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REVIEW ARTICLE **OPEN ACCESS**

Aflatoxins In Foods And Feeds: A Review On Health Implications, Detection, And Control

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ABSTRACT

Aflatoxins are mycotoxins produced by some fungi found on agricultural crops like peanuts, cottonseed, tree nuts, and maize (corn). The major molds which make aflatoxins including Aspergillus parasiticus and Aspergillus flavus that are common in the humid and warm areas of theglobe. Major types of aflatoxins include Aflatoxin B_1 and B_2 (AFB) and Aflatoxin G_1 and G_2 (AFG); their metabolites include Aflatoxin M_1 (AFM_1), Aflatoxin M_2 (AFM_2), Aflatoxicol (AFL), and Aflatoxin Q_1 (AFQ_1). Aflatoxin B_1 is considered the most toxic. Aflatoxin-producing molds may infect agricultural produces in field, during harvests, and also in storage facilities. Aflatoxin-producing molds are commonly detected in inadequately stored agricultural produce including maize, rice, cassava, cottonseed, millet, sorghum, sesame seeds, sweetcorn, chili peppers, tree nuts, sunflower seeds, spices, and wheat. Children are mostly impacted by exposures to aflatoxin; which has been linked to liver cancer, liver impairment, delay in development, and stunted growth. In developed countries, the mean exposures to aflatoxins via diet are usually below 1 ng/kg body weight (bw) per day, while estimates for many sub-Saharan African countries go beyond 100 ng/kg body weight per day. The estimates of dietary exposures to AFM1 hardly exceed 1 ng/kg body weight a day in countries. No animal species has shown immunity to the acute toxicities of aflatoxins. The major target organ in the mammals is the liver; aflatoxicosis is primarily a hepatic disease. Regular diet such as apiaceous vegetables, like parsnips, carrots, parsley, and celery can decrease carcinogenic effect of aflatoxins. A range of methods for detecting aflatoxins in foods and feeds are available. Aflatoxin control is needed in pre-harvest as wells in postharvest handling. Major lasting, sustainable solution to control pre-harvest infection ofaflatoxin is via improving capability of crops to prevent fungal infections or preventing aflatoxins productions by fungal invasion.

Key words: *Aflatoxins, Health Implications, Detection, Control Measures*

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INTRODUCTION

Aflatoxins are poisonous mutagens and carcinogens released by some molds, mostly *Aspergillus parasiticus* and *Aspergillus flavus,* that thrive in hay, grains, decaying vegetation, and soil. Aflatoxins are commonly detected in inadequately stored foods including cottonseed, millet, peanuts, rice, cassava, sesame seed, chili, pepper, sweet corn, tree nuts, sunflower seed, sorghum, spices, and wheat. When infested foods are prepared, aflatoxins find their way into overall food chain, and have been detected in human and pet food, and also in animal feedstock including livestock feeds. Animals that consume infected foods often pass aflatoxins product to milk, eggs, as well as meat [1, 2]. For instance, contaminated poultry feeds were suspected in finding of huge samples percent of eggs and chicken meat contaminated with aflatoxin in India [3]. Aflatoxins pose significant health risks to livestock and humans. They constitute substantial economic problem, resulting in at least 25 percent of global crops destroyed

per annum [4]. "Aflatoxin" is a term derived from *Aspergillus flavus*, one of the fungi that release it. Aflatoxin has been reported to be named in 1960s when it was discovered as cause of the "Turkey X disease" [5]. Aflatoxins are among the main groups of mycotoxins that are of significant concern to human and livestock.

Children are mostly at risk of exposures to aflatoxins, and have been linked to impaired development, stunted growth [6, 7], liver cancer, and liver damage. An association between aflatoxin exposure and childhood stunting [8] has been reported in various studies [9] but was not detected in all [10, 11]. Additionally, a causal relationship between aflatoxin exposure and childhood stunting has not been conclusively demonstrated by reliable studies, although studies of that nature are currently in progress [8, 12]. Adults have higher tolerance to aflatoxins exposures, but are also at risk. Aflatoxins are among most carcinogenic substances known to man. The metabolism of cancer caused by factors such as aflatoxicosis can be seen in Figure 1. No animal species is immune. After entering the body system, aflatoxins can be hydroxylated to transform into less harmful aflatoxin M_1 or metabolized to a reactive epoxide intermediate by liver.

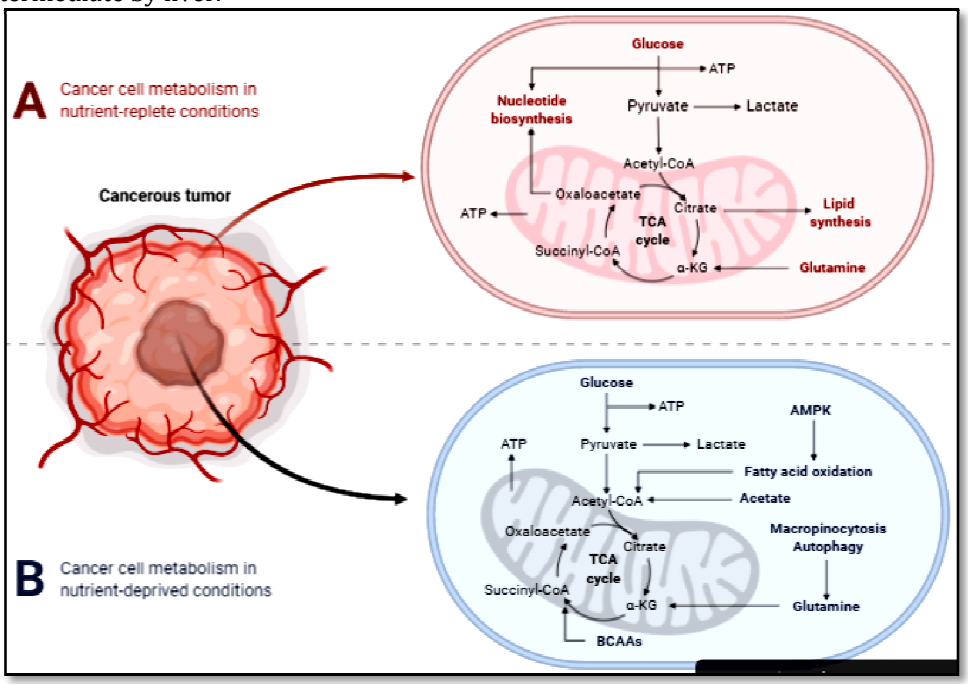

Figure 1: Metabolism of cancer caused by factors such as aflatoxicosis in (A) nutrient replete conditions and (B) nutrient-deprived conditions

Aflatoxins are mostly ingested. Although aflatoxin B_1, the most toxic aflatoxin, has the ability to penetrate via skin [2, 4, 13, 14]. FDA action level regarding aflatoxins in feed or food range from 20–300 parts per billion[14]. People are often at risk of getting aflatoxins via consuming infected plants (e.g., corns, peanut, wheat, ginger) or via eating dairy products or meat prepared with animal fed with feed contaminated with aflatoxin. Farmers and most farm workers can get exposure via breathing in the dirt created by processing and handling of contaminated feeds as well as crops. National estimates of dietary aflatoxin exposures show disparity between the developing and developed nations [4]. In developed nations, the average dietary exposure to aflatoxin is in general below 1 ng/kg body weight (bw) a day, while those of most sub-Saharan nations in Africa are above 100 ng/kg body weight a day [4], though the estimations are usually generated from few data. "The estimates of dietary exposures to AFM1 have rarely surpassed 1 ng/kg body weight per day in any country, though up to 8.8 and 6.5 ng/kg bw per day for breastfed infants and young children have been reported" [4].

MAJOR TYPES OF AFLATOXINS AND THEIR METABOLITES

Aflatoxin B_1 has been identified as the most toxic of all aflatoxins; it is released by *Aspergillusparasiticus* and *A. flavus*. "Aflatoxin M_1, metabolite of aflatoxin B_1, is present in fermentation broth of *A. parasiticus*, but it and aflatoxin M_2, the metabolite of aflatoxin B_2, are also produced when infected liver metabolizes aflatoxins B_1 and B_2."

a) "Aflatoxin B_1 and B_2 (AFB); which are produced by *A. flavus* and *A. parasiticus"*
b) "Aflatoxin G_1 and G_2 (AFG), which are produced by some Group II *Aspergillus flavus* and *Aspergillus parasiticus"* [15]

c) "Aflatoxin M_1; the metabolite of aflatoxin B_1 in animals and humans (exposure in ng levels might come from the milk of a mother)"
d) "Aflatoxin M_2; the metabolite of aflatoxin B_2 in the milk of cattle fed on/with contaminated foods"
e) "Aflatoxicol (AFL); the metabolite produced by the breakdown of the lactone ring"
f) "Aflatoxin Q_1 (AFQ_1); the major metabolite of aflatoxin B_1 AFB_1 in liver preparations (*in vitro*) of other higher vertebrates"[16].

AFM, AFL, and AFQ have possibility to be epoxide. However, they seem to have lower capability of leading to mutagenesis compared to the unmetabolized aflatoxin [15, 16].

Aflatoxins contamination circumstances

The major molds, e.g. *A. flavus, A. parasiticus, etc.*, that produce aflatoxins are popular kinds of molds known to be widely spread in nature. The molds occurrence does not often show harmful aflatoxin level, but show significant risks. These fungi can infest and infect foods before, during, and after harvest, or in storage, particularly after long-term exposures to environment with high level of humidity, or to severe circumstances including droughts.

"The native habitat of *Aspergillus* include soil, hay, grains undergoing microbiological deterioration,and decaying vegetation, but it invades all kinds of organic substrates every time conditions are favorable and to support its growth" [1, 17]. The favorable factors are high temperature and high moisture of ≥7 percent. Aflatoxins are detected in all common grains, cannabis, and peanut butter [2]. The primary foods often infected with aflatoxin are peanuts, rice, sorghum, millet, cotton seed, tree nuts, sunflower seeds, cassava, chilies, corn, spices, and wheat meant for animal and human intake. Aflatoxins transformed products can be detected in egg, meat, and milk and dairy products if animals consume infected grains [1, 17]. "A study conducted in Mali and Kenya found that the major practices for maize drying and storage were insufficient in minimizing the exposures to aflatoxins; organic produces which are not treated with fungicides can be more susceptible to aflatoxins contamination" [18].

Human exposures to aflatoxins mostly come from grains and nuts

Two fungi are mostly implicated in production of aflatoxins of health concern; *A. parasiticus* and *A. flavus.* In the conditions which are favorable, as normally occur in subtropical as well as tropical parts of the world, e.g. high humidity and temperatures, these fungi, usually seen in decaying and dead vegetations, often occupy crops [4]. "Drought stress, poor storage, and insect damage can contribute to higher occurrence of moulds including in many temperate regions" [4].

About 14 or more types of aflatoxins occur naturally, however four (including AFB1, AFB2, AFG1, and AFG2) are mostly harmful to animals and humans. "In addition, AFM1, a product of aflatoxin B1 metabolism, can be detected in milk in the areas of high aflatoxin exposures" [4]. Afterward humans can get exposure to the aflatoxin via consumption of milk or dairy products, such as breast milk and yoghurt, mostly around the regions that feed poor quality grains to animals, including livestock. Agricultural crops can be infected before, during, or after harvest. The pre-harvest infestation of food crops with aflatoxin is mostly found in tree nuts, acha, cottonseed, wheat, peanuts, maize, etc [4, 19, 20, 21, 22, 23]. Post-harvest contamination is found in many other agricultural products including rice, spices, as well as coffee [2, 23]. Inappropriate storage of food crops in conditions, such as warm and humid storage environments, which favour the growth of mould can typically result in contamination levels much higher than the ones found in the field.

Pathology of aflatoxins

No animal species has been shown to be insusceptible to toxicity of aflatoxin. However, adults are more tolerant to exposures to aflatoxins and often hardly affected by acute aflatoxicosis; children are mostly susceptible, and exposures in them can result in delayed development and stunted growths, and other symptoms [4, 7, 24]. High aflatoxin exposures produce "acute hepaticnecrosis"(acute aflatoxicosis), later leading to liver cancer or liver cirrhosis. Acute liver failure has the symptoms such as digestion alteration, bleeding, edema, mental changes, coma, and variations in nutrientsabsorption and metabolism [4, 7, 24].

Chronic, sub-clinic exposures do not result in symptom too severe like "acute aflatoxicosis." Chronic exposures increase risks of having cancer of the liver and gallbladder, as the metabolic products of aflatoxins may "intercalate into the DNA and alkylate its bases through epoxide moiety" [25]; when the intercalation and alkylation happen, it may result in *p53* gene mutations, a very vital gene in the prevention of "cell cycle" progressions when there are DNA mutations, or programmed cell death, called signaling apoptosis. "The mutation may have effect on many locations of base pair more than others, for instance, the third base of the codon 249 of the p53 gene seems to be more vulnerable to aflatoxin-mediated mutations than adjacent (nearby) bases" [26]. As with other agents of DNA-alkylation, Aflatoxin B_1 (AFB_1) can lead to "immune suppression," and its exposures are connected with an increase in viral loads in individuals who are have HIV [27].

Manifestation of diseases associated with aflatoxin is affected by some factors including nutrition, species, sex, age, and likelihood of simultaneous exposures to other toxic substances. The liver is the most affected organ in mammal; aflatoxicosis is a hepatic disease. Conditions increasing the possibility of aflatoxicosis in humans include environmental factors that support the proliferation of moulds in foods, lack of regulation system for aflatoxins control/monitoring, and limited availability of food [28].

Consistent healthy diets such as apiaceous vegetables, e.g. parsley, carrots, celery, and parsnips, can lessen toxic effect of aflatoxins [29]. "No specific antidote for aflatoxicosis yet; symptomatic and supportive care designed to reduce the liver disease severity may include active vitamin K, B vitamins, intravenous fluids with dextrose, and restricted, but high-quality protein diets with sufficient carbohydrate content" [4, 7, 24, 29].

Aflatoxin in animals

Effects of aflatoxin in chickens are reproductive efficiency, impaired productivity, liver damage, inferior eggshell quality, increased susceptibility to disease, decreased egg production, and inferior carcass quality [4]. Pig can be greatly infected by aflatoxin. Chronic aflatoxin effects in pigs are mostly manifested as damage to the liver (hepatic disease). The main symptom in cattle include decreased gaining of weight, in addition to kidney and liver impairment; secretion of milk can be decreased. "The different forms of enzymes that metabolize aflatoxins (e.g., glutathione S-transferases, cytochrome P450s) are considered responsible for different susceptibilities and vulnerabilities of different animals to the aflatoxins toxic effects" [4]. In dogs, aflatoxins have potential to cause hepatic impairment. Small level of exposures to aflatoxins need consistent intake for many weeks, or sometimes months, for symptoms of liver disease to manifest [4, 30]. Some publications have suggested the toxic levels in dog foods range from 100 to 300 parts per billion and require consistent consumption or exposures for many weeks, or sometimes months, for the development of aflatoxicosis. "Turkeys are very susceptible to the toxic effects of aflatoxicosis; recent studies have indicated that this is because of the efficient cytochrome P450 mediated aflatoxin B_1 metabolism in the turkeys' liver and deficiency in glutathione-S-transferase mediated detoxification" [31]. According to Goldblatt, "Studies on pregnant hamsters indicated a significant relationship between aflatoxin B_1 exposures (4 mg/kg for single dose) and appearance of developmental abnormalities in their offspring" [32]. Aflatoxins have been detected in many pet foods in various countries, including the US and Nigeria [2].

Detection of aflatoxins in human

Two major methods (techniques) are usually used for detecting aflatoxin level in human. The first technique is by determining AFB_1-guanine (aflatoxin B_1-guanine) adduct in the patient urine. Presence of aflatoxin B_1-guanine adduct (the breakdown product) indicates aflatoxin B_1 exposure within last 24 hours. The method measures current exposures alone. "Owing to the half-life of the metabolite, the level of aflatoxin B_1-guanine measured might vary from day to day, depending on diet, it is not efficient for assessing long-term exposures" [4]. The second method (technique) is the measurement of the level of AFB_1-albumin(aflatoxin B_1-albumin) adduct in serum of blood. This method offers additional cohesive measures of exposures to aflatoxins over many weeks, and sometimes months. Identification of aflatoxicosis in both humans and animal is challenging due to variations of the clinical symptoms as well as presence of some conditions including immune system suppression resulting from infectious diseases. "Of these two techniques commonly used to detect aflatoxin levels in humans, one measures a specific breakdown product in the urine (which is only present for a day after exposure, however), while the other measures an AFB_1–albumin level in the blood serum, giving information on exposures over weeks or months [4]. Measurements of these biomarkers are essential for studying the outbreaks in which aflatoxins infection is assumed.

Various methods for detecting the presence of aflatoxin in foods/feeds have been made for various requirements. "Aflatoxins are of major significance and the techniques for their analysis and detection have been researched extensively to develop those that are extremely specific, practical, and useful" [4]. Many methods have been made for various requirements, varying from "methods (and techniques) for regulatory controls in official laboratories (e.g., HPLC-MS) to rapid test kits used in factories and grain silos, e.g. ELISA (enzyme-linked immunosorbent assay)." "Many potential novel systems for aflatoxin detection based on the emerging technologies include hyperspectral imaging, dip-stick kits, molecularly imprinted polymers, electronic noses, and aptamer-based biosensors (they are small organic molecules which can bind specific target molecules)" [4]. Due to ease of manufacturing, stability, as well as ease of usage, latter technologies might be of importance in remote regions.

Instances of aflatoxins outbreaks

Global sources of commonly usedcooking oils (olive oil, sesame oil, peanut oil), peanut butter, as well as cosmetics are known for being contaminated with aflatoxin [33, 34]. "In some studies, liquid chromatography-tandem mass spectrometry (i.e., LC-MS/MS), and a few analytical methods, indicated a

range from 48 to 80 percent of selected product samples as having detectable amounts of aflatoxins; in many of the contaminated food products, the aflatoxin levels exceeded the safe limits set by the FDA, or other regulatory agency" [34]. Instances of aflatoxin outbreaks include;

a) 2003 Kenya: acute poisoning, over 120 confirmed deaths.
b) 2014: Bangladesh and Nepal, neonatal exposures, found in the umbilical cord blood.
c) 2019 Kenya: 5 maize flour brands recalled due to contamination [35].
d) February 2013: Iowa contamination.
e) February to March 2013: "Romania, Croatia, Serbia imported into Western Europe - 2013 aflatoxins contamination."

Aflatoxins control methods in pre- harvest and post-harvest

Aflatoxin control is needed in pre-harvest as well as in postharvest handling, and in storage. According the WHO "the most stable, long-term solution to controlling the pre-harvest aflatoxins contamination is by enhancing the capability of the food crop to resist infection from fungi and/or prevent aflatoxins production by the invading fungi" [4]. It is achieved via genetic engineering or by plant breeding of specific crops; though, the process is time consuming and laborious. Sustainable, efficient, and generally relevant strategies for pre-harvest interventions are required [4]. "One strategy which has received a significant attention for aflatoxins reduction prior to harvest is biological control using non-toxigenic *Aspergillusflavus*isolates" [4]. "These non-toxigenic strains inhabit the same niches as do the naturally occurring toxigenic strains; they are capable of competing with and displacing the toxigenic strains." The strategy is applicable on food crops including figs, pistachios, peanuts, maize, and cotton in the US, peanuts in Australia, China, and Argentina, and maize in Africa. "The strategy is also used for maize in Thailand in measuring the efficacy of this treatment both pre-harvest and postharvest; the obtained results were promising, although inconsistent" [4]. "Post-harvest interventions include the preventive measures to address proper storage conditions (temperature, mechanical or insect damage, aeration, and moisture), which influence the contamination and production of toxins by mould." Other measures, including use of enterosorbents or chemical decontamination, may be applied for removal of the aflatoxin from the foods already infected.

CONCLUSION

Aflatoxins are mycotoxins produced by some fungi found on agricultural crops like peanuts, cottonseed, tree nuts, and maize (corn). Major types of aflatoxins include Aflatoxin B_1 and B_2 (AFB) and Aflatoxin G_1 and G_2 (AFG); their metabolites include Aflatoxin M_1 (AFM_1), Aflatoxin M_2 (AFM_2), Aflatoxicol (AFL), and Aflatoxin Q_1 (AFQ_1). Aflatoxin B_1 is considered the most toxic. Aflatoxin-producing molds may infect agricultural produces in field, during harvests, and also in storage facilities. Aflatoxin-producing molds are commonly detected in inadequately stored agricultural produce including maize, rice, cassava, cottonseed, millet, sorghum, sesame seeds, sweetcorn, chili peppers, tree nuts, sunflower seeds, spices, and wheat. Children are mostly impacted by exposures to aflatoxin; which has been linked to liver cancer, liver impairment, delay in development, and stunted growth. In developed countries, the mean exposures to aflatoxins via diet are usually below 1 ng/kg body weight (bw) per day, while estimates for many sub-Saharan African countries go beyond 100 ng/kg body weight per day. The estimates of dietary exposures to AFM1 hardly exceed 1 ng/kg body weight a day in countries. No animal species has shown immunity to the acute toxicities of aflatoxins. Regular diet such as apiaceous vegetables, like parsnips, carrots, parsley, and celery can decrease carcinogenic effect of aflatoxins. A range of methods for detecting aflatoxins in foods and feeds are available. Aflatoxin control is needed in pre-harvest as well as in postharvest handling. Major lasting, sustainable solution to control pre-harvest infection of aflatoxin is via improving capability of crops to prevent fungal infections or preventing aflatoxins productions by fungal invasion.

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