Effects of Aflatoxin Contamination in Milk: A Review

1Dawit Akeberegn, 2Tewodros Alemneh and 3Derbie Zewudie

Abstract

The term Mycotoxin is derived from the Greek word ‘mycos’ meaning mould, and the Latin word ‘toxicum’, which means poison. Mycotoxins are relatively low molecular weight secondary metabolites of fungal origin that are harmful to animals and humans. Aflatoxin (AF) is one of the most common mycotoxins which can be found in milk and are generally produced in animal feed by toxigenic fungi such as Aspergillus (A) flavus, A. parasiticus and the rare A. nomius. Both A. flavus and A. parasiticus produce aflatoxins B1 and B2 which have blue fluorescence, while A. parasiticus produces aflatoxin G1 and G2 which have green fluorescence. The hydroxylated aflatoxins B1 and B2 are converted into aflatoxins M1 and M2, respectively. Aflatoxin B1 is the most toxic and widely prevalent in milk and milk products. Aflatoxin contamination in milk and its products is produced into two ways: either toxins pass to milk with ingestion of feeds contaminated with aflatoxin, or it results as subsequent contamination of milk and milk products with fungi. Like other mycotoxins, aflatoxin M1 and M2 can be detected by using chromatography (HPLC) or ELISA. Many countries have standard limits of aflatoxin M1 and M2 range between 0 to 0.5 ppb in milk and dairy products. Aflatoxin contamination of agricultural commodities poses considerable risk to human and livestock health and economic losses. Exposure of human to aflatoxins leads to several health-related problems including acute and chronic aflatoxicosis, immune suppression, liver cancer and cirrhosis, and stunted growth in children. Strict regulations and adapting good storage practices in developed countries have minimized the contamination of AFM1 in milk and dairy products.

Keywords: Aflatoxin, Dairy Industry, Health Impact, Aspergillus, Prevention

INTRODUCTION

Fungi (Moulds) are filamentous (fuzzy or dusty-appearing fungi) species that commonly occur in feedstuffs, including roughages and concentrates. Fungal growth and production of mycotoxins are usually associated with extremes in weather conditions leading to plant stress or hydration of feedstuffs, insect damage, poor storage practices, low feedstuff quality, and inadequate feeding conditions (Bhalla, 2017). Fungi can infect animals causing a disease referred to as mycosis. Aflatoxins are a group of mycotoxins which are secondary metabolites 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 (Balina et al., 2018). When aflatoxin contaminated feed is consumed by dairy cattle, the animals can be affected by the toxin. Furthermore, milk produced when toxic feed is consumed by the cow can contain aflatoxin. The aflatoxin that appears in milk is chemically somewhat different from the aflatoxin that was consumed by the cow, but the milk toxin retains the toxicity and some of the carcinogenicity of the original toxin. Aflatoxin M1 (AFM1) is the principal hydroxylated AFB1 metabolite present in milk of cows feed with a diet...
contaminated with AFB1 and excreted within 12 hours of administration of contaminated feeds (Shephard, 2008; Yohannes et al., 2018).

Aflatoxin occurs worldwide. The recent estimates suggest that there are more than five billion people worldwide at risk of chronic exposure to aflatoxins. But it occurs more frequently in tropical countries because of high temperature, moisture, and unseasonal rains. That is continued to be problems of significant public health concern as long as people consumed contaminated animal products and considered as public health important. In the developing world seriously affect people’s health and livelihoods, as freedom of choice in food is limited for a poor and food-insecure population (Williams et al., 2004; WHO, 2005). Once aflatoxins are produced by the fungi, they are heat, cold, and light stable. They persist to some extent in food even after the inactivation of the fungi by food processing methods, such as ultra-high temperature products, due to their significant chemical stability. Aflatoxins are colorless, odorless, and tasteless because even low concentrations can be important, and with the uneven distribution in commodities, aflatoxins are difficult to detect accurately (Peraica et al., 1999).

Aflatoxicosis: Backgrounds

Mycotoxins

Mycotoxins are those secondary metabolites of fungi which are associated with certain disorders in animals and humans. The manifestation of toxicity in animals is adverse as the fungal species which produce these compounds. In addition to being acutely toxic, some mycotoxins are now linked with the incidence of certain types of cancer and it is this aspect which has evoked global concern over feed and food safety, especially for milk and milk products. The term Mycotoxins is derived from the Greek word ‘mycos’ meaning mould, and the Latin word ‘toxicum’, which means poison. Mycotoxins are relatively low-molecular weight secondary metabolites of fungi that are harmful to animals and humans, and produced by various fungi which affect a wide range of agricultural products meant for human consumption and animal feed. Mycotoxins present in food products and animal feeds are an important problem concerning food and feed safety and significant economic losses are associated with their impact on human and animal health (Nogaim, 2014).

History of Aflatoxins

Aflatoxins were discovered in 1960 when more than 100,000 young turkeys died in England over the course of a few months from an apparently new disease that was termed “Turkey-X disease”. It was soon found that the mortality was not limited to turkeys. Ducklings and young pheasants were also affected. After a careful survey of the outbreaks, the disease was found to be associated with the Brazilian groundnut meal. An intensive study of groundnut meal revealed its toxic nature as it produced typical symptoms of Turkey-X disease when consumed by poultry and ducklings. A study on the nature of the toxin suggested its origin from the fungus Aspergillus flavus. Thus, the toxin was named “aflatoxin” by its origin from A. flavus. This was the event which stimulated scientific interest and gave rise to modern mycotoxicology. Research on aflatoxins led to a “golden age” of mycotoxin research during which several new mycotoxins were discovered (Angele et al., 2010; Negash, 2018). Other important mycotoxins produced by Aspergillus, Fusarium and Penicillium include ochratoxin, patulin and among all mycotoxins and polyketide compounds synthesized by fungal species, aflatoxins (the most potent hepatotoxic and carcinogenic metabolites) continue to receive major attention and are most intensely studied (Ibid.).

Aflatoxins are secondary fungal metabolites included in the class of mycotoxins. That produced by fungi genus Aspergillus species, namely A. flavus, A. parasiticus, A. ochraceoroseus, A. bombycis, A. nomius, A. fumigatus and A. pseudotamari (Cheraghali et al., 2007). Among these species A. flavus and A. parasiticus are well known and it produced during their growth under favorable conditions. 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.

These Aflatoxins are divided into six major toxins according to their fluorescent properties under ultraviolet light and their chromatographic mobility. Both A. flavus and A. parasiticus produce aflatoxins B1 and B2 which produce blue fluorescence, while A. parasiticus produces aflatoxins G1 and G2 which have green fluorescence. The four times hydrated aflatoxin B1 and B2 is converted to aflatoxin M1 and M2 respectively. They present in the milk of lactating mammals which have consumed aflatoxin contaminated feed. Aflatoxin B1 is the most toxic and the most prevalent (Yu, 2004; Lopez et al., 2002).

Epidemiology

Aflatoxicosis is the most important food borne mycotoxins. It has greatest significance in tropical developing countries (Kumar et al., 2008; WHO, 2006). Aflatoxins can affect a wide range of commodities including cereals, oilseeds, spices, and tree nuts as well as milk, meat, and dried fruit. Reports from different part of the world indicated incidence of aflatoxins vary from 40 to 92%. Especially developing countries located in the tropical regions have greatest risk. Their climate is favourable to growth of aflatoxin. Where dietary food stuffs and Staple food source commodities is highly contaminated with aflatoxins (Strosnider et al., 2006).
Table 1. Physical and Chemical Properties of Major Aflatoxins (Kumar, 2018)

<table>
<thead>
<tr>
<th>Aflatoxin Type</th>
<th>Molecular Formula</th>
<th>Molecular Weight</th>
<th>Melting point (°C)</th>
<th>UV absorption (ε)</th>
<th>Fluorescence Emission (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Amax (nm)</td>
<td>ε (L. mol⁻¹.Cm⁻¹) x 10⁻³</td>
</tr>
<tr>
<td>B1</td>
<td>C₁₇H₁₂O₆</td>
<td>312</td>
<td>268-269</td>
<td>223</td>
<td>25.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>265</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>362</td>
<td>21.8</td>
</tr>
<tr>
<td>B2</td>
<td>C₁₇H₁₄O₆</td>
<td>314</td>
<td>286-289</td>
<td>265</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>363</td>
<td>23.4</td>
</tr>
<tr>
<td>G1</td>
<td>C₁₇H₁₂O₇</td>
<td>328</td>
<td>244-246</td>
<td>243</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>257</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>264</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>362</td>
<td>16.1</td>
</tr>
<tr>
<td>G2</td>
<td>C₁₇H₁₄O₇</td>
<td>330</td>
<td>237-240</td>
<td>265</td>
<td>9.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>363</td>
<td>21</td>
</tr>
</tbody>
</table>

Aspergillus flavus and aflatoxin forms sclerotia which allow it to survive in soil for extended periods of time (Scheidegger and Payne, 2003). The sclerotia are the principal sources of primary inoculum. They are also found in foodstuffs and are not destroyed by normal industrial processing or cooking since they are heat-stable. Conditions such as high temperatures and moisture, unseasonal rains during harvest and flash floods lead to fungal proliferation and production of mycotoxins (Bhat and Vasanthi, 2003). Poor harvesting practices, improper storage and less than optimal conditions during transportation, marketing and processing can also contribute fungal growth and increase the risk of mycotoxins production (Wagacha and Muthomi, 2008). Some of their metabolites are still toxic and may be involved in human diseases. The toxic effects of aflatoxins on organs like liver, kidney and mainly their carcinogenic effects are mostly known causes of morbidity and mortality (Smith et al., 2001).

Properties of Aflatoxins

The aflatoxins have closely related structures and form a unique group of highly oxygenated, heterocyclic compounds (Balina et al., 2018). The two major types of aflatoxin were named aflatoxins B and G (blue and green) after the color of their fluorescence under long-wave ultraviolet light. This intense fluorescence forms the basis of most assay techniques for aflatoxins.

Physical and Chemical Properties of Aflatoxins

Aflatoxins are colourless to pale yellow crystals, exhibiting fluorescence under UV light. They are slightly soluble in water (10-20µg/ml) and freely soluble in moderately polar solvents such as chloroform, menthol and dimethyl sulfoxide. They are unstable in UV light in presence of oxygen, unstable in extreme pH (<3 or >10). The lactone ring opens under alkaline conditions and the aflatoxins are destroyed, but this reaction is reversible on acidification. Ammoniation results in the opening of lactone ring at high temperature, causes decarboxylation of aflatoxins and this reaction is irreversible (Physical and chemical properties of aflatoxins). Some important physical and chemical properties of major aflatoxins are shown in Table 1 (Kumar, 2018).

Intoxication

Aflatoxins, like any other mycotoxins, are a sub-class of substances which originated as a result of secondary metabolism of fungi. Unlike primary metabolites, these secondary metabolites are not essential for the growth of the fungi but have survival functions in nature (Carr and Ehrlich, 2006). The usual routes for aflatoxins exposure are ingestion of aflatoxin contaminated foods and feeds (Bennett and Klich, 2003). Diet is the major way through which humans as well as animals are exposed to aflatoxins. Apart from this, exposure to aflatoxin can be through ingestion of contaminated milk M1 (metabolite of AflatoxinB1). Moreover species susceptibility to aflatoxin mainly depends on its liver detoxification systems, genetic makeup, age and other nutritional factors (Balina et al., 2018). Wide variations of LD50 values had been obtained in animal species tested with simple doses of aflatoxins for most species. The LD50 values ranges from 0.5 to 10mg/kg body weight. The toxic properties of the aflatoxins also manifest themselves differently depending on the test system, dose and duration of exposure. Thus, they have been shown to be lethal to animals and animal cells in culture when administered acutely in sufficiently large doses and to cause histological changes in animals when smaller doses were administered subacutely. Chronic exposure for extended periods has resulted in tumor induction in several animal species (Dhanasekaran et al., 2015).
Aflatoxins in Milk and Milk Products

Aflatoxins in Milk

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).

Aflatoxins in Cheese

Occurrence of Aflatoxins in cheese can be owing to three possible causes: AFM1 present in raw milk as a consequence of carryover of AFB1 from contaminated animal feed to milk. Syntheses of aflatoxins (B1, B2, G1, and G2) by fungi that grow on cheese have low level of carbohydrate and are not very suitable substrate. The use of powdered milk contaminated with AFM1 for cheese production. Contrasting data have been reported on the influence of cheese preparation on AFM1 recovery. Studies performed in the early years showed variable losses of AFM1 during cheese production ranged from 15 to 65%, according to many studies. In contrast, later investigations of several authors, reported increases in AFM1 concentration in cheese as a function of cheese type, technologies, and the amount of water eliminated during processing. For example, Mohammadi et al. (2008) investigated some factors, which are involved in the process of making Iranian white brine cheese. They reported that some factors such as reneging temperature, press time, and saturated brine pH affected the amount of water eliminated and in turn the content of AFM1 in the cheese curds. However, many results have been drawn from experiments in which the processed milk contained the toxin at high levels, which seldom appear in the practice. Therefore, additional investigations should verify the influence of cheese making on AFM1 occurrence to avoid uncertainty in actual practice when the concentration of the toxin in the processed milk is at around the maximum permissible level of 0.05 mg/kg that is frequently recorded in monitoring programmes. The increase in AFM1 concentration in cheese has been ascribed to the affinity of AFM1 for casein, AFM1 is a water-soluble component and due to the hydrophobic sides of the casein molecule, AFM1 has affinity to casein of milk.

Therefore, they defined a factor named “Enrichment Factor” (EF) for cheeses. Further surveys should be done to find as for cheese manufacture influences on AFM1 distribution. Some tests have been carried out on several kinds of cheeses as to overall stability of AFM1 during ripening and storage, reported that the concentrations of AFM1 in Camembert cheese were higher at the beginning than at the later time of ripening. These results were in agreement with studies by Govaris et al. (2001). Such results however, conflict with reports of earlier studies that indicate different behavior of AFM1 in various other types of cheeses. Thus, in Camembert and Tilsit Cheddar and Brick cheeses stored for 3, 14 and 6.5 months, respectively, the concentration of the toxin increased during the early stage of their ripening to decrease thereafter to reach about its initial concentration at the beginning of ripening. On the other hand, the concentration of AFM1 in Parmesan cheese started high at the beginning of the ripening period, decreased until about the fifth month and then slowly increased up to the tenth month of storage.

In contrast, the AFM1 content of Mozzarella remained almost constant during storage of 4.5 months. These different profiles of AFM1 in various cheese products may be the result of several factors such as heat treatment, proteolysis, exposure of contaminated milk to light, and especially to an inadequate method of analysis (Mohammadi, 2011). Several investigations on the partitioning of AFM1 during cheese production staring with different milk contamination levels reported a wide range of distribution of AFM1 between whey and curd. On the other hand, Kaniou-Grigoriadou et al. (2005) observed that enrichment factor in the production of Feta cheese made from naturally contaminated milk ranged between 4.3 and 5.6. Meanwhile, Kamkar et al. (2008) showed that the mean concentration of toxin in curd and cheese was 3.12 and 3.65-fold more than that in whey and 1.68 and 1.80 fold more that in cheese milk, respectively. Neither ultra-filtration, nor acidic or enzymatic treatments were able to influence the toxin’s interaction with casein or whey proteins. Only the combined action of heat and low pH (as used in ricotta cheese production) was able to denature whey proteins to a point where they lost their AFM1-binding capacity. As regards the contamination level, several authors, found a maximum contamination level over 1000 ng of AFM1 per kg. This latter contamination level could be hazardous (Fallah, 2010).

Aflatoxins in Yogurt

Several studies have been conducted regarding the
effect of yogurt manufacturing on AFM1 content. Some authors reported no influence on Aflatoxin M1 content. In contrast, Bakirci (2001) detected variable increases of AFM1 content in yogurt related to the milk. The effect of fermentation was assessed by Govaris et al. (2002). They reported that AFM1 levels in all yoghurt samples showed a significant decrease from those initially present in milk. This decrease in AFM1 was attributed to factors such as low pH, formation of organic acids or other fermentation by-products, or even to the presence of lactic acid bacteria. The low pH during fermentation affects the structure of milk proteins such as the caseins leading to formation of yoghurt coagulum. The change in casein structure during yoghurt production may affect the association of AFM1 with this protein, causing adsorption or occlusion of the toxin in the precipitate. During refrigerated storage, AFM1 was rather more stable in the yoghurts with pH 4.6 than with pH 4.0. The percentage loss of the initial amount of AFM1 in milk was estimated at about 13 and 22% by the end of the fermentation, and 16 and 34% by the end of storage for yoghurts with pH 4.6 and 4.0, respectively (Ibid) Govaris et al. (2002).

Unlike cheese and milk samples, the presence of AFM1 in yogurt has not frequently been studied. Thus, more investigations are needed because: Currently, human consumption of yogurt has greatly increased; there are contradictory data on AFM1 stability over manufacture and storage in the literature; the presence of Aflatoxins in yogurt could reduce the nutritional values of its consumption.

AFM1 in Other Milk Products

Many other milk products such as cream, butter, ice cream may contain AFM1. The presence of AFM1 in these products has rarely been investigated and could be of interesting aspects for study. Some surveys conducted on the occurrence of AFM1 in milk products are reported. In a study by Bakirci (2001) the levels of AFM1 in the products made from contaminated milk namely butter, butter milk, cream, skim milk was investigated. The mean AFM1 level found in cream samples was 64.4% of AFM1 concentration of bulk-tank milk. Whereas, mean AFM1 level of skim milks was 3% higher than those of bulk-tank milk. Levels of AFM1 in butter samples in the study were less, and they were as 33.80% of AFM1 amounts of bulk-tank milk. Mean AFM1 levels obtained from buttermilk samples were similar to those of bulk-tank milk (mean 83% of it). During butter processing, protein membrane around fat globules is broken down and serum phase is separated. Due to the chemical structure of AFM1 and its affinity to casein, it adsorbs on this fraction of protein, therefore, cream contained less AFM1 than milk, and butter contained less amount of AFM1 than cream. As a result of the association effects of these factors, AFM1 concentration occurs in lipid phase (like butter and cream) less than serum phase and protein fraction (Mohammadi, 2011).

Stability and Reduction of AFM1 in Milk and Dairy Products

AFM1 is very stable at high temperatures. Several studies have investigated the distribution (stability) of AFM1 from milk to milk products. Oruc et al. (2006) found that AFM1 was stable in kashar cheese for over 60 days and in traditional white pickled cheese for over 90 days. Their results showed that the toxin was stable during cheese storage and ripening. In another study, Govaris et al. found the stability of AFM1 in yoghurt artificially contaminated with concentrations of 0.050 and 0.100 mg/L during storage for 4 weeks at 4°C and at pH values of 4.0 and 4.6. Their results show that at pH 4.6, the AFM1 levels did not significantly change (p > 0.01); however, in the yoghurt at pH 4.0, AFM1 decreased significantly (p < 0.01) after the third and fourth weeks of storage at both concentrations.

Therefore, this decrease in AFM1 may be a function of low pH. In a similar study, during the fermentation of yoghurt, the AFM1 levels decreased significantly (p < 0.01) from the initial levels present in milk. The authors concluded that this decrease in AFM1 levels may be attributed to factors such as low pH, the formation of organic acids or other fermentation by-products, and even the presence of Lactobacillus sp. (Ibid) Govaris et al. However, Bakirci has found 13% higher level of AFM1 in yogurt samples as compared to bulk-tank milk samples, but the difference of AFM1 level was not statistically significant. Cattaneo et al. (2013) observed the stability of AFM1-contaminated whey and deproteinized whey subjected to different technological treatments. During ricotta cheese production, the majority of AFM1, 94% on average, was removed in the discarded whey, so only 6% remained in the curd. Then, the use of ultrafiltration and diafiltration removed more than 90% of the toxin remaining in the whey or deproteinised whey discarded from ricotta cheese production. The spray-drying was efficient in reducing AFM1 contamination in whey, where toxin retention was approximately 60%, while in deproteinized whey, the AFM1 retention was approximately 39%. Therefore, more studies on the detoxification of AFM1 are necessary to completely destroy this lethal toxin. Therefore, to minimize the health risks associated with these toxins, most countries have implemented regulations (Iqbal et al., 2013).

Permitted Levels of Aflatoxin

Some countries have set permitted levels of aflatoxins in food to control and reduce detrimental effects of these toxins. These levels are variable and depend on
economic and developing status of the countries (Negash, 2018). 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 (Negash, 2018). In European countries, permitted levels of aflatoxin M1 in milk, milk products and baby food are 0.005mg/kg. Also, different countries have set different regulations for permitted levels of aflatoxin in livestock feed. For instance, European Union (EU) has set permitted levels of aflatoxin from 0.05 to 0.5µg/kg. Factors such as weather conditions are also effective in determining permitted levels of aflatoxin. Permitted levels of this toxin in tropical countries are higher compared to mild and cold countries (Negash, 2018).

Current Methods of Aflatoxin B1 and Aflatoxin M1 Extraction

Aflatoxin M1 is categorized as a group 2B carcinogen (probable human carcinogen), which is in the same category as chloroform and diesel exhaust. The hazard of ingesting this toxin has been combated by research towards the extraction of aflatoxin B1 from feed and aflatoxin M1 from milk. Extraction from feed has been successful, yielding many methods. Adsorbent compounds, such as NovaSiI clay, can be directly mixed with animal feed and act as a high affinity and high capacity binder when in the GI tract for aflatoxins. Green tea polyphenols (GTPs) are another type of product that can be mixed with feed. These have been shown to inhibit the chemically-induced cancer that can result from AFB1. Chlorophyllin, yet another feed component, prevents the absorption of aflatoxin within the digestive tract by sequestering it. Although these methods are useful in preventing the formation of AFM1 in the milk, the adding of compounds to feed can require expensive equipment and has shown to reduce the nutritional quality of the feed. Due to these complications, the search for a way to effectively extract aflatoxin directly from milk has been of recent interest. Research on extracting AFM1 from milk has mostly led to what doesn’t extract AFM1 from milk. Pasteurization, a heating process that milk undergoes to kill bacteria and sterilization have little effect on removing aflatoxin from milk. A study by Choudhary et al. (1998) reported that sterilization of milk at 121°C for 15 minutes only caused a 12.21% degradation of AFM1, while boiling decreased AFM1 by 14.5%. They suggested that an extended time period and increased temperatures might decrease AFM1 by a greater amount. Mohammadi (Mohammadi, 2011) continued experiments involving heat have yielded similarly disappointing results. Ultrafiltration with acidic or enzymatic treatments does not have an effect on aflatoxin M1. However, a combined method of low pH and heat was able to denature whey protein enough that they lost their affinity for aflatoxin M1. This combined method did not make much of difference, as aflatoxin is known to preferentially bind to casein. Other ineffective methods include using UV light, and ionizing radiation (Chaney, 2015).

Effects of Aflatoxin on Human and Livestock Health

Aflatoxin B1 present in livestock feed causes serious problems in genital, digestive and respiratory tracts through different mechanisms such as interference in metabolism of carbohydrates, fats and nucleic acids. Effects of aflatoxin B1 on livestock vary with concentration and time duration of contact with the toxin, strain and feed. High concentrations of this toxin are lethal, medial concentrations lead to chronic poisoning and continuous exposure to low concentration can result in hepatic cancer (Deshpande, 2002). Since about one fifteenth of consumed aflatoxin B1 is introduced into milk as aflatoxin M1 and different heat treatments used in preparing various dairy products cannot reduce quantity of aflatoxin M1, there is always a probability of poisoning by this toxin when consuming infected milk. Tumorigenesis and mutagenesis capability of aflatoxin M1 is less than aflatoxin B1 (Creppy, 2002).

Concerns about human health arise when food groups are found to contain unsafe chemicals, additives, or other contaminants. Among the different contaminants aflatoxin now a days is getting a major human health problem. Humans are exposed to aflatoxins by consuming foods contaminated with products of fungal growth. Conditions increasing the likelihood of acute aflatoxicosis in humans include limited availability of food, environmental conditions that favor fungal development in crops and commodities, and lack of regulatory systems for aflatoxin monitoring and control (Shephard, 2008). A human carcinogen such as aflatoxin B1 (AFB1), the carcinogenic potency used in calculating the population cancer risk is greater in developing countries. This is a consequence of AFB1 being synergistic with hepatitis B virus (HBV) infection, which has a greater prevalence in the developing world (ibid). However, the improvements in food safety in developed countries have eliminated acute human mycotoxicoses such as ergotism, which was previously well known in the middle Ages in Western Europe. However, such outbreaks still occur in rural communities in the developing world, as evidenced by documented cases in Ethiopia, East Africa, where there were outbreaks of gangrenous ergotism in 1978 after consumption of grain contaminated with Claviceps purpurea (Shephard, 2008). Because of these negative effects, regulatory limits for AFM1 in milk are 0.5µg/kg in the US and 0.05µg/kg in Europe (Chaney, 2015).
Economic Significance of Aflatoxicosis

The economic consequences of aflatoxicosis are the major areas of concern. Aflatoxins have negative impact on human health, animal productivity and trade. Generally, when susceptible animals are fed contaminated feeds it results in reduced growth rates, illness, and death; moreover, their meat and milk may contain toxic biotransformation products. Livestock owners often take farmers and feed companies to court legal battles can involve considerable amounts of money (Pier et al., 1990). The direct economic impact of aflatoxin contamination in crops results mainly from a reduction in marketable by rejection of products from the international market and losses incurred from livestock disease, consequential morbidity and mortality which leads to volume and value loss in the national markets which is huge economic loss (Wagacha and Muthomi, 2008). Recommended sanitary and phyto-sanitary standards set for aflatoxins adversely affect grain trade in developing countries, specifically in the international market, products that do not meet the aflatoxin standards are either rejected at the border, rejected in channels of distribution, assigned a reduced price (Gebrehiwet et al., 2007).

The crops contaminated with high levels of aflatoxins are sometimes diverted to animal feed, which resulting in reduced growth rates and illness of animals consuming toxic contaminated feeds. Many countries have established regulations to limit exposure to aflatoxin, typically expressed in parts per billion (ppb). These regulations can result in foregone trade revenues arising from increased cost of meeting the standards including cost of testing, rejection of shipments and even eventual loss of admissibility into foreign markets (Cooper and Dobson, 2007). Toxigenic fungal pathogens are important constraints to the production of the crop, affecting the quality of the seeds through spoilage, however, aflatoxin contamination is the most important quality problem in Ethiopia with serious health consequences for human and livestock for example groundnut plays an important role as a food as well as a cash crop in Ethiopia. Currently the crop is becoming one of the high value crops that are growing in the dry land areas of the Tigray region, Northern Ethiopia, but the groundnut production highly attack by aflatoxicosis (Bhat and Vasanthi, 2003).

In addition to financial losses and economic damage to agricultural and animal husbandry industries, losses due to aflatoxin contamination of foods include major pharmaceutical and health costs to treat food poisoning. Based on Food and Agriculture Organization (FAO) reports, annually, about 20% of the foods produced in the world are contaminated by mycotoxins; in which aflatoxins have a greater share than the others. Prevalence of cancer and livestock disease in farms, weakening of livestock immune system, reduction in milk production and productivity are a few examples of damages to food and livestock industry. Considering huge economic losses and public health protection, prevention and neutralization of the toxins in livestock feed and food products of animal origin such as milk is essential (Milicevi et al., 2010).

Worldwide Regulations of Aflatoxin

The reason aflatoxin is highly regulated rests highly on the impact it has on both human and animal health, most countries, established various regulations for aflatoxin levels (either total aflatoxins or for AFB1) in food and/or feed in order to limit exposure to this group of mycotoxins (Van-Egmond et al., 2007). Many countries have set a limit for a maximum tolerable level of aflatoxin in food and food stuffs and restrict the import of contaminated products to their country. Aflatoxin is becoming a major impediment to the global exchange/trade of plant and plant products. Aflatoxin regulation creates a demand for aflatoxin safe food. Different countries have different regulations for aflatoxin to protect consumers from the harmful effects of mycotoxins that may contaminate foodstuffs, as well as to ensure fair practices in food trade. The number of countries regulating aflatoxins has significantly increased over the years. Such lower limits for aflatoxin had an enormous impact on the ability of developing countries in Africa like Ethiopia to export goods. Aflatoxin is more problem for developing nations than developed countries. In the developing countries, where food supplies are already limited, legal measures may lead to lack of food and to excessive prices. Grains for animal feed in the United States are allowed 300 ppb aflatoxin (Wolde, 2017).

According to Hell and Mutegi (Hell and Mutegi, 2010) aflatoxin research in Africa is necessary to get policymakers in the Sub-Saharan region to recognize that the increased implementation of pre- and post-harvest interventions is important for increasing food security and ensuring food safety to protect the short and long term health of the population. For example, a research by Dereje et al. (2012) from Northern Ethiopia on groundnut revealed that, from the total samples analyzed, 83.9% were unsafe for direct human consumption as per the EU MTIL and 46.6% were unfit for export to EU counties (as per the EU safe limit for import of groundnut); and on the basis of the FAO MTL, 16.6% of the samples exceeded the 30 ppb limit. The average concentration for the total samples had 10 times greater than the recommended maximum aflatoxin level. Similarly, Habtamu et al. (2001) also reported that, in many parts of Africa human food staples exist which contain 10 to 30 times the recommended maximum. The maximum level of 8mg/kg of AFB1 has been established in food subjected to sorting or physical treatment before human consumption, and the corresponding 2 mg/kg of AFB1 for direct human consumption.
Ethiopia has no aflatoxin and other mycotoxin regulation (Dereje et al., 2012; Habtamu et al., 2001; PACA, 2012). This increases the exposure of humans and animals from aflatoxin contamination. However, aflatoxin regulation is not the mandatory case in Ethiopia, because almost all the effect is from indigenous contamination of commodities. Hence, more emphasis should be given for control of the toxin. Aflatoxin remains largely unregulated throughout Africa. As of 2003, aflatoxin regulations existed for five countries in Africa (Van-Egmond and Jonker, 2004). Aflatoxin regulation has great effect on international trades, especially for the developing countries like Ethiopia. For instance, (FAO, 2002) reported that developing countries account for approximately 95% of world groundnut production, but are unable to sell large quantities of groundnut on the international market because of aflatoxin contamination. Hence, high contamination of commodities in Ethiopia largely affects export, in which the country largely depends.

Symptoms and Diagnosis of Aflatoxicosis

Symptoms of Aflatoxicosis

Aflatoxicosis is the disease caused by the consumption of aflatoxins. For most producers, no visual symptoms of aflatoxicosis will be observed in the animals. However, high concentrations of aflatoxins and/or prolonged duration may cause visual symptoms in cattle, especially young calves. Beef and dairy cattle are more susceptible to aflatoxicosis than sheep and horses, although other mycotoxicoses occur in these species, such as facial eczema in sheep and leukoencephalomalacia in horses. Young animals of all species are more susceptible than mature animals to the effects of aflatoxin. Pregnant and growing animals are less susceptible than young animals, but more susceptible than mature animals. Feed refusal, reduced growth rate and decreased feed efficiency are the predominant signs of chronic aflatoxin poisoning. In addition, listlessness, weight loss, rough hair coat and mild diarrhea may occur; anemia along with bruises and subcutaneous hemorrhages are also symptoms of aflatoxicosis. The disease may also impair reproductive efficiency, including abnormal estrous cycles (too short and too long) and abortions. Other symptoms include impaired immune system response, increased susceptibility to disease and rectal prolapse (Pennington, 2012)

Diagnosis of Aflatoxicosis

Diagnosis of aflatoxicosis in milking cows is readily evident from milk samples. However, diagnosis in non-lactating cattle is more difficult because of the variation in clinical signs, gross pathology, and presence of other diseases due to suppression of the immune system. Records should be maintained for all feeds, feeding practices, milk contamination and animal health and performance for all cases of aflatoxin contamination of milk. There are simple, fast, semi-quantitative tests which can be performed to test for aflatoxin. Kits using ELISA (enzyme-linked immunosorbent assay) technology are available to test on the farm as well as commercially. The detection and quantification of aflatoxins by using ELISA has proven to be efficient, easy to use and able to detect very low levels of aflatoxin. But it has the disadvantage of requiring well equipped laboratories, well trained professional, harmful solvents and several hours to complete an assay (Dallasta et al., 2003). Rapid detection techniques are optical fiber, electrochemical transduction, low injection monitoring and biosensors. Most of these still present a lack of applications because of their practical inconveniences except biosensors. The biosensors have been designed to overcome the drawbacks that the common tools employed to detect and quantify aflatoxins presents. Apparently then measurement of aflatoxins in the future tends to be the combination of optical, immunochimical and fluorescence techniques (Carlson et al., 2000).

Control and Prevention of Aflatoxin in Animal Feed and Milk

Aflatoxins occurring naturally in foods and feeds may be reduced by a variety of procedures. Improved farm management practices, more rapid drying and controlled storage are now defined within GAP (Good Agricultural Practice) or HACCP (Hazard Analysis: Critical Control Point) (IARC, 2002). By segregation of contaminated lots after aflatoxin analyses and by sorting out contaminated nuts or grains by electronic sorters, contaminated lots of peanuts or maize can be cleaned up to produce food-grade products. Decontamination by ammoniation or other chemical procedures can be used for rendering highly contaminated commodities suitable as animal feeds.

The best control is the prevention of mycotoxins in the field, which is supported by proper crop rotation and fungicide administration at the right time. In the case of toxin manifestation, measures are required that act specifically against certain types and groups of toxins. Adsorptive compounds can be used for reduction of potency of mycotoxins in general. While adsorbents have proved to be efficient against some mycotoxin-induced toxicosis, alternative strategies such as enzymatic or microbial detoxification, have been used recently for counteracting impacts of certain fungal toxins (Binder, 2007). Prandini et al. (2009) concluded that to control AFM (1) in foods it is necessary to reduce AFB (1) contamination of feeds for dairy cattle by preventing
fungal growth and AFB (1) formation in agricultural commodities intended for animal use. Corn and corn-based products are one of the most contaminated feedstuffs; therefore risk factor analysis of AFB (1) contamination in corn is necessary to evaluate risk of AFM (1) contamination in milk and milk products. During the corn silage production, the aflatoxins production is mostly influenced by: harvest time; fertilization; irrigation; pest control; silage moisture; and storage practices. Due to the lower moisture at harvest and to the conservation methods, the corn grain is mostly exposed to the contamination by Aspergillus species. Therefore, it is necessary to reduce the probability of this contaminant through choice of: hybrids; seeding time and density; suitable ploughing and fertirrigation; and chemical or biological control. Grains harvested with the lowest possible moisture and conservation moisture close to or less than 14% are necessary to reduce contamination risks, as is maintaining mass to homogeneous moisture. Kernel mechanical damage, grain cleaning practices and conservation temperature are also factors which need to be carefully controlled. Generally, Hans and Egmond (Hans and Van-Egmond, 2013) discussed that potentially successful measures to combat and control mycotoxins include (but are not limited to) the following:

Pre-harvest

Apply crop rotation, to reduce infection pressure; Remove crop residues from field, for instance by deep ploughing, to reduce infection pressure; Use seed varieties developed for resistance to fungal infections; Apply fertilization in conformity to crop demand, to avoid plant stress; Apply good agronomic practices (irrigation, weed control, plant spacing) and avoid plant stress from high temperatures and drought; Apply proper phytosanitary measures on seeds and crops, to avoid insect damage and fungal infections; Minimize mechanical damage, to avoid plant stress and fungal infections.

Harvest

Plan to harvest at full maturity, unless extreme plant stress conditions are anticipated; Avoid delayed harvesting, to reduce risk of mycotoxin accumulation; Avoid mechanical damage of grain kernels, to avoid fungal infections during storage; Where applicable dry to moisture level required to prevent mould growth during storage as quickly as possible; Remove foreign matter and visibly infected material where applicable.

Storage

Use clean, dry and well-vented storage facilities that are protected from entry of rain, rodents and birds; Store at as low a temperature as low as possible. Where possible aerate by circulation of air to maintain uniform temperature and moisture; minimize the levels of insects and molds in the storage facility by appropriate approved methods; where applicable use appropriate approved preservatives to prevent mold growth.

Transport

Ensure that transport containers are dry and free of insects, moulds and contaminated material; Protect shipments from moisture entry and avoid temperature fluctuations that may cause condensation.

CONCLUSIONS

Aflatoxin is types of mycotoxin produced by Aspergillus mold and are considered unavoidable food and feed contaminants. Aflatoxin M1 in milk and dairy products could be a risk to human as well as animal health. High contamination in feed may result in a significant AFM1 level in milk when animals are fed with highly contaminated foodstuffs. Meeting the demands for higher milk yields and striving for increased milk production may create such situations. AFM1 appears to be a natural contaminant in milk and dairy products. The presence of AFM1 in milk and dairy from Europe was comparatively low due to strict regulations for these toxins. The most important factors that promote the production of AFs in foods and feeds are moisture and high temperature. Rapid drying of the commodity immediately after harvesting and storage under appropriate conditions are both part of proper management strategies to minimize contamination. The most popular method for AFM1 analysis in milk and dairy product is HPLC. However, analytical methods that can simultaneously detect and quantify a broad number of mycotoxins in milk with low limits of detection and quantification are needed to reduce analytical costs and to allow more frequent monitoring of mycotoxins in milk. In short, adopting good harvesting practices, improving analytical facilities, and implementing strict regulations would avoid or reduce these natural contaminants in milk and ensure the safety of milk and milk products as human food.

REFERENCES


