

The Silent Culprit: Aflatoxin Contamination in Dairy Products and Its Serious Impact on Human Health

Mahendra Pal^{1,*}, Alemayehu Bekele², Tesfaye Rebuma³, Ravindra Zende⁴, Aishwarya Nair⁴, Ramnika Aggarwal⁵

¹Narayan Consultancy of Veterinary Public Health, and Microbiology, Bharuch, Gujarat, India

²Wallaga University School of Veterinary Medicine Department of Clinical Studies, Nekemte, Oromia Region, Ethiopia

³Shaggar City administration Sebeta Sub-city Agricultural office, Sebeta, Oromia, Ethiopia

⁴Department of Veterinary Public Health, Mumbai Veterinary College, Parel, Mumbai, Maharashtra, India

⁵Department of Community and Family Medicine, All India Institute of Medical Sciences, Bathinda, Punjab, India

*Corresponding author: palmahendra2@gmail.com

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Abstract Aflatoxins are fungal toxins produced mainly *Aspergillus* spp., specifically *Aspergillus flavus* and *Aspergillus parasiticus*. The types of toxins produced by aflatoxins naturally are B₁, B₂, G₁, and G₂. Likewise, M₁ and M₂ which are found in the metabolic products of contaminated milk and dairy products. The aim of this article is to review on effects of aflatoxin in dairy cattle products and its public health significance. The outbreaks of aflatoxin are most serious in tropical and subtropical areas over the world and also in temperate regions. It is estimated that more than 5 billion people in developing nations worldwide are at risk of chronic exposure to aflatoxins through contaminated foods. Moreover, aflatoxin is third-leading cause of cancer death globally, with around 550,000-600,000 new cases occur in East Asia, and sub-Saharan Africa each year. Aflatoxins can affect a wide range of commodities including cereals, oilseeds, spices, nuts, and dried fruit, which is considered as aflatoxin B₁. When animals consume feed contaminated with aflatoxin B₁, it is bio transformed to aflatoxin M₁ by the hepatic microsomal mixed-function oxidase system and gets absorbed in the milk of mammals. Aflatoxin B₁ present in livestock feed causes different problems in genital, digestive and respiratory tracts through interference in metabolism of carbohydrates, fats and nucleic acids. It also affects milk composition, body mass gain, immunity and reproductive performance. The presence of aflatoxin M₁ poses high risk to public health by causing acute liver damage, liver cancer, toxic hepatitis, hemorrhage, immunosuppression, DNA damage, gene mutations, and can affect cell transformation. Children that have compromised immune systems are more prone to develop complications. Aflatoxins are diagnosed by various methods including thin-layer chromatography, high-performance liquid chromatography, and enzyme-linked immunosorbent assay. Aflatoxicosis can only be prevented by feeding aflatoxin free rations to dairy animal and practicing good agricultural practice in dairy production farm and processing centers.

Keywords: Aflatoxin, Contamination, Dairy products, Immunosuppression, Liver cancer, Milk, Public health significance

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1. Introduction

Aflatoxins are a type of mycotoxin synthesized by specific fungal species during their growth on various substrates. The primary fungi known for aflatoxin production include *Aspergillus flavus* and *Aspergillus parasiticus*. Other species of fungi also produce aflatoxins but at lower concentrations. The fungi occur naturally in the soil in tropical regions and infect crops while on the farm (pre-harvest) and after harvest during storage (post-harvest) and processing. Most crops infected include maize, groundnut, wheat, cassava, spices, oilseeds, beans, nuts, and dried fruits [1].

Aflatoxins are usually associated with drought stress that often occurs before harvest in various crops in the agriculture field. During the rainy seasons the poor storage conditions can aggravate the concentration of aflatoxins, mainly in humid and hot regions where humidity and high temperature are optimal for growth of molds and for production of toxins [2]. Water activity (aw) is also among the crucial environmental aspects affecting *A. flavus* growth and AF production [3]. Various factors lead to the development of favorable conditions that facilitate the fungal growth. The principal climatic circumstances, such as regular rainfall, drought, high increment of temperature between 12- 48°C and more humidity (40–89%), provide a favorable environment for the growth of molds and aflatoxins production [4].

Animals are exposed to fungal toxins such as the aflatoxins through ingestion of feeds contaminated with fungal toxin-producing molds. When lactating cows consume feed contaminated with aflatoxin B1 (AFB1), AFB1 is metabolized to form the monohydroxy derivative, aflatoxin M1 (AFM1), which is excreted into cow's milk and these milk and milk products have serious public health implications. The sources of aflatoxin contamination in animal feed differ across regions and countries. As a result, the incidence and occurrence of AFM1 contamination in milk and dairy products varies by country of origin [5]. The contamination of milk and dairy products with aflatoxin M1 is a significant public health concern, as these toxins are highly carcinogenic and can cause genetic mutations, fetal malformations, and immune suppression [6]. The resulting genetic defects can lead to fetal mis-development and abortions [7].

Currently, over 5 billion people in developing countries worldwide are estimated to be at risk of chronic exposure to aflatoxins through contaminated foods. The primary and major disease associated with aflatoxin consumption is hepatocellular carcinoma or liver cancer. This disease was reported by International Agency for Research on Cancer (IARC) [8], to be the third-leading cause of cancer death globally, with about 550,000- 600,000 new cases each year and 83% of these deaths occur in East Asia, and sub-Saharan Africa. Liver cancer has an increasing incidence that parallels the rise in chronic hepatitis B and hepatitis C virus infection [9]. Hepatitis B virus infection and aflatoxin synergistically produce 30-fold higher risk of liver cancer in hepatitis B virus-positive patients exposed to aflatoxin [10]. In addition, aflatoxin has been linked to stunting growth in children and immune system disorders. The present communication delineates the aflatoxin contamination of dairy products, and its deleterious effects on human health.

2. General Overview of Aflatoxin and their Effects

Fungal molds produce aflatoxins, which are hazardous secondary metabolites resulting in aflatoxicosis when ingested by susceptible host. The word "Aflatoxin" originates from the name *Aspergillus flavus*, the fungus responsible for its production. It was named around 1960 after its discovery as the source of a disease in turkey called turkey X disease [11].

2.1. Etiology

Aflatoxins are produced by species of *Aspergillus*, specifically *Aspergillus flavus* and *Aspergillus parasiticus* fungi, which are naturally occurring contaminants of food under favorable temperature, relative humidity and poor storage conditions. There are mainly six different types of aflatoxins-Aflatoxins-B1 (AFB1), Aflatoxins-B2 (AFB2), AflatoxinG1 (AFG1), AflatoxinsG2 (AFG2), Aflatoxin M1 (AFM1), and Aflatoxin M2 (AFM2) [12]. Aflatoxin B1 and AFB2 are produced by *A. flavus*, while AFG1 and AFG2 are synthesized by *A. parasiticus*. Aflatoxin M1 and M2 are metabolites of AFB1 in milk of cattle fed on

contaminated foods [13].

2.2. Epidemiology

Aflatoxin contamination is a persistent global hazard, primarily in underdeveloped countries of the world. Aflatoxin growth and production are greatly influenced by environmental conditions, including temperature, atmospheric CO₂, water activity and their interactions with ecology [14]. Tropical countries where the climate is conducive to aflatoxin growth are more prone to infections [15]. *Aspergillus parasiticus*, which is predominantly found on soil habitat, produces aflatoxin at temperatures between 20 and 30°C, whereas *Aspergillus flavus* is more suited to an aerial environment [16].

2.2.1. Geographical Distribution

Globally, temperate, tropical, and sub-tropical regions are susceptible to aflatoxin contamination and have reported the worst aflatoxin epidemics [17]. Sub-Saharan Africa and parts of Southeast Asia have humid and dry climates that are highly suitable for the production of aflatoxin. Animals and human health are severely impacted by Aflatoxin B1 in these areas. Agricultural produce, such as rice, peanuts, cereals, dried fruits, oil seeds, and beers contain aflatoxin fungal metabolites [18].

2.2.2. Transmission

When animals ingest contaminated feed containing AFB1, the hepatic microsomal mixed-function oxidase system bio transforms it to AFM1, which is then absorbed in the milk of mammals [19]. The main 4-hydroxylated metabolite known as AFM1 is excreted into milk of animals that consume feed contaminated with AFB1. Aflatoxin enters the human digestive system mostly through ingestion of milk and milk products containing AFM1. Aflatoxin can be also introduced to humans through the consumption of contaminated variety of agricultural commodities including maize and peanuts. They are the primary sources of human exposure to aflatoxin due to their increased vulnerability to aflatoxin contamination and increased consumption worldwide [20].

2.2.3. Risk Factors

Pathogenic risk factors: *Aspergillus flavus* usually colonizes damaged grains and corn. It can produce the enzymes pectinase and cutinase, which help penetrate the intact grain [21]. AFM1 is resistant to thermal inactivation, pasteurization, autoclaving and other types of food processing [22].

Host risk factors: Children are the most vulnerable population to aflatoxin risk, which can impact their growth rates. Children with underdeveloped immune systems are more likely to develop complications. Consumption of aflatoxin during pregnancy has been negatively correlated with the birth weight [23]. The amount of AFM1 secreted into milk varies by species. Milk from dairy cows contains 1-2% of ingested AFB1. However, average survival rates were 0.04–0.05% for mares, 0.60–0.72% (up to 2.7%) for ewes, and 2.5–2.7% for goats [24].

Environmental risk factors: Climate change is

expected to have a profound effect on our landscape world-wide. Decrease in summer precipitation and increases in temperature may impact on the interactions between different mycotoxigenic species [25]. Increasing in temperature up to 37°C along with water stress significantly reduces the production of AFB1, but the addition of carbon dioxide under the same condition enhances AFB1 production [26].

2.3. Aflatoxicosis in Dairy Cattle

Dairy cattle provide milk and milk products and thus, play an important role in supplying human nutrition throughout the world [27]. Aflatoxin ingested in the feed by cattle is physically bound to ruminal contents, and as little as 2-5% reaches the intestine. Levels of AFB1 in excess of 100 µg/kg of feed are considered to be poisonous for cattle. AFB1 causes neuronal damage in the cerebral cortex and hippocampus, leading to dysfunction of synaptic transmission, thus impairing the nervous system [28]. Aflatoxin can cause oncogenesis, chronic toxicity or per-acute signs depending on the species, age of animal, dose and duration of aflatoxin exposure [29].

AFB1 present in livestock feed leads to development of genital, digestive and respiratory tract issues through different mechanisms such as interference in metabolism of carbohydrates, fats and nucleic acids [30]. It affects milk composition, body mass gain, immunity, and reproductive performance [31]. Jaundice, weight loss, depression, hemorrhage, immunosuppression or pulmonary oedema as well as decreased milk production in dairy cattle have also been reported commonly [32].

2.3.1. Aflatoxin in Milk

The aflatoxins B1 and M1 are genotoxic and carcinogenic to both humans and animals, and it is mostly produced by *Aspergillus flavus* and *Aspergillus parasiticus* [33]. The ingested aflatoxin B1 is broken down in the rumen, while the remaining portion is absorbed in the digestive tract and hydroxylated to AFM1 in the liver through passive diffusion. Thus, cattle have the ability to convert AFB1 in the feed to AFM1 in the milk. The main oxidized metabolite AFM1, is mostly eliminated through urine and is less frequently excreted in milk [34]. The carry-over in cows milked twice daily was typically 1–2% of the ingested AFB1 for low-yielding cows (<30 kg milk yield/day) and up to ~6% for high-yielding cows (>30 kg milk yield/day). Pasteurization or the preparation of yoghurt and cheese cannot denature aflatoxin M1 [35]. Milk has the highest feed-to-tissue transfer rate of aflatoxin among animal-based meals consumed by humans. There is a significant public health risk associated with AFM1 exposure from consumption of contaminated milk products [36].

2.3.2. Aflatoxin in Grain

Aflatoxin can be present in rice, soybeans, plants, and all types of nuts, particularly peanuts and walnuts [37]. The percent occurrence of aflatoxin contamination reported across various food categories, with nuts, nut products, and seeds being the most affected, followed by fruits and vegetables and other food products in the EU countries are depicted in Figure 1. According to the

estimates from the Food and Agricultural Organization of the United Nations (FAO), mycotoxins, including aflatoxins, are present in at least 25% of the world's cereal grains [38]. Most staple foods and cereal grains like maize, wheat, oats become contaminated by aflatoxins. Maize is the third most important cereal grain in the world, preceded by rice and wheat. In Asia, Latin America, and Africa, it is preferred as a basic staple grain [39].

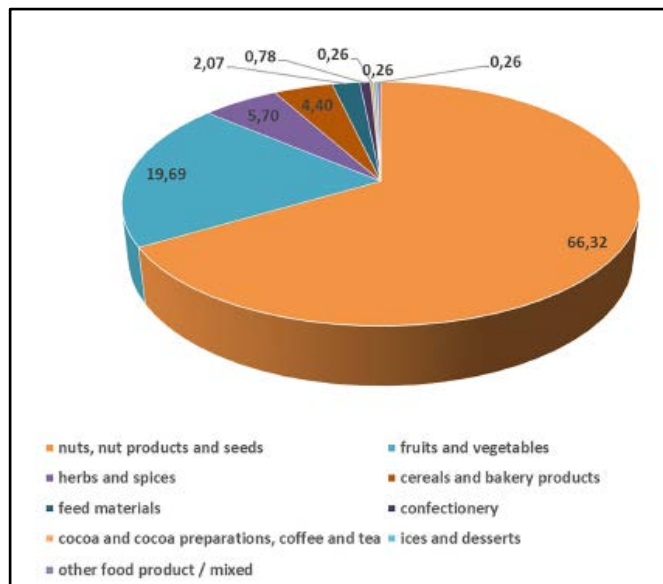


Figure 1. Aflatoxin B1 contamination (%) in food and feed sources in EU countries [Source: 3]

2.4. Public Health and Economic Importance of Aflatoxin

2.4.1. Public Health Significance

Acute aflatoxicosis is associated with consumption of high doses of aflatoxin. It is characterized by hemorrhage, acute liver damage, edema and death in humans. Conditions that increase the likelihood of acute aflatoxicosis in humans include limited food availability, environmental conditions that favor the development of fungi on crops and raw materials and the lack of regulatory systems for aflatoxin monitoring and control. Several reported cases of acute aflatoxicosis associated with consumption of contaminated home-grown maize have been documented in Africa, such as the outbreaks in Kenya in 1982, which killed 12 people, and in 2004, which sickened 317 people and killed 125 in the Central Provinces [20].

Mycotoxins are a global health issue, affecting various countries including the USA, India, and Ghana and are linked to serious health conditions, such as liver cancer, with an estimated 20,000 deaths annually in Indonesia [40]. Liver cancer, also known as hepatocellular carcinoma (HCC), is caused by prolonged exposure to aflatoxin. Aflatoxin exposure is responsible for 4.6% to 28.2% of global cases of HCC with death rate for high dose exposure is around 25% [41]. Hepatitis B and aflatoxins are especially prevalent in developing nations worldwide. People with chronic hepatitis B virus infection are most frequently affected by hepatocellular carcinoma as a result of aflatoxin exposure. Consumption of aflatoxin

increases the risk of liver cancer in people with chronic hepatitis B virus infection by up to thirty times. Aflatoxin has an impact on human immunity, though the precise mechanism of action is yet unknown [42]. However, some of the researchers described the mechanism of

development of aflatoxin toxicity after ingestion through contaminated water and feed on the gastrointestinal system, immune system and liver, leading to immunosuppression, hepatocellular carcinoma (Figure 2) [43].

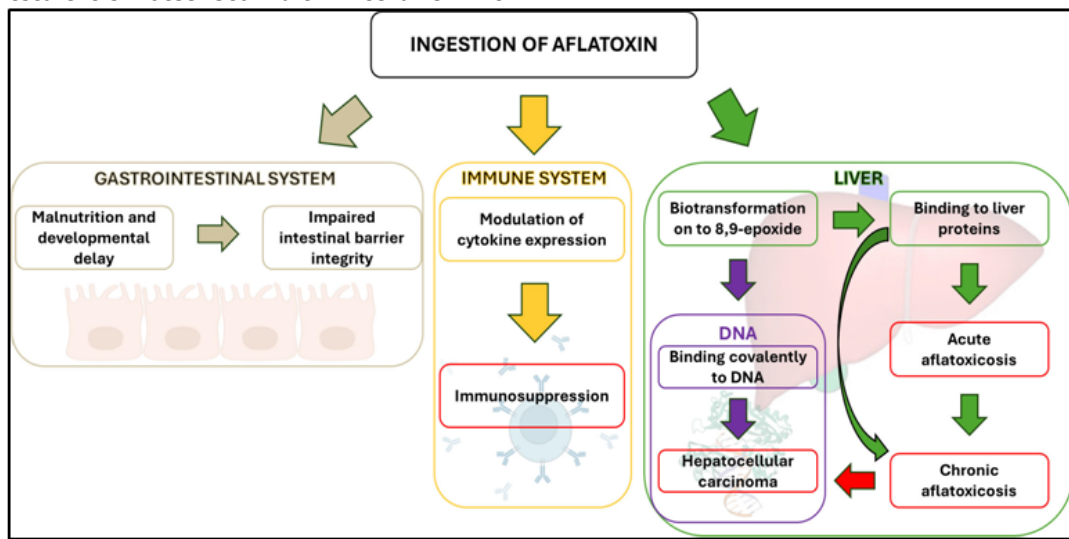


Figure 2. Mechanism of AFB1 toxicity [Source: 43]

2.4.2. Economic Impacts

The effects of aflatoxins are not only detrimental to human health, but also to animal health, productivity and trade. Exposure of susceptible animals to aflatoxin-contaminated feed, leads to slower growth rates and death. Furthermore, the meat and milk of susceptible animals may contain toxic biological metabolites. The aflatoxin exposure is reported to be highest in Nigeria whereas it is lowest in USA, with varying prevalence rate in other countries as depicted in Table 1. The direct economic consequences of aflatoxin contamination in crops are mainly due to rejection in marketable products by the international market, as well as losses caused by livestock disease, resulting in serious illness and death, which results in a loss of volume and value in the domestic markets, resulting in a significant economic loss [44].

Table 1. Economic impact, aflatoxin exposure and health characteristics of selected nations

Country	% population living below national poverty line [45]	GDP per capita, 2010 USB (PPP) [46]	Aflatoxin exposure, ng/kg bw/day [47]	% stunted children [45]
Argentina	NA	15030	0-4	8
China	5	7240	17-37	22
France	NA	34250	0.3-1.3	NA
Gambia	58	1479	4-115	28
India	29	3176	4-100	48
Kenya	52	1783	3.5-133	36
Nigeria	34	2357	139-227	43
Philippines	37	3604	44-54	34
Spain	NA	29649	0.3-1.3	NA
Tanzania	36	1484	0.02-50	44
Thailand	13	8479	53-73	16
USA	NA	47702	0.26	4

Note: GDP: gross domestic product per capita, NA: not available, PPP: purchasing power parity

2.5. Diagnostic Tests for Aflatoxins

Diagnosing aflatoxicosis is challenging due to the variability in clinical symptoms, gross pathological changes, and secondary infections resulting from immune suppression. In addition, presence of more than one mold or toxin in the contaminated feed, often makes a definitive diagnosis of aflatoxicosis difficult. The prognosis of aflatoxicosis depends upon the severity of liver damage [48]. Two techniques most often used to detect levels of aflatoxins in humans, one measures a breakdown product in urine (only present for 24 hours after exposure), and the other measures the level AFB- albumin compound in the blood serum, providing information on exposure over weeks or months [49].

Currently, AFM₁ analysis is done by various methods including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA). ELISA methods potentially have advantages over the other procedures because of their sensitivity, low cost, accurate and the use of safe reagents [50]. Recent techniques applied for detection of aflatoxins include chromatography coupled with tandem mass spectrometry, chromatography with targeted high resolution mass spectrometry, multiplex biosensors including lateral flow immune- assay and assays with antibody analogues [51]. Aflatoxin toxicity is characterized by its toxic effects on the liver and presented with symptoms similar to liver injury. The list of differential diagnoses of acute aflatoxicosis is as follows: Drug intoxication (acetaminophen, tetracycline, halothane, isoniazid, and ecstasy), Amanita species poisoning Infection (hepatitis-A/B/C/D/E, cytomegalovirus, and Epstein Barr virus), immunological (autoimmune hepatitis), metabolic (alpha-1 antitrypsin deficiency, Wilson disease), and Veno-occlusive diseases [52].

2.6. Treatment, Prevention and Control of Aflatoxicosis

Aflatoxin toxicity has no specific antidote. Administration of L-methionine (200mg/kg) and sodium thiosulfate (50mg/kg), at eight-hour intervals, administered for 7 to 14 days has proven to be therapeutic. Incorporation of protein, vitamins and antioxidants in the diet can also be beneficial. Chemoprotection and enterosorption are effective strategies to reduce the biological impact of aflatoxins. Various compounds, including esterified glucomannans and yeast extracts, have been utilized as chemopreventive agents, either enhancing aflatoxin detoxification or inhibiting aflatoxin epoxide formation, thereby reducing or blocking AFB1-induced hepatocarcinogenesis. Compounds such as oltipraz and chlorophyll are utilized to reduce the biologically effective dose [53]. Feeding rations free of aflatoxin can help avoid aflatoxicosis. Comprehensive sampling and testing operations are necessary to prevent aflatoxin contamination. Excellent agricultural practices and the biological control of fungal growth in fields is necessary. Field crop rotation, appropriate insect and weed control, and antifungal chemical treatments (e.g., acetic and propionic acids) [54]. It is advisable to dry the produce well, store properly, and transport using dry, clean containers following harvest [55].

2.6.1. Dairy Cattle Management

Ingestion of aflatoxin has dangerous effect on livestock and humans when ingested is excess of the recommendations [56]. In the United States, the Food and Drug Administration (FDA) limits the amount of aflatoxin in milk to 0.5 parts per billion and to 20 parts per billion in other foods for human consumption. No reports of aflatoxin in dairy products in the United States were listed in recently compiled review articles including worldwide data on aflatoxin in milk and milk products [57].

Reduction of aflatoxin in additives, inhibitors reduce the sources of contamination. The addition of bacteria used as silage inoculants under experimentally varied conditions reduced AFB1 [58]. Moreover, the cows treated with clay capsules added to the rumen led to the reduction of aflatoxin excreted in the milk and feces of cows [59]. Vaccination of dairy animals against AFB1 reduces AFs contamination and enhances animal health thereby, increasing food safety. Systemic vaccination of dairy cows and heifers has recently proved to be effective in reducing AFB1 carry-over as AFM1 in milk [60].

2.6.2. Hygienic and Husbandry Practices

Dairy cattle can be exposed to aflatoxin through corn grain, corn silage, and corn processing by-products that contaminated with aflatoxin [61]. Aflatoxins contamination can be reduced by proper storage and handling, detoxification, developing a systematic inspection and clean-up program, minimizing dust accumulation in milling and mixing areas, prevention of rodent and insects into storage areas and treatment of contaminated grains with ammonia [62]. The addition of antifungal agents and antioxidants such as selenium, vitamins A, C, and E to stored grains minimize aflatoxin contamination [63].

2.7. Status of Aflatoxin in Ethiopia

Ethiopian researchers have been investigating mycotoxins for more than four decades, with the earliest study on aflatoxins published in 1981. Several researchers have since reported the presence of mycotoxins in diverse food and feed commodities. According to a recent survey, *Aspergillus* species dominated in 90 maize grain samples collected the West Showa and East Wallega zones, wherein the detections of AFB1 ranged between 3.9 and 381.6 g/kg. Spices and mixes used in the food are also at high risk of contamination with Aflatoxin. A variety of spices and legumes are mixed together to make shiro. 'Shiro' is consumed by all Ethiopians, so detecting AFB1 in it is of concern. Ethiopian barley, sorghum, wheat and teff contain mycotoxins caused by *Aspergillus*. Chronic aflatoxin exposure led to approximately 11–288 cases of hepatocellular carcinoma in Ethiopia, and the synergetic effects of AFs and hepatitis B virus were responsible for 21–643 cases, with chronic hepatitis B virus prevalence in Ethiopia being among the highest among African countries (6–7%) [64]. In Ethiopia, the groundnut samples have been contaminated to an extent much higher when compared to international FAO and WHO standards (which are 15 g/kg). An investigation of the aflatoxin content of groundnut (*Arachis hypogaea*) in relation to the shelling and storage practices of Ethiopian farmers was conducted, which revealed that aflatoxins (causative agent: *Aspergillus flavus*) were detected in 80.69% of the total samples [65].

Ethiopia ranks among Africa's top countries in the livestock population, and has a growing dairy industry. Milk produced from rural areas account 97% of the total national milk production and 75% of commercialized milk production. Out of the total milk produced in the rural area, 85% is used for household consumptions. The rural milk production is characterized by indigenous breed and the common feed types are natural grass hay, crop residues and free grazing grass which are difficult to monitoring. Therefore, people in the country have high possibility of exposing to AFM1, since the use of cow raw milk and its products are common practice in the dairy farmers. However, limited evidence is available regarding to the extent of aflatoxin contamination and exposure of feeds, crops and farm animals and human [66]. Studies conducted in the Bishoftu, Sululta and Debirebirhan sites of Ethiopia indicated that 93% of the tested samples were contaminated with AFM1 [67]. The central highlands of Ethiopia also exhibited that 71% of the tested milk samples collected from three study sites namely Bishoftu, Holeta and Hawassa tested were found positive for aflatoxin. However, a significant share of milk production in Ethiopia comes from rural areas, and there is limited published data on aflatoxin levels in cow milk.

3. Conclusion and Recommendations

Aflatoxins are considered as a silent killer of humans as well as animals, and can cause both acute and chronic health problems. Chronic aflatoxin exposure can have a range of harmful effects on animals and human health. Aflatoxin-containing compounds are potent carcinogens

that can impair various organ systems, particularly the liver and have been linked to a variety of malignancies. Humans have been reported to develop cancer after exposure to AFB1, and this exposure significantly increases the risk of liver cancer. Along with weakening their immune systems, it has mutagenic, hepatotoxic, carcinogenic, and teratogenic effects flavonoid and the associated health disorders in human and animals. The economic impact of the disease in developing countries is not only limited to the high cost of production but also the cost lost from international trade.

Based on the above conclusion the following recommendations are to be suggested:

- ✓ There should be proper monitoring of temperature and relative humidity of grain and surrounding atmosphere from harvest to storage especially in the initial stage of storage.
- ✓ Feed should be purchased from reputable persons and companies which have been experienced in aflatoxin prevention and who have a proven record of properly monitoring their feed products.
- ✓ Awareness should be created on public about aflatoxicosis and its economic impact.
- ✓ The entrance of rodent and insect in grain storage areas should be controlled.
- ✓ Regular testing of feed and water offered to the animals is recommended.

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Contribution of Authors

All the authors contributed in the manuscript.

Conflict of Interest

There was conflict of interest among the authors.

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