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Research article

Aflatoxines Contaminations Levels in Animal Feeds and Milk around Addis Ababa, Ethiopia

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Aflatoxins are toxic and carcinogenic metabolites produced by a variety of fungi. Risk of aflatoxin contamination of commodities in the world, especially in Africa which in turn in Ethiopia were increasing. Aflatoxins are natural toxins that contaminate various types of feedstuffs and food leading to health risk in both humans and animal feeds. Milk is a highly nutritious food, and it is a source of necessary macro- and micronutrients for the growth, development and maintenance of human health. This work was conducted to review the level of aflatoxin in animal feeds and milk from high potential place around Addis Ababa district, most milk products supplied for the great city of Ethiopia, Addis Ababa. Aflatoxin B1 and M1 are the major carcinogenic type frequently found in animal feed and milk, respectively thus posing a significant impact on human health. The results of the present review have shown that aflatoxin contamination in samples of animal feeds and milk were alarmingly high in major both samples. This is evidently posing a dangerous problem to the feed and milk industry as well as human health. Animal feeds specially prepared from industrial by products like noug cake highly contaminated with aflatoxin B1 which leads to contaminate produced milk also. Many researches indicated that animal feeds are highly affected by aflatoxin B1 which is the most common aflatoxin in the county and Aflatoxin M1 was also the greater aflatoxin which is found greater than the standard in the produced milk. This indicates that a lot of developing countries including Ethiopia are at risk of aflatoxin contamination in milk. Lack of awareness on aflatoxin contamination increases the risk of damage to human and animals. High economic losses due to aflatoxin occur in the country livestock feeds and milk.

Key words: Aflatoxin, Feedstuffs, Milk, Carcinogenic, Aflatoxin B1and Aflatoxin M1

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INTRODUCTION

Food safety and security are among the major problems in the current climate of increasing population. These are mainly determined by three key aspects viz., enough food availability, and access to safe food and utilization of the food in terms of quality, nutritional and cultural purposes for a healthy life (FAO, 1996). The failure of any of these aspects leads to food insecurity and malnutrition that further influences human health, in addition to the socio-economic aspect of society. In addition, food and feed contamination by mycotoxins are one of the key factors responsible for creating food insecurity (Udomkun *et al.,* 2017)

A toxin can be defined as a substance that is synthesized by a plant species, an animal or by micro-

organisms, that is harmful to other organisms (Turner *et al*., 2009). Mycotoxins are substances produced by fungi that are poisonous or 'toxic' to mammals. Mycotoxins are classed as 'secondary' metabolites, because they are not considered essential for the 'primary' purpose of growth and reproduction in fungi. Nevertheless, secondary metabolites have important roles, such as helping the fungus to invade plant tissue and as defence against insect predators or competing fungi.

Milk is a highly nutritious food containing many macro- and micronutrients that are essential for the growth and maintenance of human health. The health of human populations is often reflected in the condition of their food-producing ecosystems. Moreover, the implementation of food regulations may be directly linked with the quantity and quality of available food. Therefore, consumers from developing countries, especially from rural areas, face issues related to food security and food safety because they depend on locally produced foods (Marroquin-Cardona, Johnson, Phillips and Hayes, 2014). The presence of aflatoxin M1 (AFM1) in milk and dairy products is an important issue, especially for developing countries (Prandini *et al*., 2009).

Milk has the greatest potential for introducing AFM1 into the human diet and the possible presence of AFM1 in milk and their products represents a worldwide concern, mainly because the major consumers are children, who are more susceptible to immunosuppressive, mutagenic, teratogenic, and carcinogenic effects (Sefidgar *et al,* 2011). The source of AFM1 in milk has been reported to be Aflatoxin B1 (AFB1) present in feed of lactating animals, which gets transformed to 4-hydroxylated metabolite in liver and is excreted in milk.

Dairy plays an important role in the Ethiopian agricultural sector and the national economy (Azage *et al*., 2013). The sector is a source of livelihoods for a vast majority of the rural population in terms of consumption, income generation and employment. Estimates by the nation's Central Statistical Agency (CSA, 2014) indicate that there are about 55 million cattle, of which 44.6% are males and 55.4% are females. The same source further indicated that 2.8 billion litters of milk were produced in 2012-13, out of which 42.3% was used for household consumption. Milk is a common health drink consumed by people of all age groups especially children. Milk is a product of biological evolution; its role in human nutrition is well known and its biochemical complex which appears to be the only material to function solely as a source of food (Fallah, 2010).

Aflatoxins

Aflatoxins are a group of mycotoxins mainly produced by several fungus species in the genus Aspergillus. It includes A. flavus and A. parasiticus, A. pseudotamarii, and A. nomis species. Among these species A. flavus and A. parasiticus are well known. These organisms invade crops and grow on foods during storage if temperature and humidity levels are favorable. The relative proportions and amounts of the various aflatoxins on food crops depend on the Aspergillus species present, pest infestation, growing and storage conditions, and other factors. Although these species have similar geographical ranges, A. parasiticus is less widely distributed and A. flavus is the most widely reported fungus in foodstuffs. Aflatoxins are metabolized in ruminants by the liver and excreted in the bile. The major aflatoxins produced in feed stuffs are B1, B2, G1, G2, M1 and M2. Both A. flavus and A. parasiticus produce aflatoxins B1 and B2, and A. parasiticus also produces aflatoxins G1 and G2 (Ramesh *et al*., 2003). Many metabolites of aflatoxin have been discovered, but four occur naturally, namely aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), and aflatoxin G2 (AFG2) (Wu *et al*., 2013). The members of blue fluorescent (B) series are characterized by a fusion of a cyclopentenone ring to the lactone ring of the coumarin moiety, whereas the green fluorescent (G) toxins contained a fused lactone ring (Kensler *et al*., 2011). Of these aflatoxins, AFB1 is the most potent and carcinogenic toxicant as it has been classified by the International Agency for Research on Cancer (IARC) as Group 1 carcinogen that is linked to the development of hepatocellular carcinoma (IARC, 2002). Furthermore, AFB1 can also cause teratogenic and mutagenic effects to animals and possibly in humans (Wild and Turner, 2001).

B1 and M1 Aflatoxins

Among these toxins, Aflatoxin B1 (AFB1) is considered the most recurrent and also the most harmful. Its carcinogenicity and immunosuppression capacity have been extensively reported in all kind of animals, including poultry, trout, cattle and rats with different incidence across species, gender and age. The toxicity in humans has been assessed in association with different outbreaks of acute intoxication, especially in developing countries. Many epidemiological studies focused on the connection between aflatoxins assumption through contaminated food and health problems. Several in vitro studies demonstrated that the carcinogenicity of AFB1 is prevalently exerted upon activation by Cytochromes P450 (CYP450) in the liver and elucidated the mechanism of its toxicity. Immuno-response modulation has been observed on murine macrophages after AFB1 exposure; in fact, some authors showed an anti-proliferative action not related to apoptotic pathways and a reduction in NO levels upon exposure to not cytotoxic concentrations.

Aflatoxin M1 (AFM1) the principal hydroxylated metabolite of AFB1, found in milk (hence the designation M) of mammals fed upon contaminated feedstuff. Carry-over of AFB1 as AFM1 in the milk of dairy cows has been established to range from 0.3% to 6.2%. However, AFM1 was also found in lactating mother's milk. Several studies reported carcinogenic and immunosuppressive effects similar to that of AFB1, on both humans and other animals, even if with a less potent effect. AFM1 exerted even in absence of the metabolic activation typically needed to AFB1, thus pointing out that caution should be put when classifying AFM1 as essentially detoxification product of AFB1 metabolism. However, AFM1 is the only mycotoxin for which maximum residue limits (MRLs) in milk were established.

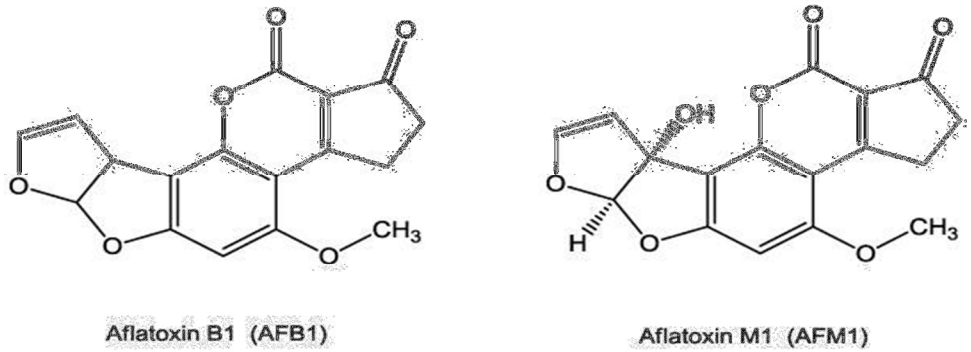

Figure 1: Chemical structures of Aflatoxin B1 and Aflatoxin M1.

Properties of aflatoxins

Aflatoxins are produced by fungi in the genus Aspergillus that grow on grains and other agricultural crops. They exist as colour less to pale-yellow crystals at room temperature. They are slightly soluble in water and hydrocarbons, soluble in methanol, acetone, and chloroform, and insoluble in non- polar solvents. Aflatoxins are relatively unstable in light and air, particularly in polar solvents or when exposed to oxidizing agents, ultraviolet light or solutions with a PH below 3 or above10. Aflatoxins decompose at their melting points, which are between 237 °C (G1) and 299 °C (M1), but are not destroyed under normal cooking conditions. They can be completely destroyed by autoclaving in the presence of ammonia or by treatment with bleach. Physical and chemical properties of aflatoxins are listed in the following (IARC, 2002).

Physical properties of aflatoxins

Aflatoxins are crystalline odourless solids when isolated and the colour range from pale white to yellow. The melting points range from 268 °C for B1 down to 190 °C for G2. The optimal water activity for growth of *A. flavus* is high (about 0.99). The maximum is at least 0.998 whereas the minimum water activity for growth has not been defined. In general, production of toxins appears to be favoured by high water activity. Aspergillus flavus is reported to grow within the temperature range 10-43 °C. The optimal growth rate occurs at a little above 30 °C, reaching as much as 25 mm per day. The aflatoxins are produced by *A. flavus* over the temperature range 15-37 °C. It is not possible to specify an optimum temperature for the production of the toxins, although production between 20-30 °C is reported to be significantly greater than at higher and lower temperature (Waliyar and Reddy, 2003).

Chemical properties of aflatoxins

Aflatoxins belong to the group of difuranocoumarins. The compounds are usually soluble in methanol, chloroform, acetone and acetonitrile which are slightly polar but insoluble in non-polar solvents. Aflatoxins react with alkaline solutions causing the hydrolysis of the lactones moiety. This hydrolysis is reversible since it has been shown that racialization occurs following acidification of basic solution containing aflatoxin. At higher temperatures above 100°C, ring opening followed by decarboxylation occurs and the reaction may proceed further, leading to the loss of methoxy group from the aromatic ring (Scott *et al*., 1993).

In the presence of mineral acids aflatoxins B1 and G1 are converted into aflatoxin B2A and G2A, due to acid catalysed addition of hydroxyl group across the double bond in the furan ring. In the presence of acetic anhydride and hydrochloric acid, the reaction proceeds

further to acetoxy derivative. Similar adducts of aflatoxin B1 and G1 are formed with formic acid-thionyl chloride and trifluroacetic acid. Many oxidizing agents, including sodium hypochlorite, potassium permanganate, chlorine, hydrogen peroxide, ozone and sodium per borate, react with aflatoxin molecule in some way as indicated by the loss of fluorescence in ultraviolet light at 365nm (Health and Hibert, 2005).

Hydrogenation of aflatoxin B1 and G1 yields aflatoxins B1 and G1 respectively. Further reductions of aflatoxin B1 by three moles of hydrogen yields tetra hydroxyl aflatoxin. Reduction of aflatoxin B1 and B2 with sodium boro hydride yields aflatoxin R-B1 and R-B2 respectively. These arise as a result of opening of the lactones ring followed by reductions of the acid group and reduction of the keto group in the cyclopentene ring (Waliyar and Reddy, 2003).

Occurrence of Aflatoxin contamination in animal feed and milk

Mycotoxins are ubiquitous. They can occur in cereals, cereal products and foods, feeds, animal products and soil. Animal feeds commonly harbor mycotoxins are wheat bran, noug cake, pea hulls and maize grain. Concentrated animal feedstuffs harbor the growth of mycotoxins. Mycotoxins can be transferred from feed to food of animal origin, as this food represents a significant route of exposure for humans. There are six common mycotoxins that affect animals: aflatoxins, fumonisins, ochratoxins (which like aflatoxins affect liver function), trichothecenes, and zearalenone. Diagnosis of aflatoxin exposure in animals is difficult especially in large farms that use mixed feed, which may contain highly varied combinations of feedstuffs.

Importantly, it has been demonstrated that simple measures can significantly reduce the risk of mycotoxin exposure on farm. Storage of grain at appropriate moisture content (below 130 g/kg), inspection of grain regularly for temperature, insects and wet spots will limit the possibility of fungal development in feeds and feedstuffs as discussed before. The risk of feed contamination will be reduced in animal units with rapid turnover of feed because there will be less time for fungal growth and toxin production (Bryden, 2012). Aflatoxin is just one of many mycotoxins that can adversely affect animal health and productivity. Care regarding animal feed must be extended not only to the nutritional and economic value, but also to food quality (Gncalez *et al*., 2004).

Mammals that ingest AFB1-contaminated diets eliminate into milk amounts of the main hepatic 4-hydroxylated metabolite known as “milk toxin” or AFM 1. AFM1 residues in milk are a variable percentage (0.3-6%) of AFB1 ingested. AFM1 is usually considered to be a detoxification product of AFB 1, however its acute toxicity is nearly equal to that of AFB 1; as regards the potential carcinogenic hazard, it is about one order of magnitude less than that of AFB 1; the International Agency for Research on Cancer classified AFM1 as a possible human carcinogen (group 2B). Maize grain is normally utilized in the feed rations for dairy cows at the rate of 5-6 kg per cow per day. The feeding of dairy cows with contaminated maize led to the severe widespread contamination of milk with AFM 1.The problem was immediately identified by manufacturers of milk for human consumption and by health inspectors (Pietri and Piva, 2007).

There are several different types of aflatoxins strains. The most common naturally produced are B1, B2, G1, and G2 and two additional strains, M1 and M2 are the metabolic products of contaminated food or feed and are found in milk and other dairy products (Lopez *et al*., 2002). Among these several type of aflatoxin strain, Aflatoxin B1 is the most potent mycotoxin (toxic substance produced by a mold). This type of toxin increases the apparent protein requirement of cattle and is a potent cancer causing agent (carcinogen). When significant amounts of aflatoxin B1 are consumed, the metabolite M1 appears in the milk within 12 hours (Shephard, 2008).

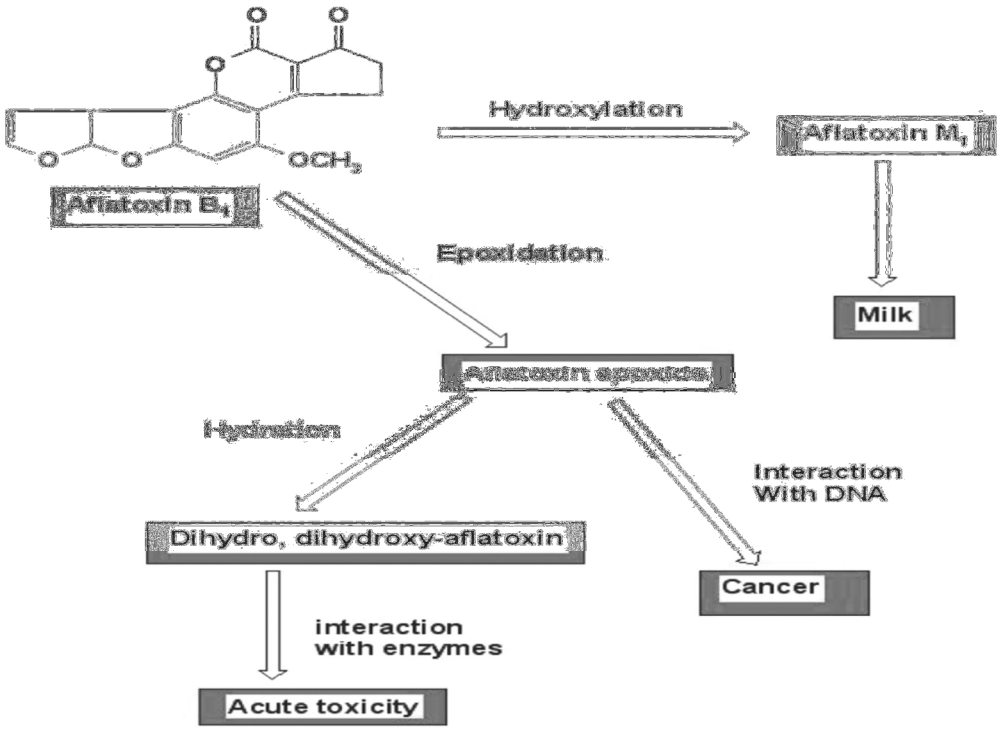

Figure 2: Some metabolic products from aflatoxin B1.

Factors affecting aflatoxin Contamination

Field and postharvest practices can predispose crop produce to aflatoxin contamination. The risk of contamination is greater in developing countries where peasant farmers who constitute the majority face financial challenges and have little or no access to improved technology. The factors that influence mycotoxin production are either biological (biotic), environmental (abiotic) or nutritional (Dorner, 2013). Some of the biotic factors include cultivar susceptibility and growth stage, insect and bird damage and presence of other fungi or microbes and strain variation in the fungus while abiotic factors include mechanical damage, moisture, temperature, pH and other crop stresses such as drought, soil type, suitability of substrate, excessive rainfall, gaseous exchange andgaseous environment and preservatives and crowding of plants (Whitlow *et al.,* 2010)). Nitrogen stress is another biotic factor which can also predispose crops to aflatoxin contamination.

Relative humidity between 83%-88% and appropriate level of CO_2 and O_2 has also been reported to influence the mould growth and aflatoxin production. For instance 20% CO_2 and 10% O_2 in air depress the aflatoxin production (Bankole and Adebanjo, 2003). As biological factors, the preferred carbon sources for aflatoxin production are glucose, sucrose or fructose. Also, zinc and manganese are essential for aflatoxin biosynthesis. But a mixture of cadmium and iron depress the mould growth and hence aflatoxin production (Gilbert and Anklam, 2002).

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

Preventing exposure to aflatoxin

Pre-harvest

Pre-harvest contaminations of food stuffs by aflatoxins were started with this stage. Environmental conditions such as drought during the grain growth stage (Mehan *et al.,* 1988), insect damage in the field (Lynch RE, Wilson, 1991), variety, and soil characteristics have their own contribution (9). In developing countries, many of these pre-harvest opportunities are not exploited by producers to minimize contamination. Insect damage in the field is not controlled by pesticides or by cultural practices; drought is a common phenomenon, and most crops are produced without irrigation as an option. Harvesting is usually done without machinery, and drying is usually

carried out very inefficiently and is dependent on the weather. Adverse weather at harvest results in slow and inadequate drying and brings attendant risks of contamination (Wright and Nageswara, 2000).

Storage

Most of livestock feeds and human foods are contaminated with aflatoxin during the time of storage. To keep the quality of feedstuff and food in storage there must be the following considerations, it is necessary to prevent biological activity through adequate drying, elimination of insect activity that can increase moisture content through condensation of moisture resulting from respiration, low temperatures, and inert atmospheres (Smith *et al*., 1995). In other words, the conditions needed to prevent the development of contamination are known, but it is not always easy to produce them in storage systems in developing countries because of most people in rural areas grow and store their own food; in consequence, most food is stored in small, traditional granaries, and there is little investment in the management of the conditions. Studies of grain quality in such storage structures show a steady increase in the aflatoxin content over time, which reflects the failure to maintain appropriate conditions (Hell *et al*., 2000).

Processing

Processing of different food products can be used to reduce the aflatoxin content and there are three main approaches exist: dilution, decontamination, and separation. Dilution is the easiest means of satisfying the requirement to mix grain low in aflatoxin with grain exceeding the regulated limits which is already stated as a rule. Thus, although the concentration is reduced, but still consumers are exposed to overall aflatoxin burden as before mixing is done. Decontamination is the development of methods which are used to treat the contaminated commodities of food or feeds may be by denaturing the aflatoxin. For example treating the contaminated foodstuffs with ammonia, alkaline substances and ozone can denature aflatoxins (Phillips *et al*., 1993). With regard to separation is separating of contaminated grain from the bulk and can cause successful reduction of aflatoxin contamination achieved depending on the heavy contamination. Further removal of aflatoxin contaminated seeds may be achieved by colour sorting, which, in the case of peanuts, is most effective when the seeds are blanched.

Minimization of Aflatoxin Contamination levels in Livestock Feeds and Foods

Different scientific methods focused on three approaches to control aflatoxin contamination: prevention of contamination of food and feed by the fungi that elaborate the toxins decontamination, and inhibition of aflatoxin absorption in the gastrointestinal tract. Although preventing fungal contamination of food and feed commodities can be considered as the most rational approach, its implementation is difficult in tropical areas where favourable environmental and climatic conditions promote the fungal growth (Fuffa and Urga, 2001) In addition, aflatoxins are extremely durable and unavoidable under most conditions of storage, handling, and processing of foods or feeds (Martins *et al.,* 2017).

Various physical, chemical, and biological methods have been proposed in the past for the decontamination of aflatoxins in feed commodities and food through elimination, inactivation, or reduction of the levels (Tripathi and Mishra, 2009). Physical methods, including cleaning, washing, aqueous extraction, dehulling and milling, has been shown to be effective, to a certain extent, in reducing aflatoxin in feeds and food products (Rafaat et al., 2014). Another promising approach is using microbiological actors for reducing aflatoxins contamination in food commodities and animal feeds. As Raffta et al., 2014 reported that, yoghurt fermented by 50% yoghurt culture (S. thermophilus and L. bulgaricus) and 50% L. plantrium recorded the highest reduction in the level of AFM1 at the end of storage period. Different strains of lactic acid bacteria inoculums were used to reduce the AFM1 level in yogurt samples. This study showed the highest reduction percentage in AFM1 by certain species of LAB at the end of the storage period. Strains of probiotic bacteria were also used for the reduction of AFM1 in milk in an in vitro digestive model where up to 25.43% reduction was reported (Elsanhoty et al., 2014). Though the use of defined starter cultures to initiate fermentation and thereby reduce aflatoxin is effective, it is difficult to implement in developing countries like Ethiopia where dairy production is dominated by traditional methods (Tolosa et al., 2016).

Permitted Levels of Aflatoxin

Most developed countries have set permitted levels of aflatoxins in food for human consumption and livestock feed to control and reduce detrimental effects of these toxins. These levels are variable and depend on economic and developing status of the countries. In US, Food and Drug Administration (FDA) has permitted a total amount of 20 ng/g in livestock feed and 0.5g/kg or 50 ng/l in milk. In European countries, permitted levels of aflatoxin M1 in milk and milk products are 0.005mg/kg. Also, different countries have set different regulations for permitted levels of aflatoxin in livestock feed, different livestock based products and also for others foods which are consumed by human beings. For instance, European Union (EU) has set permitted levels of aflatoxin from 0.05 to 0.5µg/kg for aflatoxin in livestock feed.

Table 1. FDA maximum level of aflatoxin in animal feeds and food

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

Source: Rehrahie, 2018

Effects of Aflatoxin on Human and Animal Health

Aflatoxicosis is a condition caused by aflatoxins in both humans and animals. It occurs in two general forms (Bankole *et al*., 2003) the acute primary aflatoxicosisis produced when moderate to high levels of aflatoxins are consumed. Specific acute episodes of disease may include hemorrhage, acute liver damage, edema, alteration in digestion, absorption and/or metabolism of nutrients, and possibly death (Thrasher, 2012). The chronic primary aflatoxicosis results from ingestion of low to moderate levels of aflatoxins (USAID, 2012). The effects are usually subclinical and difficult to recognize. Some of the common symptoms are impaired food conversion and slower rates of growth with or without the production of an overt aflatoxin syndrome (WHO, 2000).

They are responsible for damaging up to 25% of the world"s food crops, resulting in large economic losses in developed countries and human and animal disease in under-developed countries (Abbas *et al.,* 2005).

The problem of aflatoxin has resulted in reduction of livestock productivity (Bennett and Klich, 2003) and it has also led to higher susceptibility to infectious diseases in livestock and kidney and liver cancers in human beings. Clinical signs of aflatoxicosis in animals include gastrointestinal dysfunction, reduced reproduction performance, and reduced feed utilization efficiency, anaemia, and jaundice. Young and nursing animals may be affected as a result of the conversion of aflatoxin B1 to the metabolite aflatoxin M1 excreted in milk of dairy cattle (Rios *et al*., 2013).

Table 2. Levels of aflatoxin (Mainly Aflatoxin B1 and Aflatoxin M1) in animal feeds and milks from areas around Addis Ababa districts

Location	Sample type (feed or Milk)	Number of sample	Detection Method	Ranges of Concentration of Aflatoxin	Level of in % contaminated sample	Reference
	Maize feed	17	ELISA		88%	Ayalew, 2006
	barley, sorghum, tef and wheat	352	ELISA	trace to 26 µg/kg	8.8%	
Holetta (total of 43+15=58 samples of feeds and 3-6 month storage time tested-aflatoxin-B1)	Roughage feeds(Grass hay)		ELISA	0.33 – 2.47 µg/kg	13.8%	Rehrahie *et al.,* 2017
	Concentrate feeds(With oil seed Cake)		ELISA	9.19 – 11.39 µg/kg	29.3%	
	Without oilseed cake		ELISA	3.94 – 6.54 µg/kg	24.1%	
	Wheat bran		ELISA	1.51 – 5.71 µg/kg	10.3%	
DZ (total of 48 samples and 3-6 months storage time tested aflatoxin-B1)	Roughage feeds (Wheat straw)		ELISA	0.33 – 2.47 µg/kg	2.1%	
	With oil seed Cake)		ELISA	16.2 – 18.8 µg/kg	34.5%	
	Without oilseed cake		ELISA	8.14 – 11.14 µg/kg	20.8%	
	Wheat bran		ELISA	4.33 – 8.53 µg/kg	8.3%	
Hawassa (total of 54 samples and less than one month storage time tested aflatoxin-B1)	Roughage feeds (Grass hay)		ELISA	Trace – 1.83 µg/kg	1.9%	
	With oil seed Cake)		ELISA	5.33 – 10.93 µg/kg	7.4%	
	Without oilseed cake		ELISA	0.4 – 3.6 µg/kg	11.1%	
	Wheat bran		ELISA	0.2 – 1.81 µg/kg	9.3%	
Debre Ziet, tested aflatoxin-M1	milk	15	ELISA	0-0.1403 µg/L	2 samples free (13%), 4 samples (27%)≤0.05 µg/L) and others are below 0.5µg/L	Rehrahie *et al.,* 2017
Holetta, tested aflatoxin-M1	milk	15	ELISA	0.0015-0.146 µg/L	No free sample, 4 samples (27%)≤0.05µg/L and others are below 0.5µg/L	Rehrahie *et al.,* 2017
Hawassa, tested aflatoxin-M1	milk	15	ELISA	0-0.11 µg/L	15 samples (100%) ≤0.05 µg/L)	
Addis Ababa, tested aflatoxin-B1	Concentrated (wheat bran and noug) feed sample from milk producers	27	ELISA	9 samples <20ppb,8 samples ranges 20-100ppb and 10 samples are >100ppb	37% from 27 sample were >100ppb	Daweit *et al.,2016*

Table 2. Continues

Debre Ziet, tested aflatoxin-B1	Concentrated feed sample from milk producers	23	ELISA	5 samples <20ppb,7 samples ranges 20-100ppb and 11 samples are >100ppb	48% from 23 samples were >100ppb	
Sebeta, tested aflatoxin-B1	Concentrated feed sample from milk producers	9	ELISA	6 samples <20ppb,1sample ranges 20-100ppb and 2 samples are >100ppb	22% from 9 samples were >100ppb	
Senedafa, tested aflatoxin-B1	Concentrated feed sample from milk producers	31	ELISA	26 samples <20ppb,4 samples ranges 20-100ppb and 1 sample >100ppb	3% from 31 samples were >100ppb	
Sululeta, tested aflatoxin-B1	Concentrated feed sample from milk producers	10	ELISA	5 samples <20ppb,1sampe ranges 20-100ppb and 4 samples are >100ppb	40% from 10 samples were >100pbb	
A total of 114 feed samples from dairy farmers and 42 feed samples from feed producers, processors and traders, from above 5 town	**Concentrate feed sample + wheat bran + Noug cake**	**156**	**ELISA**	**All the feed samples were contaminated with AFB1 ranging between 7 and 419 mg/kg**	**16 samples are contained by AFB1 less than or equal to 10 mg/kg and 41 samples greater than 100 mg/kg.**	
From 5 location	wheat bran		ELISA	31 mg/kg		
From 5 location	Noug cake		ELISA	290-397 mg/kg		
Addis Ababa, , tested aflatoxin-M1	milk samples from milk producers	27	ELISA	3 samples <0.05ppb,8 samples ranges 0.05-1ppb,12 samples ranges 0.1-0.5ppb and 4 samples are >0.5 ppb	14.8% from 27 samples were >0.5ppb	Daweit *et al.*,2016
Debre Ziet, tested aflatoxin-M1	milk samples from milk producers	23	ELISA	2 samples <0.05ppb,8 samples ranges 0.05-0.1ppb ,9 samples ranges 0.1-0.5ppb and 4 samples are >0.5 ppb	17.4% from 23 samples were >0.5ppb	
Sebeta, tested aflatoxin-M1	milk samples from milk producers	9	ELISA	No sample <0.05ppb ,6 samples ranges 0.05-0.1ppb ,1 sample in range of 0.1-0.5ppb and 2 samples are >0.5 ppb	22.2% from 9 samples were >0.5ppb	

Table 2. Continues

Sendafa, tested aflatoxin-M1	milk samples from milk producers	31	ELISA	3 samples <0.05ppb,21 samples ranges 0.05-0.1ppb,5 samples ranges 0.1-0.5ppb and 2 samples are >0.5 ppb	6.4% from 31 samples were >0.5ppb	
Sululta, tested aflatoxin-M1	milk samples from milk producers	10	ELISA	No samples were <0.05ppb,3 samples ranges 0.05-0.1ppb,3 samples ranges 0.1-0.5ppb and 4 samples are >0.5 ppb	40% from 10 samples were >0.5ppb	
A total of 100 milk samples from dairy farmers and 10 milk samples from milk traders, from above 5 town	**Milk samples**	**110**	**ELISA**	**0.028 - 4.98 mg/L**	**9 (8.2%) ≤ 0.05 mg/L, 29 (26.3%) < 0.5 mg/L**	
West Gojjam, tested aflatoxin-B1	Pre-harvest maize sample	15	HPLC-FLD	5 (33.3%) samples have <20 µg/kg and 10 (66.7%) samples have < 4 µg/kg		Masresha *et al.,* 2016
West Gojjam, tested aflatoxin-B1	Post-harvest maize sample	15	HPLC-FLD	11(73.3%) samples have <20 µg/kg and 13 (86.7%) samples have <4 µg/kg		
Butajira, tested aflatoxin-M1	Milk	10	HPLC-FLD	0.31±0.90 µg/L	58% of samples contaminated with aflatoxin M1	Yohannes *et al.,* 2018
Agena, tested aflatoxin-M1	Milk	10	HPLC-FLD	0.07±0.26 µg/L	23% of samples contaminated with aflatoxin M1	
Emdibir, tested aflatoxin-M1	milk	10	HPLC-FLD	0.02±0.29 µg/L	10% of samples contaminated with aflatoxin M1	
Arkit, tested aflatoxin-M1	milk	10	HPLC-FLD	0.04±0.31µg/L	9% of samples contaminated with aflatoxin M1	
Wolkite, tested aflatoxin-M1	milk	10	HPLC-FLD	ND	ND	

Table 2. Continues

Butajira	Mix feed sample	10	HPLC-FLD	G2 (1.14 µg/kg), G1 (17.1 µg/kg), B2 (3.27 µg/kg) and B1 (31.2 µg/kg)	52.27% of samples contaminated with aflatoxin M1	
	Furshika (maize grain + semi grinded wheat grain)		HPLC-FLD	ND for all aflatoxins		
	Mix feed sample	10	HPLC-FLD	G2 and B2 are ND, G1 (4.05 µg/kg) and B1 (2.56 µg/kg	6.61% of samples contaminated with aflatoxin M1	
Agena	Furshika (maize grain + semi grinded wheat grain)		HPLC-FLD	ND for all aflatoxins		
	Areke Atela	10	HPLC-FLD	G2 and B2 are ND,G1 (1.13 µg/kg) and B1 (1.88 µg/kg)	9.08% of samples contaminated with aflatoxin M1	
	Mix feed sample			G2 (ND), G1 (1.27 µg/kg),B2 (0.81 µg/kg) and B1 (10.06 µg/kg)		
Emdibir	Maize stored 6-8 months	17	ELISA	15 samples below 5 µg/ kg, except in one sample from Adama which had 27 µg/ kg		
	peanut	52	ELISA	38 samples contaminated with B1 aflatoxin	0.57 (new harvest) - 447.02 ppb (stored for 3 months)	
Derie Dewoa, Adama and Ambo	Groundnut	168		Ranges in between 0.1- 397.8 ppb	All samples were found 100 % positive with B1	Amare , 2010
	Noug cake (average moisture content) = 15.27%	12	HPLC	G2 (33.06 ng/g), G1 (213.58 ng/g),B2 (319 ng/g) and B1(408.63 ng/g)	Total aflatoxin 974.27ng/g	Eshetu, 2010
Tigray	Noug cake (average moisture content) = 11.93%	12	HPLC	G2 (16.396 ng/g), G1 (490.3 ng/g), B2 (36.64 ng/g) and B1 (302.96 ng/g)	Total aflatoxin= 846.3ng/g	Dereje Assefa, 2012)

Table 2. Continues

Sululeta	Noug cake (average moisture content) = 11.55%	12	HPLC	G2 (24.279 ng/g),G1 (298.53 ng/g),B2 (63.57 ng/g) and B1 (293.988 ng/g)	Total aflatoxin= 680.37ng/g	Mulugeta *et al.*, 2017
Deber Ziet	wheat bran (average moisture content) = 8.65%	12	HPLC	G2 (<LOQ),G1 (10.316 ng/g), B2 (10.058 ng/g) and B1 (16.161 ng/g)	Total aflatoxin= 36.535ng/g	
Deberbrhan	Wheat bran (average moisture content) = 8.6%	12	HPLC	G2 (<LOQ),G1 (18.06 ng/g), B2 (15.34 ng/g) and B (<LOD	Total aflatoxin= 33.4 ng/g	
Sululta	Wheat bran (average moisture content) = 6.75%	12	HPLC	G1 (<LOQ),G1 (5.75 ng/g),B2 (<LOQ) and B1<LOD	Total aflatoxin= 15.75ng/g	
Debre Zeit	Atela	6	HPLC	G1, G2, B2 and B1 were below limit of quantification.		

Were LOD- limit of detection, ND- non detectable, HPLC- high performance liquid chromatography's, ELISA-Enzyme Linked Immunosorbent Assay, HPLC-FLD- high performance liquid chromatography-flourcns

CONCLUSION

Aflatoxin B1 in animal feed and M1 in animal milk could be health risk to human. High contamination of aflatoxin in animal feed (AFB1) may results in a significant AFM1 level in milk when animals are feed with highly contaminated foodstuffs. Ethiopia is most favourable for aflatoxicogenic fungi and aflatoxin contamination, especially AFB1 which leads to AFM1. Different reports show that concentrated animal feeds specially concentrated animal feeds which contain the so called noug cake are the most contaminated animal feed in the country. Crating awareness on all stockholders should be done on grains people engaged in feed processing, feed marketing proper handling and management is a key issue for minimizing aflatoxins contaminations and to employ better feed storage practices and hygienic feeding practices. In short, adopting good harvesting practices, improving analytical facilities, and implementing strict regulations would avoid or reduce these natural contaminants in feed and milk and ensure the safety of milk and milk products as human food. Collaboration between the agricultural and public health communities, between the local, regional, national, and international governing bodies, and between different disciplines within public health and agricultural is necessary to reduce aflatoxin exposure. Furthermore, investigations are recommended to be carried out routinely to study contamination of food and feed commodities by all types of mycotoxins and around the country.

CONFLICT OF INTEREST

The author has not declared any conflict of interests.

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