

# Review On Occurrence and Public Health Significance of Aflatoxin in Dairy Products and Feed

Felmata Kenei<sup>1\*</sup>, Mezene Woyessa<sup>1</sup>, Abdu Mohammed<sup>2</sup>, Kumela Lema<sup>3</sup>, and Mokonin Gemmeda<sup>3</sup>

<sup>1</sup>Department of Veterinary Medicine, School of Veterinary Medicine, Wallaga University, P.O. B. 395, Nekemte, ETHIOPIA

<sup>2</sup>Department of Veterinary Clinical Science and laboratory technology, School of Veterinary Medicine, Wallaga University, P.O. B. 395, Nekemte, ETHIOPIA

<sup>3</sup>Department of Animal Science, Collage of Agriculture, Wallaga University, P.O. B. 395, Shambu, ETHIOPIA

\*Corresponding Author: [falmike.or@gmail.com](mailto:falmike.or@gmail.com)

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## Abstract

*Aflatoxins are toxic compounds that are produced by certain strains of fungi, namely, Aspergillus flavus and Aspergillus parasiticus. This fungus invades stressed crops in the field or proliferates in improperly stored feed. Aflatoxin is an important problem in Dairy products and feed. The presence of this mycotoxin in these products is an important issue, especially for children and infants, who are more susceptible than adults. Animals are exposed to aflatoxin by consuming feeds contaminated by aflatoxin-producing fungal strains during growth, harvest, or storage. When cows are fed contaminated feed, aflatoxin B1 (AFB1) is converted by hydroxylation to aflatoxin M1 (AFM1), which is subsequently secreted in the milk of lactating cows. The purpose of this review is to know the level of presence of Aflatoxin in milk products and feed and also to highlight its public health significance. Aflatoxins have been implicated in human health disorders, including hepatocellular carcinoma, aflatoxicosis, chronic hepatitis, immune suppression, stunted growth in children and many others. Several researchers show that AFB1 is the most common aflatoxin in the world from most commodities at a much higher level. AFM1 was also detected as greater than the standard from milk and milk products. This indicates that many developing countries are in danger of aflatoxin contamination.*

**Keywords:** Aflatoxin, Milk, Occurrence, Public Health.

## Introduction

Aflatoxins were discovered in 1960, when over 100,000 young turkeys died in England over a few months from what appeared to be a new disease called "Turkey-X disease" (Negash, 2018). Following a thorough examination of the outbreaks, the disease was linked to Brazilian groundnut meal. An extensive study of groundnut meal revealed its toxic nature, as it caused typical symptoms of Turkey-X disease in poultry and ducklings when consumed. A study of the toxin's nature suggested that it came from the fungus *Aspergillus flavus*. As a result of its origin from *A. flavus*, the toxin was dubbed "aflatoxin" (Angele *et al.*, 2010; Negash, 2018).

Recent estimates suggest that more than five billion people worldwide are at risk of chronic exposure to aflatoxins. Aflatoxicosis is caused by consuming aflatoxin-contaminated feed (Negash, 2018). As long as people consumed contaminated animal products and considered public health to be important, this has remained a major public health concern (Seid and Mama, 2019). Mycotoxins are fungus secondary metabolites linked to specific human and animal diseases. Furthermore, some mycotoxins are now known to be acutely toxic; linked to the prevalence of specific cancer types, and this feature has caused concern worldwide (Celik *et al.*, 2005). It is estimated that aflatoxin (AFs) and other mycotoxins contaminate one-quarter of the world's food and feed along the food chain (Marin *et al.*, 2013). Fungal contamination can occur at any stage of the food production chain, including field growth, commodity harvesting, transportation, and storage (Kamika *et al.*, 2016).

The problem of aflatoxin contamination of food is a common problem in tropical and subtropical regions of the world, especially in developing countries such as sub-Saharan countries, where there are bad practices and warm environmental temperatures and humidity favor the growth of fungi. (Trasher, 2012). Unlike other African countries, few studies have been conducted on aflatoxin and aflatoxin-induced fungal contamination of food in Ethiopia (Chala *et al.*, 2013). A recent study in Ethiopia shows that milk and milk products in a milk warehouse in Addis Ababa are highly contaminated with aflatoxins ranging from 0.028 to 4.98 mg/L (Gizachew *et al.*, 2016).

Milk and milk products are the main components of the human diet that can be exposed to aflatoxins in the human body, which poses a risk to consumers, especially infants and young children (Hampikyan *et al.*, 2010). The source of AFM1 in milk is reported to be dietary aflatoxin B1 (AFB1) from lactating animals, which is converted to a hydroxylated metabolite in the liver and excreted in milk (USAID and Danya International, 2012). In general, aflatoxin M1 is a serious problem for human consumption in milk and milk products. The presence of this mycotoxin in these products is a major concern, especially for children and babies, who are more sensitive than adults. Therefore, the objectives of this article are:

- To review the aflatoxin content in dairy products and feed.
- To emphasize its public health importance and level of intervention in the prevention and control of the problem.

## Aflatoxicosis

### *Mycotoxins*

Mycotoxins are secondary fungi metabolites associated with certain diseases in animals and humans. Toxicity in animals is harmful because these compounds are produced by fungal species. In addition to acute toxicity, some mycotoxins are now also associated with certain forms of cancer. This aspect has raised global concerns about feed and food safety, especially the safety of milk and milk products (Nogaim, 201).

The term mycotoxins are derived from the Greek word "mycos" meaning mold and the Latin word "toxicum" meaning poison. Mycotoxins are relatively low molecular weight secondary metabolic products of fungi harmful to animals and humans, produced by various fungi, and affecting many agricultural products intended for human consumption and animal feed. Mycotoxins in food and feed are a major food and feed safety problem and their impact on human and animal health is associated with significant economic losses (Akebergn *et al.*, 2019)

### *Aflatoxins*

Aflatoxins are a group of mycotoxins produced mainly by several species of fungi of the genus *Aspergillus*. This

includes *A. flavus* and *A. parasiticus*, *A. pseudotamarii* and *A. called* species. Among these species, *A. flavus* and *A. parasiticus* are known (Bhat and Vasanthi, 2003). Aflatoxin has six forms; they are AFB1, AFB2, AFG1, AFG2 and AFM1. AFB1 and AFB2 are produced by both *A. flavus* and *A. parasiticus*, while AFG1 and AFG2 are produced only by *A. parasiticus* (Iamanaka *et al.*, 2007). Investigation of the nature of the toxin revealed that it originates from the fungus *Aspergillus flavus*. Thus, the toxin was named "aflatoxin" based on its origin in *A. flavus*. This was the event that sparked scientific interest and gave birth to modern mycotoxicology. Aflatoxin research ushered in a "golden age" of mycotoxin research, during which several new mycotoxins were discovered (Angela *et al.*, 2018; Negash, 2018).

The relative proportions and amounts of different aflatoxins in food plants depend on the species of *Aspergillus* present, pest infestation, growth and storage conditions, and other factors. Although the geographic distribution of these species is similar, *A. parasiticus* is less common and *A. flavus* is the most common fungus in food. In ruminants, aflatoxins are metabolized in the liver and excreted in the bile. These aflatoxins are divided into six major toxins based on their ultraviolet fluorescent properties and chromatographic mobility. Both *A. flavus* and *A. parasiticus* produce aflatoxins B1 (AFB1) and aflatoxins B2 (AFB2), which produce blue fluorescence, while *A. parasiticus* produces aflatoxins G1 (AFG1) and aflatoxins G2 (AFG2), which exhibit green fluorescence. Quadruple-hydrated aflatoxin B1 and B2 are converted to aflatoxin M1 and M2, respectively. They are found in the milk of lactating mammals that have eaten food contaminated with aflatoxin. Aflatoxin B1 is the most toxic and widespread (Lopez *et al.*, 2002; Yu, 200).

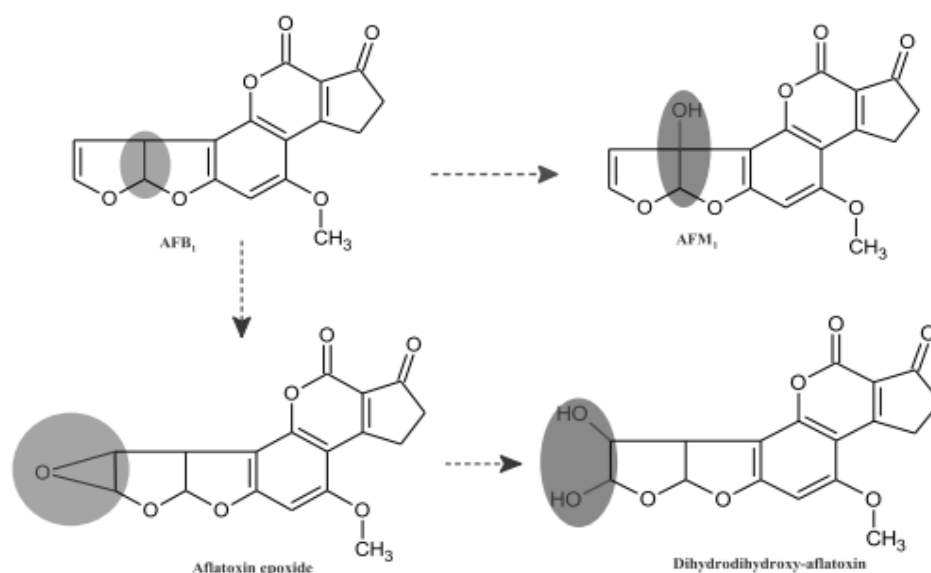


Fig. 1: Aflatoxin B1 metabolism in the liver (Iqbal, *et.al*, 2015).

### Properties of Aflatoxins

Aflatoxins are produced by fungi of the genus *Aspergillus*, which grow on grains and other crops. They are less colorful or 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, especially in polar solvents or when exposed to oxidizers, ultraviolet light, or solutions with a pH below 3 or above 10. Aflatoxins decompose at melting temperatures ranging from 237 °C (G1) to 299 °C (M1), but not destroyed under normal cooking conditions. They can be completely destroyed by autoclaving in the presence of ammonia or treatment with bleach. The physical and chemical properties of aflatoxins are listed below (Wang *et al.*, 2010)

**Physical Properties of Aflatoxins:** When isolated, aflatoxins are crystalline, odorless solids that vary in color from pale white to yellow. Melting temperatures range from 268 °C for B1 to 190 °C for G2 (Seid and Mama, 2019). The optimum water activity for the growth of *A. flavus* is high (about 0.99). The maximum is at least 0.998, while the minimum water activity for growth is not defined. In general, high water activity seems to favor the production of toxins. *Aspergillus flavus* has been reported to grow between 10 and 3 °C. The optimum growth rate occurs slightly

above 30 °C, up to 25 mm per day. *A. flavus* produces aflatoxins in the temperature of 15-37 °C. The optimum temperature for toxin production cannot be determined, although production between 20 and 30 °C has been reported to be significantly greater than at higher and lower temperatures (Pitt and Miscamble, 1995).

**Chemical Properties of Aflatoxins:** Aflatoxins belong to the group of difuranocoumarins. The compounds are generally soluble in methanol, chloroform, acetone, and acetonitrile, which are slightly polar but insoluble in non-polar solvents. Aflatoxin reacts with alkaline solutions, causing hydrolysis of the lactone moiety. This hydrolysis is reversible, as recycling has been shown to occur upon the acidification of aflatoxin-containing basic solutions. At elevated temperatures above 100°C, ring-opening occurs, followed by decarboxylation, further reaction, and loss of the methoxy group from the aromatic ring (Taylor, *et al.*, 1993).

In the presence of mineral acid, aflatoxins B1 and G1 are converted to aflatoxins B2A and G2A by acid-catalyzed addition of hydroxyl groups across the double bond of the furan ring. In the presence of acetic anhydride and hydrochloric acid, the reaction proceeds to acetoxy derivatives. Similar adducts of aflatoxins B1 and G1 are formed with thionyl formate and trifluoroacetic acid. Many oxidizing agents, such as sodium hypochlorite, potassium permanganate, chlorine, hydrogen peroxide, ozone, and sodium perborate, exhibit some form of fluorescence as indicated by loss of fluorescence in UV light at 365 nm, reacts with aflatoxin molecules (Seid and Mama, 2019).

Hydrogenation of aflatoxin B1 and G1 yields aflatoxins B1 and G1, respectively. Further reductions of aflatoxin B1 by three moles of hydrogen yield tetra hydroxyl aflatoxin. Reduction of aflatoxin B1 and B2 with sodium borohydride yields aflatoxin R-B1 and R-B2, respectively. These arise due to the 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).

### **Factors Associated with Aflatoxin Production and its Occurrence**

The production of aflatoxin is equally influenced by physical and biological factors. They are reported to be produced between 25°C - 35°C optimum temperature and acidic pH. Relative humidity between 83%-88% and an appropriate level of CO<sub>2</sub> & O<sub>2</sub> has also been reported to influence mold growth and aflatoxin production. For instance, 20% CO<sub>2</sub> and 10% O<sub>2</sub> in the air depress aflatoxin production (Bankole, and Adebajo, 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 mold growth, producing aflatoxin (Gilbert and Anklam, 2002).

**Agro-ecology:** The contamination of crops by aflatoxin can be affected by a variety of factors, including agroecology, pre-and post-harvest conditions, and crop cultivar. Mutegi *et al.*, (2009) showed an association between aflatoxin concentration and agroecological zone and crop cultivar. The association between improved cultivars and aflatoxin concentration is highly significant with improved cultivars reducing the risk of contamination to half. In the same study area, AFB1, AFB2, AFG1, and AFG2 production was assessed by isolating 1458 cultures of *Aspergillus flavus* or *A. parasiticus* from selected samples. The incidence of *A. flavus* L-strain was highest (78%) > *A. flavus* S-strain (68%) > *A. niger* (65%). The probability of containing total aflatoxin exceeding 10 µg/kg was three times higher in samples from the Busia districts than from the Homa Bay districts.

There was a negative correlation between the incidence of AFB and crop rotation, between *A. flavus* and grading, being a member of a producer marketing group, and planting improved cultivars. Therefore, it was reported that the modification of these factors might reduce the hazard of aflatoxin contamination in groundnut (Mutegi *et al.*, 2012). In another study, by (Bumbangi *et al.*, 2016), 92 groundnut samples were collected from open markets and supermarkets in Zambia for aflatoxin analysis. Aflatoxin concentrations ranged from 0.014 to 48.67 ppb in 55% of the samples.

**Storage:** Kachapulula *et al.*, (2017) revealed an association of agroecology, fungal morphology, and storage conditions with the concentration of aflatoxin contamination in maize and groundnut. Out of the 334 groundnut and maize samples collected from 27 districts of Zambia, aflatoxin concentrations > 10 µg/kg (maximum permissible limit in Zambia) were found in 17% of the samples. Out of the samples with aflatoxin concentration ≥ 10 µg/kg, 38% were from Agroecology I, which is the warmest region, and 8% from Agroecology III, the coolest. A lower concentration of aflatoxin was found in maize (16µg/kg) than in the groundnut (39 µg/kg). There was a 1,000-fold

increase in safe crops that had been stored under poor storage conditions at temperatures  $> 31^{\circ}\text{C}$  and relative humidity of 100% for a week. During the marketing of groundnut seeds, different risk factors can increase aflatoxin concentration. According to (Manizan *et al.*, 2018) the storage of peanuts for 3 months could cause market loss due to fungi (37%) and high temperature and humidity (4.5%). The incidence of those fungi leads to the occurrence of mycotoxins, including aflatoxin.

**Threshing:** The threshing method is one of the factors associated with aflatoxin contamination. For example, from the research done by (Taye *et al.*, 2018), sorghum grain samples threshed on bare ground, canvas, concrete asphalt, and cow-dung-painted ground have different concentrations of aflatoxin. The results showed that *Aspergillus* species were found in all samples from different threshing methods but were higher in bare-ground threshed samples. As the storage time increased, there was a gradual increase in the aflatoxin B1 concentration: the highest was in bare-ground-threshed samples ( $1.97\mu\text{g}/\text{kg}$ ) and the lowest in canvas-threshed samples ( $0.70\mu\text{g}/\text{kg}$ ).

### **Occurrence of Aflatoxin M1 in Milk and Milk Products**

**Aflatoxin M1 in Milk:** According to estimates made by Pei *et al.* (2009), between 0.3-6.2% of AFB1 consumed through animal feed is converted into AFM1 and secreted through milk. Different animals within the same species, such as cows, buffaloes, goats, and sheep, may convert at different rates. Additionally, AFM1 is found in milk almost 12 to 24 hours after an animal first consumes AFB1 (Goncalves *et al.*, 2017). Diet, breed, health, lactation, stage, and digestion rate of the animal are some of the variables that affect the conversion rate of AFB1 to AFM1 (Duarte *et al.*, 2013). The carryover rate of dietary AFB1 into AFM1 in milk was observed to be 2.2% in cows. Among goats, the amount of AFM1 excreted via milk was estimated to range between 0.18% and 3% of the ingested AFB1 (Virdis *et al.* 2008), while the presence of AFM1 in sheep's milk ranged between 0.08% and 0.33% of consumed AFB1 (Battacone *et al.*, 2005). The concentration of AFM1 was observed to decrease down to undetectable levels after 84 h and 120 h of AFB1 ingestion in goats and cows, respectively (Battacone *et al.* 2012; Sumantri *et al.* 2012). Additionally, it has been noted that levels of AFM1 excreted through milk have a linear relationship to levels of AFB1 consumed (Goncalves *et al.*, 2017). AFM1 may not be distributed uniformly throughout milk, with casein and milk serum containing the majority of the protein while the fat fraction of milk may only contain a small amount (Granados Chinchilla, 2016).

**AFM1 in Milk Products:** AFM1 may be present in numerous other milk products, including cream, butter, and ice cream. The presence of AFM1 in these products has not been extensively studied but may have interesting research implications. The levels of AFM1 in dairy products made from contaminated milk, such as butter, buttermilk, cream, and skim milk, were examined in a study by Bakirci (2001). The average AFM1 concentration in cream samples was 64.4% that in bulk-tank milk. Meanwhile, the mean AFM1 level in skim milk was 3% higher than in bulk-tank milk. AFM1 levels in the study's butter samples were lower and were 33.80% higher than those in bulk tank milk. The protein membrane surrounding the fat globules in butter is damaged during processing and the serum phase.

**Status of Aflatoxins In Ethiopian:** Contrary to other African nations, Ethiopian food contamination with aflatoxin and aflatoxigenic fungi has only been the subject of a small number of studies. One of these was the contamination of groundnut samples obtained from significant groundnut production districts in eastern Ethiopia, according to the report by (Chala *et al.* 2013). Aflatoxin contamination was found in 93 out of 120 groundnut samples in the study, with levels ranging from 15 to 11,900 g/kg. The high aflatoxin contamination in groundnut seeds and cake was confirmed by a subsequent study conducted in the same districts and published by Mohammed *et al.* in 2016. During the cropping seasons of 2013/14 and 2014/15, 160 groundnut seed samples and 50 groundnut cake "Halawa" samples were gathered for that study. Aflatoxins (B1, B2, G1, and G2) ranging from 0.1 to 2526 ng/g were detected in the samples. It is mandatory to set rules and regulations regarding aflatoxin concentration in beer and other food commodities in Ethiopia (Nigussie, *et al.*, 2018). Furthermore, for the first time in 2015, aflatoxin contamination in milk and dairy feeds has been reported. All 110 milk samples from dairy farmers and milk traders as well as 156 feed samples from dairy farmers, feed producers, traders, and processors were found to be contaminated with AFM1 and AFB1 in that Addis Ababa study (Gizachew *et al.*, 2016). In milk and feed samples, AFM1 concentrations ranged from 0.028 to 4.98 g/L and AFB1 concentrations from 7 to 419 g/kg.

In a study conducted to assess the occurrence of different mycotoxin types in barley, sorghum, teff, and wheat from Ethiopia, AFB1 was detected in all cereal samples. AFB1 concentration ranged from  $< 1$  to  $26\mu\text{g}/\text{kg}$  in 8.8% of the 352 samples. The maximum concentration ( $26\mu\text{g}/\text{kg}$ ) was detected in sorghum; the reason for this could be the

underground storage of sorghum, which leads to a high seed moisture content (Ayalew *et al.*, 2006). In another study comparing aflatoxin concentrations in fresh and stored sorghum samples, a higher concentration of AFB1 was detected in stored than in fresh sorghum samples. The aflatoxin analysis of sorghum samples collected from eastern Ethiopia revealed a lower concentration of AFB1 (17 µg/kg) in fresh sorghum than in samples stored for 5–6 months (33.1 µg/kg) (Taye, *et al.*, 2016). A study by (Chala *et al.* 2013) also confirmed the detection of AFB1, AFB2, AFG1, AFG2, and AFM1 in sorghum samples and AFB1 and AFG1 in finger millet samples. These findings suggest the wide aflatoxin contamination of different Ethiopian foods and hence an urgent implementation of rules and regulations regarding aflatoxin concentration is required to prevent further health issues related to aflatoxin toxicity.

### **Public Health Significance of Aflatoxin**

In both human and animal populations, aflatoxins are linked to toxicity and carcinogenicity (Murray, 2009). Acute aflatoxicosis causes death, while chronic aflatoxicosis causes longer-lasting pathologic changes, such as cancer and immunosuppression. Aflatoxin B1 primarily targets the liver, and liver damage has been observed in rodents, poultry, and nonhuman primates after ingestion. Humans have experienced acute hepatitis as a symptom of acute aflatoxicosis (Murray, 2009). Aflatoxin-contaminated maize consumption in 1974 in India led to an outbreak of hepatitis that resulted in the deaths of 100 people. The livers of those who passed away contained high concentrations of aflatoxin B1 (Magnussen and Parsi, 2013).

### **Toxicity of Aflatoxins and Acute Aflatoxicosis**

According to Ali (2019), AFB1 is the most common aflatoxin, a powerful hepatocarcinogen in humans and other species, and has been categorized as a group 1 carcinogen. Due to aflatoxin B1's potent human carcinogenic effects, the majority of research on mycotoxins has concentrated on this substance. According to Wild and Turner (2002), CYP3A4, 3A5, and 1A2 are the primary human cytochrome P450 (CYP) enzymes involved in the liver's metabolism of human AFB1. Various animal species exhibit diversity in their AFB1 metabolism (Wu *et al.*, 2009). And the most critical reaction is bioactivation to (endo-, exo-) AFB1-8,9-epoxide, a highly reactive metabolite that covalently binds to DNA and induces mutations or forms adducts with proteins. A recent study indicated that residual AFB1 in the liver negatively affects the p53 and protein Rb pathways in hepatocellular carcinoma (Ramalho, *et al.*, 2018).

Hepatitis also affects aflatoxin exposure in humans. It has been demonstrated that AFB1 and hepatitis B virus (HBV) are synergistic causative agents of hepatocellular carcinoma. Infection by HBV directly or indirectly sensitizes hepatocytes to the carcinogenic effects of AFB1 (Ali, 2019). In an epidemiological study, a higher concentration of AFB1 adducts was found in chronically infected Gambian children and adolescents with HBV than uninfected individuals (Turner, *et al.*, 2000). AFB1 possesses toxic effects with a range of consequences; large doses cause acute toxicity and death whereas, chronic sublethal doses induce tumors and impair growth. There is limited information on acute aflatoxin toxicity in humans. Acute poisoning in humans has been reported in developing countries, for example, the severe acute aflatoxicosis outbreak in Kenya in 2004 with a mortality of 39.4% (Lewis, *et al.*, 2005; Probst, *et al.*, 2007). Abdominal pain, vomiting, fatty liver, and necrosis are common acute poisoning in humans. Other symptoms include depression, anorexia, diarrhea, jaundice, and photosensitivity (Marin, *et al.*, 2013). In human beings, prolonged consumption of aflatoxin-contaminated food has been linked to liver cancer (Wu, and Santella, 2012). impaired immune function, decreased reproductive functions, visceral encephalopathy, and pulmonary interstitial fibrosis (Marin, *et al.*, 2013; Ali, 2019). Acute aflatoxicosis in humans has been reported in many countries, including developing countries. Vomiting, abdominal pain, pulmonary edema and fatty infiltration, and necrosis of the liver were the clinical manifestations of aflatoxicosis (Wu, *et al.* 2014).

### **Child Growth Impairment**

Childhood stunting has been linked to aflatoxin exposure. When a child's height for his or her age is at least two standard deviations below the WHO growth reference for a nation or region, the condition is known as stunting. Stunting is significant from the standpoint of public health due to its correlation with outcomes like susceptibility to infectious diseases and cognitive impairment (Wu *et al.*, 2014). The possibility of compromised intestinal integrity, endothelial cell toxicity, which changes barrier function, and immune suppression are viable hypotheses (Ali, 2019), even though the mechanism of the association between aflatoxin exposure and child impairment is not fully understood.

Numerous studies have been done to confirm the link between aflatoxin exposure and a reduction in a child's ability to grow, with the exposure starting during pregnancy. As an illustration, Turner *et al.*, (2007) research on the impact of maternal aflatoxin exposure on infant growth in the Gambia. Infants in their first year of life gain weight (0.8 kg) and height (2 cm) as a result of a drop in the aflatoxin-albumin concentration in the mother's blood (110 to 10 pg/mg). Additionally, an inverse relationship between maternal aflatoxin exposure and baby birthweight was hypothesized by Shuaib *et al.* (2010). AFB1-lys levels in the study's 785 pregnant Ghanaian women ranged from 0.44 to 268.73 pg/mg albumin. Mothers with very high AFB1-lys have a higher chance of giving birth to a baby with a low birth weight. In a longitudinal study on Benin children aged from 16 to 37 months, an association between the aflatoxin-albumin concentration and growth rate was found. After an 8-month follow-up period, a mean 1.7 cm growth reduction was observed in the high aflatoxin exposure group (Gong, *et al.*, 2004). McMillan *et al.*, (2018) The association between the number of 'wasted' children and being fed aflatoxin-contaminated flour was highly significant. Aflatoxin concentration ranged from 2 to 82 µg/kg in the weaning flour samples (Okoth, and Ohingo, 2004).

### **Hepatocellular Carcinoma**

According to the World Health Organization WHO (2008), liver cancer, also known as hepatocellular carcinoma (HCC), causes between 550,000 and 600,000 new cases worldwide each year (Ferlay *et al.* 2004). Humans and many other animal species develop liver cancer after being exposed to aflatoxins. "Naturally occurring mixes of aflatoxins" have been categorized as a Group 1 human carcinogen by the International Agency for Research on Cancer (Anfossi, *et al.*, 2011). According to Grace and Unnevehr (2013), aflatoxins and the hepatitis B virus (HBV) significantly increase a person's risk of developing hepatocellular carcinoma in developing nations. Hepatocellular carcinoma is more likely to develop in people exposed to both aflatoxins and HBV than it is in people who are only exposed to aflatoxin (Wu *et al.*, 2014). There is a difference in the incidence of hepatocellular carcinoma in the world and the highest burden is in Asia and Sub-Saharan Africa (Liu and Wu, 2010). In China, there are >750,000 new cases each year and the annual death rate is >300,000.

Through cross-sectional, ecological, case-control, and prospective cohort investigations in highly exposed populations, the relationship between aflatoxin exposure and human hepatocellular carcinoma has been examined (Wu *et al.*, 2014). The liver is the organ that AFB1 is metabolized in. AFB1 is metabolized by cytochrome-P450 enzymes into reactive genotoxic intermediates (aflatoxin B1-8, 9-oxide, AFBO) or is hydroxylated (to AFQ1 and AFM1) and demethylated (to AFP1) to become less toxic than AFB1 after consumption of food contaminated with aflatoxin. If this is not repaired before DNA replication, the DNA adducts interact with the guanine base of the DNA and cause mutational effects in the p53 tumor suppressor gene, resulting in hepatocarcinogenesis (Hamid, *et al.*, 2013). Therefore, inhibition of this route using different natural chemicals is used as an intervention method to prevent hepatocellular carcinoma (Figure 2). A rat study revealed that exposure to AFB1 can cause pathological changes including edema of the heart, for example (Kudayer, *et al.*, 2019). Aflatoxin may be responsible for as much as 28.2% of all HCC cases worldwide, according to Liu and Wu's (2010) research. At its lower estimate, aflatoxin contributes to only 4.6% of all annual HCC cases. This wide range is a result of the data on cancer risk factors, HBV prevalence, aflatoxin exposure, and other risk factors in various parts of the world being extremely uncertain and variable. Sub-Saharan Africa, Southeast Asia, and China are the regions of the world that are most severely affected (Liu and Wu, 2010).

### **Immunomodulation**

The immune system of people is impacted by aflatoxin exposure, according to numerous studies. Only a small portion of the immune system's response cells were active in subjects exposed to high dietary aflatoxin levels in a Ghanaian study (Jiang *et al.*, 2005). Similar to this, higher levels of serum aflatoxin-albumin adducts were linked to lower levels of secretory IgA in the saliva of Gambian children (Turner *et al.*, 2003). Aflatoxin-induced immunosuppression increases the risk of secondary infection, according to studies done on lab animals.

According to a recent study, mice that have already been exposed to the parasite *Toxoplasma gondii* can become infected when exposed to aflatoxin (Bondy and Pestka, 2000). One month before the feeding studies began, mice were given an injection with the parasite. Gavage was used to administer aflatoxin (0.1 mg/kg body weight) every day for 50 days. In the brains of immunocompetent control animals, the parasite created cysts, but the infection was dormant. The brain lesions and ruptured cysts in mice treated with aflatoxin, on the other hand, were signs of

impaired immune function. Studies on poultry have demonstrated that exposure to aflatoxin at diet levels of 200 ppb AFB1/d for up to 40 weeks decreases antibody titers to vaccines in addition to increasing infectivity (Gabal and Azzam, 1998).

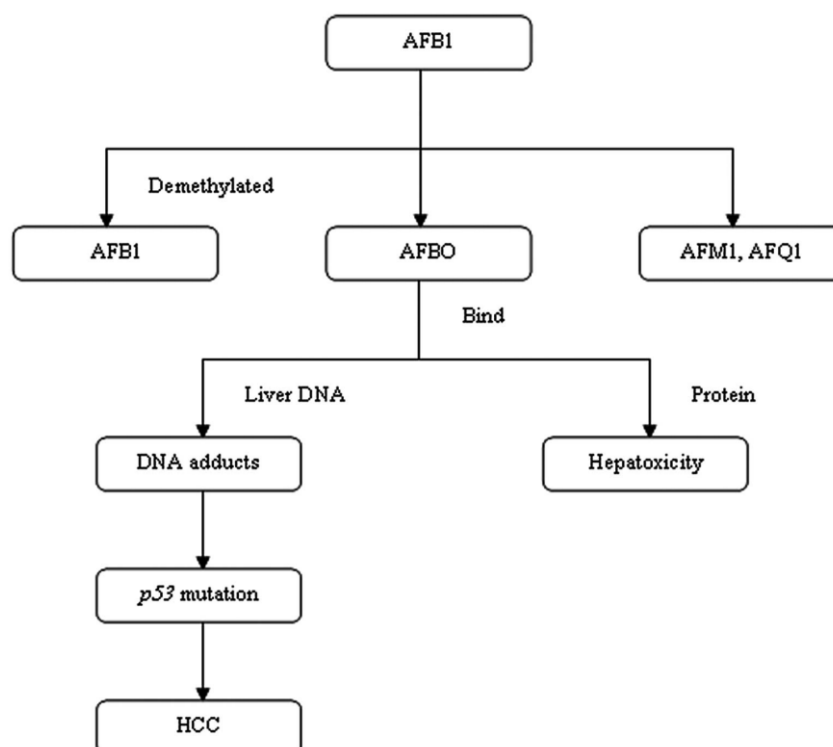


Fig. 2: AFB1 biotransformation to AFM1 and AFQ1, or demethylation to AFP1 Source: (Hamid *et al.*, 2013)

## Control and Prevention of Aflatoxin in Animal Feed and Milk

Numerous techniques can be used to lower aflatoxins that naturally occur in foods and feeds. Modern definitions of GAP (Good Agricultural Practice) or HACCP (Hazard Analysis Critical Control Point) include improved farm management practices, quicker drying, and controlled storage (IARC, 2002). Contaminated lots of peanuts or maize can be cleaned up to produce food-grade products by segregating contaminated lots after aflatoxin analyses and by sorting out contaminated nuts or grains by electronic sorters. Highly contaminated commodities can be made suitable for animal feed by decontamination using chemical processes like ammonization. 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 have required that act specifically against certain types and groups of toxins (WHO, 2008).

Absorptive substances can be used to lessen mycotoxins' overall potency. Alternative tactics, such as enzymatic or microbial detoxification, have recently been used to mitigate the effects of some fungal toxins, even though adsorbents have demonstrated their effectiveness against some mycotoxin-induced toxicosis (Binder, 2007). Prandini *et al.* (2009) concluded that to control AFM (1) in foods it is necessary to reduce AFB1 contamination of feeds for dairy cattle by preventing fungal growth and AFB (1) formation in agricultural commodities intended for animal use. During 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 contamination by *Aspergillus* species. Therefore, it is essential to lessen the likelihood of this contaminant through the selection of hybrids, seeding time and density, appropriate plowing and fertirrigation, as well as chemical or biological control. To lower the risk of contamination, it is essential to harvest grains with the lowest moisture possible, conserve moisture close to or below 14%, and maintain mass to homogeneous moisture. Mechanical damage to the kernel, grain cleaning procedures, and conservation temperature are additional elements that need to be carefully managed. 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 lower the risk of infection and remove crop residues from the field, for example, by deep plowing, to lower the risk of infection; Utilize seed varieties created to be resistant to fungi; Apply fertilizer in accordance with crop needs to prevent plant stress; Use effective agronomic techniques (weed control, plant spacing, irrigation) to prevent plant stress caused by drought and high temperatures; To prevent insect damage and fungus infections, take the appropriate phytosanitary precautions when handling seeds and crops; Reduce mechanical damage to a minimum to prevent fungal infections and plant stress (Hans and Van-Egmond, 2013).

**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 (Hans and Van-Egmond, 2013).

**Storage:** According to the USAID desk review, maintaining storage areas that are clean, dry, insect- and rodent-free is essential for preventing the growth of aflatoxin. For consistent, long-term use, it is crucial to make storage options affordable and available. The various pre- and post-harvest methods are generally described in the USAID synthesis (USAID and Danya International, 2012).

**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 (Hans and Van-Egmond, 2013).

## Conclusion and Recommendations

Aflatoxin is a particular class of mycotoxin made by *Aspergillus* molds. The most well-known and extensively studied mycotoxin is aflatoxin. Animals and humans worldwide frequently suffer from chronic consumption of food contaminated with aflatoxin, particularly in underdeveloped nations where food and food products are improperly harvested, processed, and stored, increasing the likelihood of mold growth. Public health is endangered by the presence of AFM1 in milk and dairy products. Aflatoxin contamination, particularly AFB1, and aflatoxin-producing fungi are most prevalent in Ethiopia. Additionally, the majority of the country's goods have contamination levels that are far above the norm for the world. AFM1 contamination in milk and milk products is also higher than average. Aflatoxin poisoning results in a number of diseases in both humans and animals. Age, gender, exposure level, duration of exposure, health status, immune system strength, diet, and environmental factors all affect how much a toxin affects people. Aflatoxin has severe negative effects on health and generates significant financial losses in Ethiopia and many other developing nations. On the basis of the foregoing conclusion, the following recommendations are suggested:

- Natural food chain contaminants containing Aflatoxin should be reduced through hygiene measures and a multifaceted awareness approach.
- Strategies should be in place to deal effectively in developing countries where exposure to Aflatoxins is intertwined with problems of food insecurity and scarcity.
- Collaboration through 'ONE HEALTH' approach, between the local, regional, national, and international governing bodies, and between different disciplines to reduce aflatoxin exposure

## Contribution by Authors

Equal contribution

## Conflict of Interests

There is no conflict of interest.

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