proportion of concentrated ingredients such as oil cakes during the ration formulation, with care to avoid Aflatoxin contamination.

On the other hand, the protein and fat content of "noug cake" ranged from 28–38% and 2.1–12.6%, as reported by Adugna (2008), which was in agreement with the current findings. Feyissa et al. (2015) conducted a study throughout Ethiopia on the nutrient content of oilseed cakes and reported 28.54% CP content for "noug cake"; Souliana et al. (2020) also reported on the DM, CF, and ash content of "noug cake", 8.6%, 92.1%, and 7%, respectively, and both authors were in agreement with the current findings. A similar result was also reported by Mulugeta (2017), who said that out of twelve “noug cake” samples analyzed, the moisture content was in the range of 9.25–23.6%. However, Vakili et al. (2015), Mokubedi et al. (2019), Dewa and Tikau (2019), Ofori et al. (2019) and Demissie (2020) reported differently with the current findings, ash of 4.77% for starters, CP 15% and fiber 7% for finisher feed, and an ash of 10.3% for grower feed, CP 16.15% for broiler finisher, and CP 14.68% for layer feed, respectively.

The types and proportions of materials used during feed formulation, the efficiency and technology levels of manufacturing plants, as well as the management of the feed plant, are all possible reasons for such variations in the nutrient quality of feeds. As a result, feed processors are one of the most significant organizations responsible for ensuring that their feed is fit for its purpose and complies with regulatory standards. Variations in the nutrient content of the “noug cake” samples, on the other hand, could be related to the type of oilseed utilized, the method and effectiveness of extracting oil from the seeds, or other factors.

5.3 KAP Survey

The findings of this study on the knowledge, attitude, and practices (KAP) concerning Aflatoxin were similar to the previous findings by Demissie (2020) that fifteen manufacturers never analyses their compound feeds, 24 manufacturers never analyses raw materials, and two companies analyses their feeds. Mulugeta (2017) assessed the KAP of feed producers, dairy farmers, and feed traders towards Aflatoxin around Addis Ababa, and he reported, in agreement with the current findings, that about all the feed producers (five in number) respondents were aware of mold growth and the formation of mycotoxin, whereas all (twenty-five in number) feed sealers were not. Merwe et al. (2019) studied mycotoxin contamination in 933 samples of feed in Ethiopia and revealed findings that were similar to the current findings about storage conditions. According to the author's study, storage conditions at feed distribution facilities were typically good.

The knowledge concerning Aflatoxin was not satisfactory in the oilseed cake factories; oilseed cakes may be handled and stored improperly, resulting in contaminated feed. This could be one reason why "Noug Cake" has the largest concentration of AFs right now. On the other hand, high awareness of aflatoxins' production was observed in the majority of the feed manufacturing plants. This may have resulted from quality control qualified experts (with a Bachelor's degree in animal production) employees in the feed majority of manufacturing plants. Feed ingredients in some of feed manufacturing factories can be stored for more than a month. This approach can be problematic in feed handling if it is not well maintained, as fungus can quickly develop in storage, resulting in the production of Aflatoxin.

Cleaning and visual examination of the feed storage prior to and after storage, as noted by the respondents, are crucial in feed handling because fungus can easily grow in the storage and cause Aflatoxin production. Only two feed producers (8%) test their finished products and raw materials for aflatoxins in their own laboratories, according to the report. Because Aflatoxin is odorless, tasteless, and colorless (and hence difficult to detect with the naked eye), contaminated raw materials can be transformed into compound feed products, causing animal and human health issues.

6. CONCLUSSION AND RECOMMENDATION

In this study, the majority of the current manufactured commercial feed samples (more than 70%) were contaminated with AFB1, and more than 45% were prevalent. The current study makes evident that Aflatoxin (AF) contamination in commercial feeds, notably in AAS, is alarming since in AAS, ES, and SE, in that order, the individual sample prevalence of AF was higher, as for broiler finishers, starters, layers, dairy, growers, and "noug cake". Furthermore, except layer feed, AAS had the highest individual levels of Aflatoxin in each feed type. Among total samples, the highest unfit samples were noted, in descending order, for CP, fiber, CF, and DM content. The most unfit samples for: DM and fiber content were found in AAS, ES, and SE; CP content in ES, SE, and AAS; CF content in SE, AAS, and ES, in descending order. Oilcake producers lacked adequate AF knowledge, and only a few feed processors had their own laboratory to test for aflatoxins in feed. The mean AF level of each poultry feed at the AAS study location exceeded the limit, and in neither study location did the mean CP requirements fit the limit. No significant difference in the mean nutrient content, mean AF level and prevalence of AF in feeds was observed among the study locations, except for the mean CF, mean AFB1 and TAF level and prevalence of AFB1 in broiler starter feed; mean TAF level and mean fiber content in layer grower and mean TAF level in layer feed. The highest AF level, prevalence, and positive samples in feeds investigated in this study are animal and public health concerns. In light of this:

- Compound feed processors must implement strict quality control in feed; should indeed test the levels of aflaoxin in all materials used as feed, including the nutrient content as necessary; and need to follow good manufacturing practices (GMP), hazard analysis, and critical control points (HACCP), as well as binders, blending, and decontamination technologies.
- Regulations must be properly applied by the responsible authorities in order to ensure that commercial feeds meet quality and safety standards.
- It is critical to maintain training aimed at minimizing Aflatoxin in feeds.
- Further research is also needed on the Aflatoxin that is contained in ingredients used in commercial feed.

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8. APPENDIX

Appendix 1: Interview form

1	GENERAL INFORMATION									
i	Administrative zone Town									
ii	Date of interview									
iii	Farm/feed producer									
	Gender	O	Male	O	Female					
i	Age(year)	O	<30	O	30 - 39	O	40 -	O	50 -	O
ii	Marital status	O	Married	O	Single					
iii	occupation	O	Household	O	Employee	O	other			
iv	Level of education	O	Elementary	O	High School	O	Bachelor degree			O
v	Year of experience									

2 KNOWLEDG, ATTITUSE AND PRACTICE	
	Put cross mark (×) in the relevant boxes to indicate your response
1	Are you aware of Aflatoxins (its cause, health impact, productivity impact, its contamination in feed, its occurrence in livestock products, preventive measures and any solutions) O Yes O no
2	Do you heard of about mandatory Aflatoxin max limits of in feed O Yes O no
3	Do you know VDFACA O Yes O no

4	Do you have selection criteria for contamination of			O Yes O No	
5	If your answer for question 4 is yes, which criteria you practiced?				
	a) Mould growth b) insect infestation c) color change d) other (write) ___________				
6	How long is feed stored in your practice?				
	a) <1 month	b) 1-3 month	c) 3-6 month	d/) 6-12 month	e) >12
7	Have you got a problem when you store feed for a longtime?				
	a) Yes, the problem was mould		b) Not stored for long time		
	c) Yes, insect infestation problem		d) No problem		
8	Do you practice adequate drying of feed before storage?			O Yes O No	
9	Is there a practice of selling mouldy feed?			O Yes O No	
10	If your answer for question number 9 is yes what are the reasons for selling mouldy				
	a) No effect on human and animal		b) No effect on livestock productivity		
	c) I consider only my profit		d) Other (write)		
11	Do you have practice of protecting feed from mould growth?			O Yes O No	
12	If your answer to question number 11 is yes, which measures you practice				
	a) Adequate storage	b) Receiving mould free ingredients		c) Regular	
	d) Adequate drying of feed	e) Low moisture in feed		f) Other (write)--------	
13	Do you have practical solution for Aflatoxin contaminated feed			O Yes	
14	If your answer to question number 13 is yes, which practice used as a solutions				
	a) Biding agent	b) dilution	c) reprocessing	d) other(write)-------------	
	e)	f)	g)	h)	

PRACTICE (P)		Yes, excellentl	Yes, moderate	no
1	Does your storage design not favor mould growth?	O	O	O
2	Do you have practices of inspecting the store before storing feed?	O	O	O
3	Do you store feeds (bags) on palates	O	O	O
4	Does your storage temperature and humidity not favor mould growth?	O	O	O
5	Do you regularly control the moisture content in feed	O	O	O
6	Do you have control system of rodent and insects in the storage	O	O	O
7	Do you check mould occurrence before receiving ingredient	O	O	O
8	Do you check Aflatoxin level before distribution of feed	O	O	O

Appendix 2: ANOVA results

ANOVA result on Total Aflatoxin contamination in feeds

Feed type	**Mean**	**SD**	**Median**	**Min**	**Max**
Broiler finisher feed	28.9	8.4	55.3	0.0	393.0
Broiler starter feed	21.4	9.1	63.4	0.0	268.7
Layer feed	16.1	5.9	15.0	0.0	389.0
Layer grower feed	12.4	7.4	24.9	0.0	233.0
Lactating dairy feed	21.45	5.61	38.74	0	426
"noug cake"	6.67	14.62	0	0	549.14

ANOVA result on Aflatoxin B1 contamination in feeds

Feed type	**Mean**	**SD**	**Median**	**Min**	**Max**
Broiler finisher feed	15.0	8.1	30.0	0.0	190.0
Broiler starter feed	11.9	6.9	28.4	0.0	128.0
Layer feed	8.3	4.5	7.0	0.0	174.0
Layer grower feed	6.8	5.5	12.5	0.0	116.0
Lactating dairy feed	10.2	5.4	19	0	316
"noug cake"	5.01	10.36	0.00	0.00	375.00

ANOVA result on Total Aflatoxin value of starter feed among study locations

location	Mean	Std.Dev	Median	Min	Max	P value
AAS	157.215	0.86573	172.911	75.8086	268.729	0.0139
ES	3.67267	5.52382	1	0	63.4171	

ANOVA result on Aflatoxin B1 value of starter feed among study locations

location	Mean	Std.Dev	Median	Min	Max	P value
AAS	75.8699	0.80174	85.7337	34.98	128	0.01273
ES	2.0798	3.91367	0	0	28.4143	

ANOVA result on Total Aflatoxin value of finisher feed among study locations

location	Mean	Std.Dev	Median	Min	Max	P value
AAS	130.036	3.6478	230.97	8	393	0.02811
ES	10.8588	7.78627	13.5602	0	218.34	

ANOVA result on Aflatoxin B1 value of finisher feed among study locations

location	Mean	Std.Dev	Median	Min	Max	P value
AAS	52.5657	8.36063	119	0	190	0.05568
ES	6.4844	5.95533	6.21863	0	109.42	

ANOVA result on Total Aflatoxin value of layer feed among study locations

location	Mean	Std.Dev	Median	Min	Max	P value
AAS	52.6359	1.44252	63	11.3057	139.043	0.1039
SE	2.59669	6.34404	0.48324	0	163	
ES	23.5266	5.12533	23.8782	0	389.006	

ANOVA result on Aflatoxin B1 value of layer feed among study locations

location	Mean	Std.Dev	Median	Min	Max	P value
AAS	24.0602	1.5835	30	4.09714	63.7457	0.1526
SE	1.81255	4.30473	0.48324	0	74	
ES	10.5364	4.5327	10.7196	0	173.977	

ANOVA result on Total Aflatoxin value of grower feed among study locations

location	Mean	Std.Dev	Median	Min	Max	
AAS	63.5649	2.10999	53	14.59	233	0.03646
SE	1.52678	6.94636	0	0	102	
ES	9.31502	6.86874	6.61577	0	125	

ANOVA result on Aflatoxin B1 value of grower feed among study locations

location	Mean	Std.Dev	Median	Min	Max	P value
AAS	24.9539	3.37273	26	0.84	116	0.06057
SE	1.15983	4.5948	0	0	46	
ES	5.45171	5.18087	4.83095	0	55	

ANOVA result on Total Aflatoxin value of lactating feed among study locations

location	Mean	Std.Dev	Median	Min	Max	P value
AAS	30.3923	4.75011	38.74	1.36857	426	0.1432
SE	18.6819	6.72275	40.3522	0	182	
ES	17.0989	7.3444	38.9231	0	125	

ANOVA result on Aflatoxin B1 value of lactating feed among study locations

location	Mean	Std.Dev	Median	Min	Max	P value
AAS	17.583	4.87416	26.2571	1	316	0.7664
SE	9.75376	5.40501	19	0	80	
ES	5.53853	7.11294	3.58258	0	60	

ANOVA result on Total Aflatoxin value of "noug cake" among study locations

location	Mean	Std.Dev	Median	Min	Max	P value
AAS	6.80953	16.5425	0	0	549.143	
SE	7.00521	35.7018	0	0	512	0.1923
ES	5.7082	13.757	5.7082	0	44	

ANOVA result on Aflatoxin B1 value of "noug cake" among study locations

location	Mean	Std.Dev	Median	Min	Max	P value
AAS	4.69324	10.3451	0	0	226.963	
SE	6.21765	29.6751	0	0	375	0.1923
ES	4.65685	10.5958	4.65685	0	31	

ANOVA result on Total Aflatoxin value of total samples among study locations

location	Mean	Std.Dev	Median	Min	Max	P value
AAS	38.9694	7.61731	65.453	0	1708	0.2462
SE	5.09569	8.18989	0.48324	0	512	
ES	8.47397	6.89132	3	0	389.006	

ANOVA result on Aflatoxin B1 value of total samples among study locations

location	Mean	Std.Dev	Median	Min	Max	P value
AAS	16.2867	7.02764	27.3123	0	1433	
SE	3.48359	5.94519	0.04881	0	375	0.4619
ES	4.73595	5.35271	1	0	251	

ANOVA result on Nurtient Content in finisher feed among the study locations

Nutrient	location	Mean	SD	Min	Max	P value
Dry matter	AAS	90.837	0.439	90.33	91.11	0.0196
	SE	89.663	0.212	89.49	89.9	
	ES	89.891	0.531	89.32	90.62	
Crude Protien	AAS	18.56	1.786	17.45	20.62	0.7973
	SE	19.333	0.252	19.1	19.6	
	ES	18.614	1.842	16.15	20.41	
Crude fat	AAS	5.99	1.011	5	7.02	0.6295
	SE	5.68	0.661	5.1	6.4	
	ES	5.383	1.002	3.97	6.77	
Fiber	AAS	5.973	0.689	5.33	6.7	0.0292
	SE	3.497	0.285	3.21	3.78	
	ES	4.838	1.14	3.71	7.37	
Ash	AAS	10.907	3.079	8.68	14.42	0.678
	SE	10.333	0.611	9.8	11	
	ES	11.274	1.157	9.29	13.16	

ANOVA result on Nurtient Content in starter feed among the study locations

Nutrient	Location	Mean	SD	Min	Max	p value
Dry matter	AAS	90.547	0.551	90.07	91.15	0.5222
	SE	89.8	0.566	89.4	90.2	
	ES	90.253	0.757	89.09	91.17	
Crude Protien	AAS	20.547	2.496	18.33	23.25	0.4476
	SE	22.65	0.212	22.5	22.8	
	ES	20.372	2.191	17.28	23.28	
Crude fat	AAS	6.71	0.755	5.91	7.41	0.3745
	SE	5.45	0.495	5.1	5.8	
	ES	5.838	1.165	4.49	7.56	
Fiber	AAS	6.067	0.673	5.56	6.83	0.0183
	SE	4.725	0.205	4.58	4.87	
	ES	4.617	0.571	3.58	5.14	
Ash	AAS	11.983	1.641	10.61	13.8	0.2016
	SE	8.695	5.791	4.6	12.79	
	ES	12.327	0.732	10.94	12.99	

ANOVA result on Nurtient Content in layer feed among the study locations

Nutrient	location	Mean	SD	Min	Max	P value
Dry matter	AAS	90.552	0.167	90.33	90.73	
	SE	90.383	0.623	89.17	90.9	
	ES	90.07	0.603	89.19	91.42	0.3046
Crude Protien	AAS	18.535	2.308	16.06	21.64	
	SE	17.903	2.655	14.61	21.32	
	ES	17.42	1.435	15.27	19.46	0.647
Crude fat	AAS	5.43	0.48	4.95	5.97	
	SE	5.78	0.937	4.61	7.45	
	ES	5.759	0.774	4.41	7.14	0.7467
Fiber	AAS	5.383	0.389	4.89	5.8	
	SE	5.703	0.751	4.79	6.57	
	ES	4.959	0.498	4.06	5.71	0.0609
Ash	AAS	11.43	1.18	10.54	13.1	
	SE	11.568	1.623	9.47	14.37	
	ES	12.216	0.782	10.66	13.49	0.4113

ANOVA result on Nurtient Content in layer feed among the study locations

Nutrient	location	Mean	SD	Min	Max	P value
Dry matter	AAS	90.372	0.45	89.88	90.8	
	SE	90.308	0.582	89.68	90.93	
	ES	89.903	0.559	88.93	90.9	0.2217
Crude Protein	AAS	16.516	1.311	15.49	18.78	
	SE	16.884	2.175	15.08	20.42	
	ES	17.582	2.523	14.74	23.65	0.6558
Crude fat	AAS	5.72	0.959	4.76	6.88	
	SE	5.66	1.069	4.34	7.02	
	ES	6.06	0.908	5.17	8.27	0.7026
Fiber	AAS	5.446	0.507	4.68	5.99	
	SE	5.792	0.43	5.1	6.2	0.2234
	ES	5.073	0.913	3.51	6.7	
Ash	AAS	11.61	0.655	11.18	12.75	
	SE	11.25	2.101	9.64	14.56	
	ES	10.39	1.752	8.58	13.79	0.3773

ANOVA result on Nurtient Content in lactating dairy feed among the study locations

nutrient	location	Mean	SD	Min	Max	P value
Dry matter	AAS	88.47	0.65	87.5	88.88	0.6311
	SE	88.786	0.492	88.15	89.5	
	ES	88.861	0.795	87.61	89.77	
Crude Protein	AAS	15.217	2.17	14.02	18.47	0.6671
	SE	16.181	1.359	14.73	18.02	
	ES	15.993	1.801	12.64	18.3	
Crude fat	AAS	5.88	1.74	4.09	7.56	0.7986
	SE	6.319	1.076	4.78	8.02	
	ES	5.83	1.666	3.85	9.29	
Fiber	AAS	12.363	1.927	10.09	14.45	0.7814
	SE	13.266	1.884	11.56	17.09	
	ES	12.822	2.245	8.55	15.2	
Ash	AAS	8.053	1.131	7.19	9.62	0.1778
	SE	9.976	1.343	8.5	12.56	
	ES	9.189	1.847	6.7	12.26	

ANOVA result on Dry matter Content of feeds

Compound Feed type	N	Mean	SD	Min	Max
Broiler Finisher feed	13	90.142	0.625	89.32	91.11
Broiler Starter feed	9	90.351	0.675	89.09	91.17
Layer feed	21	90.271	0.554	89.17	91.42
Layer Grower feed	22	90.11	0.608	88.93	90.95
“noug cake”	13	91.651	1.40	88.86	93.65
Lactating Cow feed	21	88.779	0.649	87.5	89.77

ANOVA result on Crude protein Content of feeds

Compound Feed type	N	Mean	SD	Min	Max
Broiler Finisher feed	13	18.781	1.793	16.15	20.94
Broiler Starter feed	9	20.43	2.137	17.28	23.28
Layer feed	21	17.815	1.92	14.61	21.64
Layer Grower feed	22	17.298	2.252	14.74	23.65
“noug cake”	13	30.635	4.484	22.01	36.64
Lactating Cow feed	21	15.875	1.643	12.64	18.47

ANOVA result on Crude fat Content of feeds

Compound Feed type	N	Mean	SD	Min	Max
Broiler Finisher feed	13	5.599	0.981	3.97	7.02
Broiler Starter feed	9	6.129	1.087	4.49	7.56
Layer feed	21	5.688	0.738	4.41	7.45
Layer Grower feed	22	5.824	0.907	4.34	8.27
“noug cake”	13	8.258	3.357	4.56	15.79
Lactating Cow feed	21	5.967	1.416	3.85	9.29

ANOVA result on Fiber Content of feeds

Compound Feed type	N	Mean	SD	Min	Max
Broiler Finisher feed	13	5.076	1.102	3.71	7.37
Broiler Starter feed	9	5.1	0.918	3.58	6.83
Layer feed	21	5.276	0.62	4.06	6.57
Layer Grower feed	22	5.395	0.765	3.51	6.7
“noug cake”	13	22.15	5.044	15.51	29.39
Lactating Cow feed	21	12.81	1.966	8.55	17.09

ANOVA result on Ash Content of feeds

Compound Feed type	N	Mean	SD	Min	Max
Broiler Finisher feed	13	11.271	1.612	8.68	14.42
Broiler Starter feed	9	12.212	1.018	10.61	13.8
Layer feed	21	11.885	1.13	9.47	14.37
Layer Grower feed	22	10.915	1.657	8.58	14.56
“noug cake”	13	8.52	5.617	4.86	22.32
Lactating Cow feed	21	9.311	1.639	6.7	12.56

Appendix 3: Fishers exact test result among the study locations

	Fisher’s exact test	P-value
Feed type	TAF	AFB1
“noug cake”	1	1
Dairy feed	1	1
Layer grower feed	0.3997	0.3997
Layer feed	0.1482	0.1482
Broiler starter feed	0.0476	0.047
Broiler finisher feed	0.5649	0.5649