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Aflatoxin B1: Mechanism, Oxidative Stress and Effects on Animal Health

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Abstract

Aflatoxins (AFs) are secondary fungal metabolites also known as mycotoxins which are produced by fungi of the Aspergillus genus, particularly Aspergillus flavus. the most common type of AF are AFB1, AFB2, AFG1, AFG1, AFM1 and AFM2. AFs are known to contaminate a large portion of the world's food supply.

AFB1 is the most carcinogenic of AF. AFB1 contamination of agricultural commodities poses a considerable risk to human and livestock health and high economic losses occur in the country crops and animals. Human exposure to AF leads to a variety of health-related disorders, including acute and chronic aflatoxicosis, immunosuppression, liver cirrhosis, liver cancer, growth retardation, and others.

One of the causes of AFB1-induced toxicity is oxidative stress, which leads to the improved generation of reactive oxygen species and oxidative DNA damage. These radicals initiate a damaging process in biological systems.

This review relates the metabolic transformation of AFB1, its mechanism of oxidative stress, and its effects on animal health

Keywords: Aflatoxin; Biotransformation; Oxidative Stress; Toxicity; Health

Introduction

Molds, which are common in our daily life and can reproduce in almost all kinds of foodstuffs, have been a research topic that has been emphasized in recent years. Molds that grow in raw and processed materials under suitable conditions cause deterioration of the product by changing its quality and quantity, on the other hand, they create toxic substances harmful to human and animal health [1].

Aflatoxins (AFs) are various toxic carcinogens and mutagens produced by certain molds, especially Aspergillus species. The synthesis of AFs, which are mycotoxins that contaminate feed and food, depends on mechanisms triggered in response to environmental stimuli such as pH, light, food sources, and oxidative stress [2,3].

There are more than 20 derivatives of AF produced by different types of fungi. For example, Aspergillus flavus can synthesize AFB1 and AFB2, while Aspergillus parasiticus can synthesize AFB1, AFB2, AFG1 and AFG2. AFB1=AFM1>AFG1>AFB2=AFM2>AFG2 from strongest to weakest in terms of toxicity (Figure 1). The potency of AFB2 is only 1-10% of AFB1; this is related to the conversion of ingested AFB2 to AFB1 and then to active metabolites in the body. AFB1 is the most common in food and among the most potent genotoxic and carcinogenic AFs. AFM1 is a major metabolite of AFB1 in humans and animals, which may be present in milk from animals fed with AFB1 contaminated feed [1,3,4].

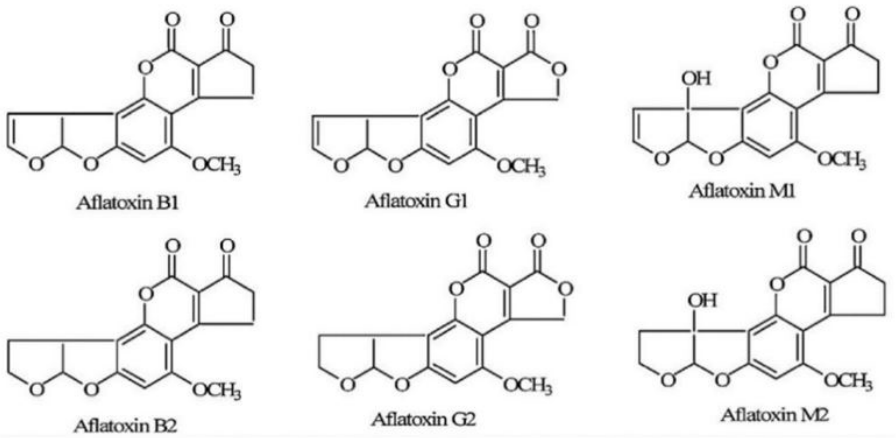

Figure 1: Chemical structure of AF's (3)

AFs often contaminate various staple foods such as straw, wheat, rice, maize, sorghum, millet, peanuts, capsicum, cottonseed, tree nuts, sesame seeds and sunflower seeds during storage and poor processing conditions. Fungal growth can occur on food at any point in the pre or post-harvest stage, making it difficult to control contamination [5]. Humans and animals are exposed to carcinogenic AFs through contaminated food, feed, drinking water, and air. AFs not only contaminate foodstuffs, but are also found in edible tissues, eggs and milk when contaminated feed is consumed by animals. Farmers and other agricultural workers can be exposed to breathing dust generated during the transport and processing of contaminated crops and feeds [6,7].

AFB1 is of great concern worldwide due to its proven carcinogenic properties in humans and its toxic effects due to its frequent occurrence in many foodstuffs. Among all AFs, AFB1 is highly toxic, mutagenic and carcinogenic to many species, including humans, pigs, birds, fish and rodents. AFB1 has been classified as a group I human carcinogen by the International Agency for Research on Cancer (1, 2). Studies have shown that chronic exposure to AFB1 can lead to numerous diseases in humans and animals, including immunosuppression, nutrient malabsorption, infertility, endocrine problems, as well as teratogenic effects associated with congenital malformations and hepatocellular carcinoma. AFB1 causes chromosomal aberrations, micronuclei, sister chromatid exchange, chromosomal strand breaks, and inserts in fish, birds, and mammalian cells [3,8,9].

Biotransformation, Toxicity And Mechanisms of Action Of Aflatoxins

AFs are highly liposoluble compounds. AFs are readily absorbed from the site of exposure usually through the gastrointestinal tract and respiratory tract into the bloodstream. Humans and animals get exposed to AFs by two major routes: 'direct ingestion of AF-contaminated foods or ingestion of AFs carried over from feed into milk and milk products like cheese as well as other animal tissues' 'by inhalation of dust particles of AFs especially AFB1 in contaminated foods in industries and factories. AFs are absorbed through cell membranes after entering the body [3,10].

AFs undergo biotransformation mainly in the liver. Liver biotransformation of AFB1 is related to its toxic and carcinogenic effects. When AFB1 is taken with food and feed, it is rapidly absorbed from the digestive system, binds to serum albumins, passes into the portal circulation and is transported to hepatocytes [11,12]. Orally ingested AFB1 is metabolized in the liver by the action of the cellular cytochrome P450 (CYP450) enzyme system and aryl hydrocarbon hydroxylase enzyme, forming reactive intermediates such as lipid peroxidation (LPO) and AFB1-8,9-epoxide, which cause cellular injury. Epoxidation of AFB1 to exo-8,9-epoxide is a critical step in the genotoxic pathway of the carcinogen [13,14].

There are Two Types of Biotransformation: Phase I and Phase II.

Phase I: Phase I is mostly mediated by CYP450 enzyme systems. AFB1 is oxidized to various products by CYP450 subfamilies and specific isoforms of enzymes. Only one of these, the AFB1-8,9-epoxide, appears to be mutagenic and the others are detoxification products. The putative AFB-8,9- epoxide is generally considered to be the active electrophilic form of AFB1 that can attack nucleophilic nitrogen, oxygen, and sulfur heteroatoms in cellular components. CYP450-mediated oxidation to the highly reactive AFB1-8,9-epoxide is considered the primary bioactivation pathway for AFB1. This conversion of AFB1 to epoxide is the reaction step that enables covalent binding to cellular macromolecules (eg. DNA and/or protein) to occur. This reaction may involve several CYP450 isozymes, including 1A2 and 3A4. The CYP450 3A4 enzyme, which can both activate and detoxify AFB1, is present in the liver and small intestines. CYP450 3A4 and CYP450 1A2 enzymes catalyze the biotransformation of highly reactive AFB1 (in exo-8,9-epoxide) [7,14,15]. AFB1-exo-8,9-epoxide is highly unstable and elicits the biological effects of AF, especially with its covalent binding affinity to cellular macromolecules such as DNA. This highly reactive AFB1-8,9-epoxide substance makes changes in DNA by adding DNA bases, especially to the N7 position of guanine. It is thought that AFB1-N7-guanine, which binds to DNA, has an important role in the carcinogenic and mutagenic effect. Another reason for DNA damage is the formation of reactive oxygen species (ROS), which provide oxidation of DNA bases. These free radicals cause damage to chromosomes. Superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\cdot}OH$) are formed from ROS in cells. LPO and oxidative DNA damage indicate the presence of AFB1-mediated toxicity (Figure 2) [14,16-18].

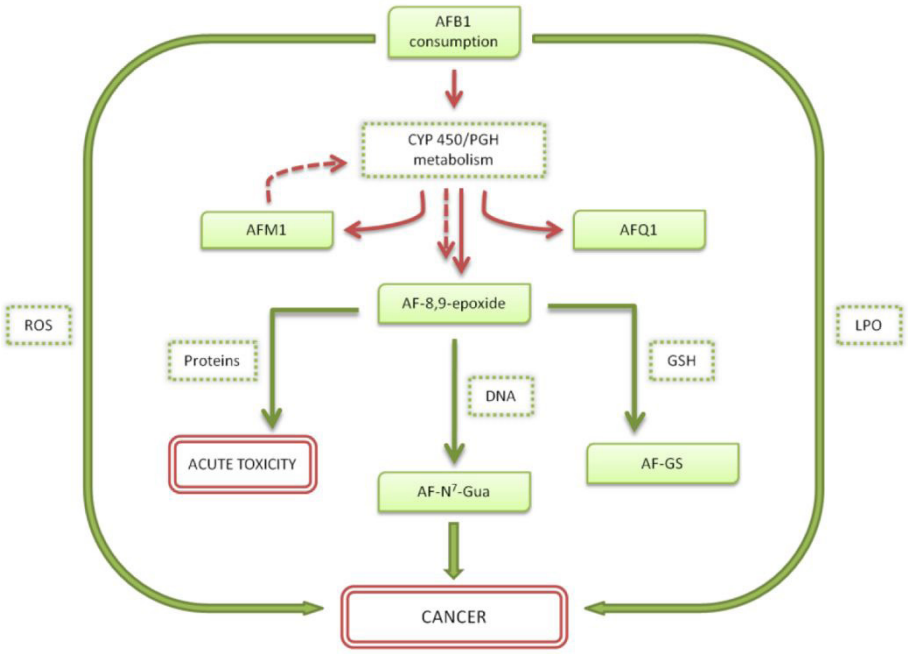

Figure 2: Schematic representation of AFB1's relationship with ROS and the occurrence of cancer [36]

Phase II: Some of the oxidative metabolism products of AFB1 form a substrate for phase II detoxification enzymes. Phase II reactions leading to AF detoxification include conjugation with glucuronic acid, sulfate, and GSH. AFB1 metabolites of phase I metabolism undergo phase II enzymatic metabolite mainly by glutathione-S-transferases (GST), which catalyze conjugation reactions [3]. After a phase I oxidation, AF can be easily conjugated with SH groups (in phase II reactions) allowing further detoxification and elimination of the toxin [15, 19]. AFB1-8,9 exo and endo-epoxides can neutralize the toxic power of AFB1-8,9-epoxide by conjugating AFB1-8,9-epoxide to GSH as a result of the formation of AFB-mercapturic by conjugation with GSH as a result of reactions catalyzed by the GST enzyme in the liver. GSH plays an important role in the detoxification of AFB1. Low GSH levels can increase the toxic effects of AFB1. However, in the case of long-term and excessive intake of AFB1, the detoxification function will be insufficient and serious health problems may be observed (Figure 3). Monkeys are more resistant to AF carcinogenesis as GST activity is 3-5 times higher in monkeys than in rats. In humans with lower GST activity or AFB1-8,9-epoxide conjugation, AF detoxification is less effective than in rats and monkeys [3,7].

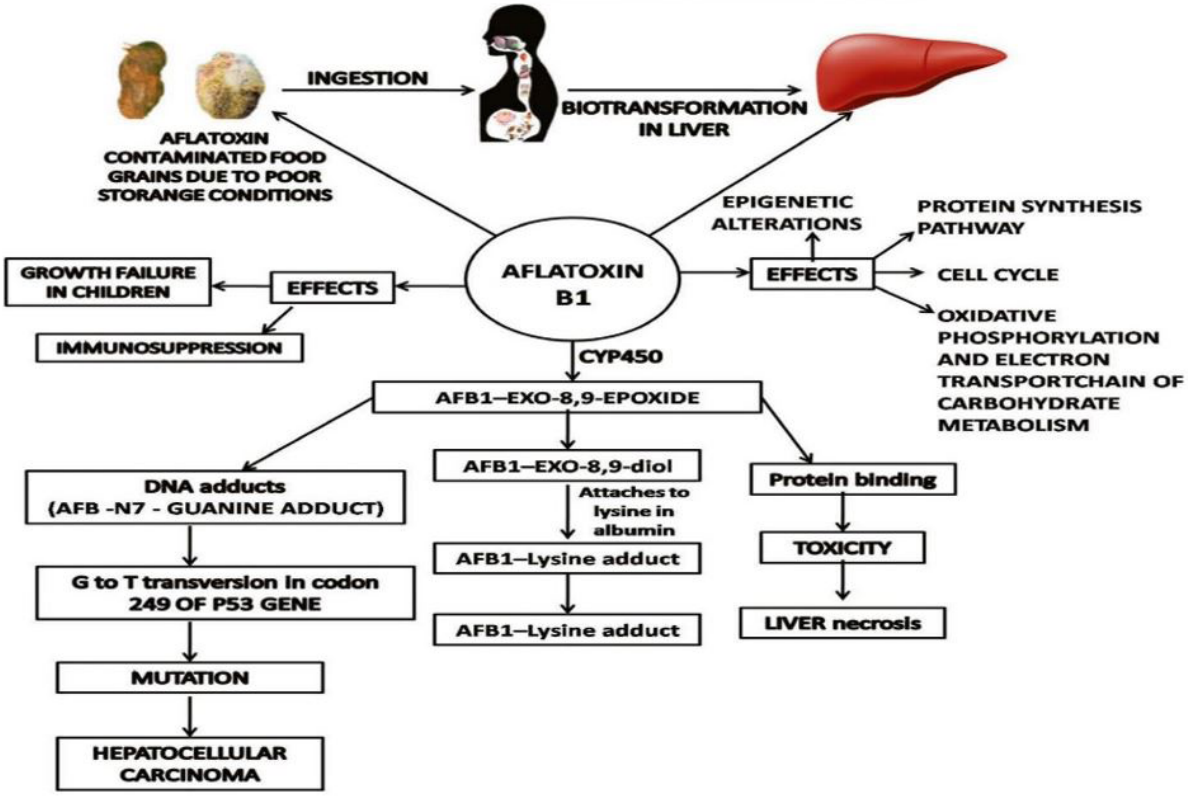

Figure 3: Effects of AFB1. AFB1 reacts with DNA, RNA, proteins and other compounds to form adducts. These AFB1 adducts cause many genetic mutations and epigenetic alterations leading to the deregulation of many cellular metabolic pathways affecting the growth and normal functioning of cells [63]

AFB1 is also metabolized into several hydroxylation products through the CYP450 system. These include AFM1, AFQ1 and AFP1. AFM1 is a major metabolite produced by CYP1A5 and is commonly detected in humans and animals exposed to AFB1. AFM1 is the most carcinogenic of the hydroxylated metabolites which have been shown to induce tumors in rainbow trout and rats (20, 21). This is supported by the DNA binding effect of AFM1 which has been demonstrated in rats, mice, and pig and has even been identified to form N7 guanine adducts similarly to AFB1 [22,23]. AFM1 is commonly found in the milk of dairy cattle and humans, leading to many potential routes of dietary exposure.AFM1 is also excreted in high levels in urine following AFB1 exposure and thus has to become an additional biomarker of AFB1exposure (Figure 4) [24, 25].

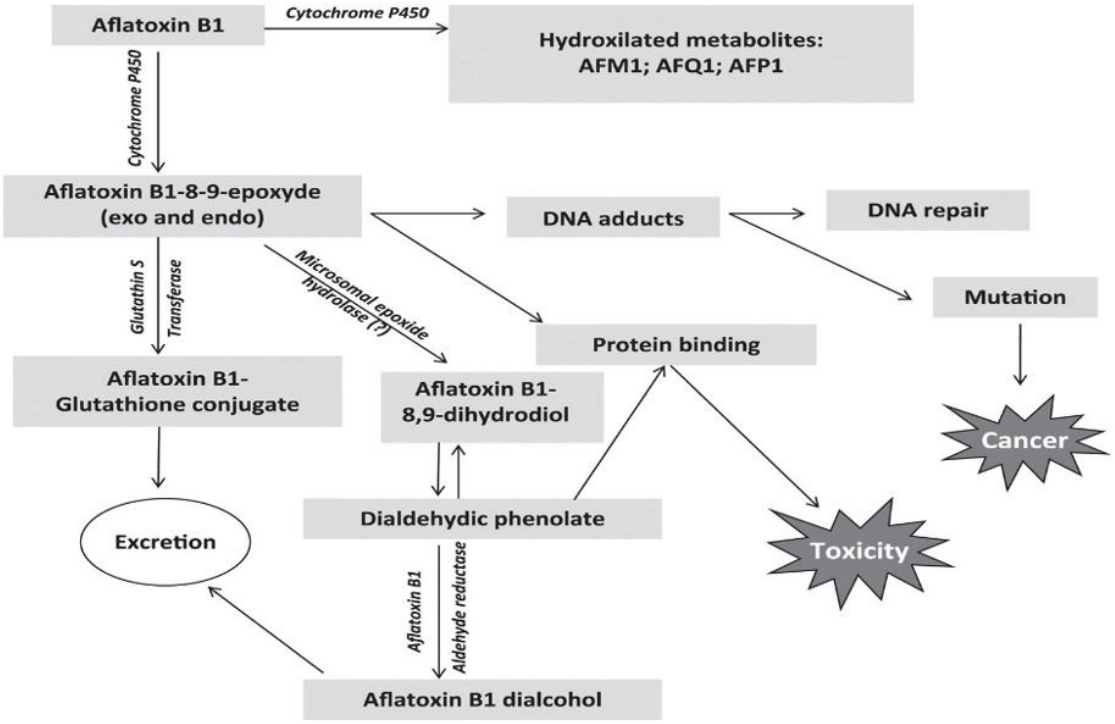

Figure 4: AFB1 biotransformation pathways [64]

Aflatoxin B1 and Oxidative Stress

AFB1 causes an increase in ROS that leaves cells vulnerable to nucleic acids, proteins or lipid oxidation [26-28]. The effects of AFB1 toxicity on the formation of AFB1-8,9-epoxide in the liver and $O_2^{\cdot-}$, ·OH. It has been reported to occur with the formation of intracellular ROS such as AFB1-8,9-epoxide has been shown to increase LPO, followed by loss of membrane stability and blockade of membrane-bound enzyme activity [28-30]. Thanks to its capacity to generate ROS, AFB1 can promote ROS-mediated oxidative damage to proteins. At the same time, AFB1 can inhibit some (serine) proteolytic enzymes responsible for the degradation of damaged proteins and consequently may have relevant implications in hepatocarcinogenesis [7,31,32]. In studies on rats and other animals, AFB1 has been shown to cause changes in oxidative stress markers in biological materials [7,28,33-37]. AFB1 reacts with DNA, proteins and lipids, which cause mutations in the structure of DNA, especially ·OH, which binds to the structure of proteins and triggers structural changes in proteins, thus oxidation of proteins and LPO [28,33,34,35,37]. AFB1 toxicity causes a significant increase in LPO, enzymatic (glucose-6-phosphate dehydrogenase (G6PD), glutathione peroxidase (GSH-Px) and glutathione reductase (GR), catalase (CAT) superoxide dismutase (SOD) and GST) and non-enzymatic antioxidants involved in antioxidant defense (GSH, vitamin C and vitamin E) changes accompanied by a decrease were observed [34,38,39,40].

Role of Aflatoxins in Hepatic Injury and Other Organs

As one of the most potent hepatocarcinogens, AFB1 is a major contributor to the worldwide occurrence of hepatocellular carcinoma (HCC). Because human exposure to AF occurs so frequently, chronic liver damage occurs due to the ingestion of this toxin. AFs have been reported to cause liver cirrhosis as well as liver cancers [5]. In studies where different doses of AFB1 were applied, MDA levels, which are indicators of LPO, increased significantly and decreased antioxidant activities such as GSH, GST, CAT, GSH-Px, SOD and G6PD have been reported in rat liver, kidney and heart tissues. Table 1 shows that lycopene, vitamin E (α tocopherol)and propolis have protective effects against AFB1-induced hepatotoxicity, nephrotoxicity and cardiotoxicity [7,13,17,18].

Table 1: The effects of lycopene, Vitamin E and propolis in experimental studies using AFB1 at different concentrations. Data Sources in the Table [7,13,17,18]

	AFB1 (0.5 mg/kg/ day, orally, 7 days)	**AFB1 (1.5 mg/ kg/ day, orally, 3 days)**	**AFB1 (2.5 mg/ kg, a single, i.p.)**	**AFB1 (1 mg/kg/ day,orally, 7 days)**	**AFB1 (0.5 mg/ kg/day, orally for 7 days)+ Likopen (5 mg/kg/ day, orally, 15 days)**	**AFB1 (1.5 mg/ kg/ day, orally for 3 days)+Likopen (5 mg/ kg) every other day, orally 15 days)**	**AFB1 (2.5 mg/ kg a single i.p)+Vit E (100 mg/ kg/day, orally, 20 days)**	**AFB1 (1mg kg/day, orally, 7days)+ Propolis (100 mg/kg/ day,orally, 10 days)**
	Liver Heart, Kidney	**Heart, Kidney**	**Liver Heart Kidney**	**Erythrocyte, Liver**	**Heart, Kidney**	**Heart, Kidney**	**Liver, Heart Kidney**	**Erythrocyte Liver**
MDA	increase	increase	increase	increase	decrease	decrease	decrease	decrease
GSH	decrease	decrease	decrease	decrease	increase	heart unchanged kidney increase	increase	increase
CAT	decrease	decrease	decrease	decrease	liver increase heart increase kidney unchanged	heart increase kidney unchanged	liver increase heart unchange kidney unchanged	increase
GSH-Px	decrease	decrease	decrease		increase	increase	liver uncahnged heart increase kidney increase	increase

GST	heart unchanged liver decrease kidney decrease	heart decrease kidney unchanged	decrease	liver increase	increase	heart increase kidney unchanged	increase	liver decrease
SOD	decrease	decrease	decrease		liver increase heart, unchange kidney unchange	unchange	liver increase heart unchange kidney increase	increase

One of the most common mutations found in human hepatocytes exposed to AFB1 is a G→T transversion on codon 249 of the p53 gene causing a 249Arginine →249 Serin of the p53 protein [11,41,42]. As a tumor suppressor protein, p53 regulates many cellular functions such as cell cycle progression, DNA repair, apoptosis, and autophagy [43]. Many cancers, including HCC, have mutations of p53, which is thought to alter tumor suppressive functions, allowing the damaged cells to be come cancerous [44].

Health Effects of Aflatoxins on Human and Livestock (Aflatoxicosis)

AFB1 present in livestock feed causes different problems in genital, digestive and respiratory tracts through different mechanisms such as interference in the metabolism of carbohydrates, fats and DNA. Effects of AFB1 on livestock vary with concentration and time duration of contact with feed, the toxin and strain. High concentrations of AF are lethal, medial concentrations lead to chronic poisoning and continuous exposure to a low concentration can result in hepatic cancer. Exposure to AFB1 with food suppresses the immune system in animals, leading to increased susceptibility to infections. In addition, exposure to riboflavin and light increases toxicity in vitamin B12, carotene and protein deficiency [45,46].

Affected Rates of Aflatoxins by Animal Species

Table 2 shows the susceptibility of different animals to AF's. The effects of AF depend on genetic factors (species, breed strain); physiological factors (age, nutrition, exercise); and environmental factors (climatic, husbandry, housing). Developing fetuses are very susceptible to even low levels, and young and fast-growing animals are more affected than adults. Males are more susceptible than females. One measurement of the toxicity of poison is the LD50. This is the amount of toxin that will kill 50 percent of the animals exposed to it. This data provides an approximate yardstick of which animals are most vulnerable to AFB1 [47].

Table 2: AFB1 LD50 values by species

Species	LD50 (mg/kg)
Rabbit	0.3-0.5
Cat	0.55
Dog	1
Sheep	2
Calf	1.5
Guinea pig	1.4-2
Chicken (21days)	18
Turkey (15 days)	3.2
Ducklings (cubs)	0.3-0.6
Rat Newborn Rat Weaned Rat	9 1.5 7.3
Male Monkey Macaque, Female	2.2 8
Pig	0.62

Acceptablelim of AFB1 in foods in tended for human consumption range from approximately 0-40 parts perbillion (ppb) whereas levels in animal feed are allowed to be much higher, reaching upwards of 300 ppb. Generally tolerable feed AF levels are ≤50 ppb in young birds, ≤100 ppb in adult birds, <100 ppb in calves, and <300 ppb in cattle. Even when the level of AFs in feed is as low as 10-20 ppb, their metabolites (AFM1 and AFM2) excreted in milk are measurable; therefore, feed raw materials containing AF should not be fed to dairy cattle. For AFM1, maximum allowable levels range between 0.02 and 5 ppb, with 0.05 ppb the most common. However, AFs in milk are of concern because milk consumption is often higher among infants and children, who are also more vulnerable [48-50].

AF in poultry feed can produce the metabolite aflatoxin in eggs. AFs may be carried over from feed to eggs at ratios of 5.000-125.000:1 diet to egg ratio. AFB1 was detected at levels of 0.05 to 0.16 ppb (mean 10 ppb) in eggs from hens on the 500 ppb diet [51,52].

Aflatoxin Sensitivities of Animals and Aflatoxins in Animal Source Foods

AFs and their metabolites are present in the animal source. Given relatively low quantities of animal source food consumed, meat and eggs are unlikely to present a major contribution to the overall consumption of AF in the diet. AF may also be present in yogurt and other dairy products. Recent studies have shown that other toxic metabolites (aflatoxin) can also be significantly excreted in AFs excreted in milk, eggs, urine, semen, bile and fecal milk [53, 54].

The effects of AFs on farm animals depend on many factors such as animal type, sex, breed, age, type and amount of toxin ingested, exposure time, and stress factors. Animals such as duck, trout, cat, dog and turkey are most susceptible; animals such as horses, cattle, sheep, goats, rats, guinea pigs and quail are moderately susceptible; mice and monkeys are known as the least sensitive animals. AF contaminations lead to a decrease in feed quality and problems such as a decrease in the utilization rate of nutrients or reproductive abnormalities, resulting in low yields in animals. Intoxication of farm animals as a result of natural contamination in feed has great economic importance. Pig, cattle and livestock are domesticated species with significant economic losses in factors such as immunity, decrease in live weight gain, nutritional deficiency and production in general, and it is reported that the poultry sector is more affected by aflatoxicosis. While monkeys and humans are more sensitive to acute poisonings and partially more resistant to carcinogenic effects; In animals such as rats, the situation is reversed. Horses are more susceptible to AF than ruminants [54,55].

Dairy cattle, calves and pregnant animals are highly susceptible to AF, and in the rumen of ruminants AFs are degraded at a low rate and converted to aflatoxicol, which is less toxic than the parent compound. Only 10% of the parent compound is degraded to aflatoxin. AFs generally cause chronic poisoning in cattle. In chronic aflatoxicosis cases, jaundice, loss of appetite, hair growth, decrease in feed consumption and feed efficiency, decrease in milk yield and abortion are seen. The most important symptom of chronic aflatoxicosis cases is a regression in growth. The reason for this is disorders in protein, carbohydrate and fat metabolism. In addition, AFs cause disturbances in rumen functions such as digestion of cellulose, production of volatile fatty acids and decrease in motility in ruminants. AFs suppress the immune system in chronic poisonings and lead to the emergence of many diseases [56]. It has been stated that 100 μg/kg AF given with feed reduces milk yield and 120 μg/kg AF reduces fertility. Most AFs consumed by dairy cows are degraded by the microbial flora in the cow's rumen. AFs are also eliminated through urine and feces. However, a small amount of AFB1 is metabolized to AFM1 in the liver and excreted in the milk of dairy cows. The amount of AFM1 excreted in milk is only around 1-2 percent of the total amount of AFB1 ingested. This metabolite has been estimated to have around 3 percent of the mutagenicity of AFB1, however, it is still toxic, and its potential to inflict chronic disease has not been evaluated. When AFB1 is given by adding 1-3% to cattle feed, approximately 1.7% is extracted as AFM1 with milk. It has been reported that AF was detected in milk within 24 hours after consumption of feed containing AF. It was stated that AF excretion in milk ceased within 2-3 days after feeding was stopped [57]. gave 0.35 mg/kg AFB1 to dairy cattle for 3 days and detected 0.1 μg/kg AFM1 in milk. Holstein cattle 13 mg/bovine AFB1 orally for 7 days and stated that they found AFM1 between 1.05-10.58 ng/l in their milk. The amount of AFM1 allowed in milk in Turkey is 50 ng/l (Figure 5) [20,58].

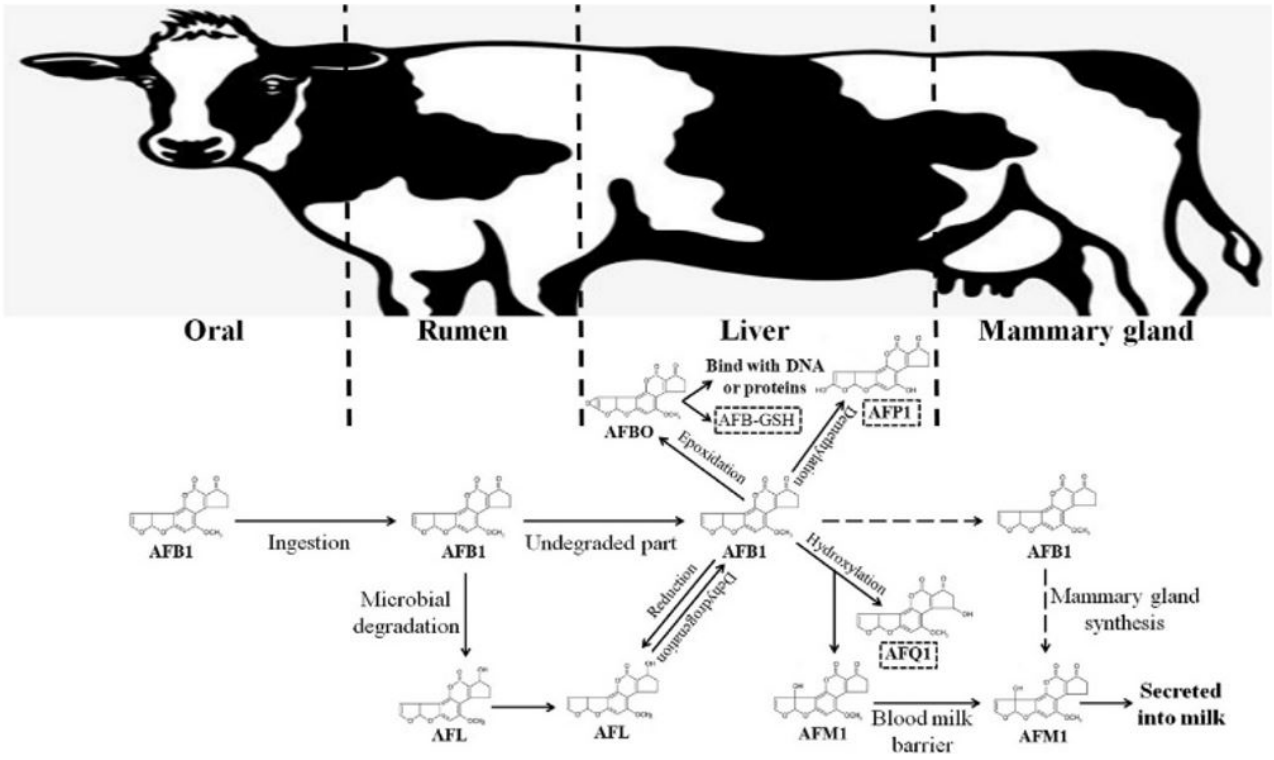

Figure 5: The metabolism and biotransformation pathways of AFB1 in lactating dairy cattle. AFB1 ¼ aflatoxin B1; AFBO ¼ AFB1-8,9-epoxide (highly toxic, mutagenic, and carcinogenic); AFB1-GSH ¼ aflatoxin glutathione adduction; AFL ¼ aflatoxicol; AFM1 ¼ AFM1 (highly toxic and excreted in milk); AFP1 ¼ AFP1; AFQ1 ¼ AFQ1 [65]

AFs have proven negative impacts on animal health. Death from poisoning if large amounts are consumed (aflatoxicosis), decrease in productivity when lower amounts are consumed, cancers in some animals, immunosuppression predisposing to infectious diseases, vaccine failure due to inadequate immune response. The most important economic losses are experienced in the poultry sector, as poultry is highly susceptible to AFs [59]. Comparative toxicological studies in poultry species have shown that ducks and turkeys are the most susceptible to AF, quails are moderately susceptible, and chickens are the most resistant. In chickens, inhibition of DNA biosynthesis is more effective because reactive metabolites and metabolic activation are formed more effectively in the liver [60]. The presence of AFB1 in the poultry diet stimulates the production of CYPP450 isoenzymes, thereby making AFB1 AFBO; It converts AFB1 to the more toxic form AFB1-8.9-epoxide, causing oxidative damage and organ failure, low productivity, reduced reproductive performance, high susceptibility to diseases and accumulation of AFB1 in eggs and meat, which can be harmful to consumers' health. AFB1 affects the accumulation of carotenoids in chicken tissues. In poultry, aflatoxicosis also causes fatigue, loss of appetite, decreased growth, feed efficiency and egg production, and increased mortality. They also cause a decrease in body weight, suppression of the immune system, liver dysfunction and disorders in blood coagulation. AFB1 reduced jejunal mucosa lutein content by 35% and serum lutein content by 70% in young birds; This suggests that AFB1 inhibits the absorption, transport and storage of carotenoids. It has been reported that many deaths in waterfowl are due to acute aflatoxicosis [3,55,61].

In case of AFs, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was the most frequently used test, followed by, the lactate dehydrogenase (LDH) release and the neutral red uptake (NRU), while HepG-2 and Caco-2 were the most frequently used cell lines. Results showed a decrease in cells' viability, as well as increase in the apoptotic cell ratio, increased ROS production and cell cycle arrest. IC50 values for aflatoxins range from nM to μM, depending on experiment conditions, time of incubation and cell line used (full results are presented in Table 3).

The European Union (EU) maximum levels (MLs) for food-crops are listed in Table 4. For the sum of T-2 and HT-2, the EC prescribes indicative levels for cereals and cereal products [62] (Table 4).

Table 3: Cytotoxicity of aflatoxins tested on different cell lines

Type of Test	Cell Line	Results	Reference
CCK-8	BME	Aflatoxins AFB1 and AFM1 exhibited cytotoxic properties in a dose- and time-dependent manner at various concentrations after 24 and 48 h of incubation. They also induced apoptosis and increased the ratio of cells in the G1 and G2 phases.	(66)
MTT, LDH release	Caco-2, Hep-G2, SK-N-SH	Both aflatoxins AFB1 and AFM1 decreased the viability of cells by damaging the cell membrane.	(67)
MTT	Caco-2	Aflatoxin AFM1 inhibited cell viability in a dose- and time-dependent manner after 24, 48 and 72 h of incubation.	(68)
MTT, LDH release	BRL 3A	AFB1 reduced cell viability in a dose- and time-dependent manner. AFB1 also increased LDH activity, apoptotic cell ratio and ROS production.	(69)
MTT, NRU	Caco-2, Raw264.7, MDBK	AFB1 exhibited cytotoxic properties against MDBK, reducing cell viability by 21% after 48 h of incubation with AFB1 at a concentration of 3.8 μg/mL. No significant decrease in cell viability was observed in Raw264.7 and Caco-2 cell lines.	(70)
Cell Proliferation Reagent WST-1	BME-UV1	Aflatoxin B1 is cytotoxic against the BME-UV1 cell line in a doseand time-dependent manner, with LC50 values of 687 and 180 nM after 24 and 48 h, respectively.	(71)
MTT, NRU	BME-UV1	Aflatoxin caused a decrease in cell viability in a dose- and timedependent manner. NRU tests showed that after 72 h of incubation, cell viability was decreased by more than 70% in all concentrations tested. The MTT test also showed a significant decrease in cell viability in all concentrations tested after 24 h of incubation.	(72)
MTT	Caco-2	Aflatoxin B1 and M1 exhibited cytotoxic properties against the Caco-2 cell line. The MTT assay showed a significant dose- and time-dependent decrease in cell viability, both differentiated and undifferentiated cells, when treated with mycotoxins. It was shown that aflatoxin B1 is more cytotoxic than aflatoxin M1.	(73)
MTT, LDH release	PK-15	Aflatoxin B1 exhibited dose- and time-dependent cytotoxic properties. The MTT test showed that after 48 h of incubation, the IC50 for aflatoxin B1 was 38.8 μM. Regarding the LDH release, an AFB1 in concentration of 24.9 μM caused an increase in LDH release by 30% after 24 h of incubation.	(74)

Cell Proliferation ELISA BrdU Kit, Flow cytometry	MAC-T	Incubation with AFB1 significantly decreased cell proliferation in a dose-dependent manner. Since the ratio of cells in sub-G1, S and G2/M phases was elevated, it was assumed that AFB1 inhibited cell proliferation by inhibiting the cell cycle. Flow cytometry also showed that incubation with AFB1 induced apoptosis in MAC-T cells.	(75)
High content screening	BF-2	AFB1 reduced cell viability in a dose-dependent manner, with IC50 estimated at 11.11 μM. Moreover, AFB1 generated strong oxidative stress.	(76)
Cell Proliferation Reagent WST-1	HepG-2, BEAS-2B	AFB1 decreased HepG-2 cell viability, with a IC50 estimated at 1 μM; however, after exposure of BEAS-2B cells to AFB1, cell viability was at 90% compared to the control group in all tested concentrations.	(77)

Table 4: Maximum levels for most often regulated mycotoxins in food crops

Mycotoxin	**Food crop**	**Established levels (mg/kg)**	
Aflatoxins	**Codex Alimentarius standard**	AFB1	Total
	Almonds, Brazil nuts, hazelnuts, peanuts, pistachios for processing	-	15
	Almonds, Brazil nuts, hazelnuts, pistachios for direct human consumption	-	10
Aflatoxins	**European Union maximum and guidance levels**	AFB1	Total
	Groundnuts (peanuts), hazelnut, Brazil nuts, other oilseeds for processing	8	15
	Almonds, pistachios, apricot kernels for processing	12	15
	Tree nuts, other than the tree nuts above for processing	5	10
	Groundnuts (peanuts), other oilseeds, other three nuts below for direct human consumption	2	4
	Almonds, pistachios, apricot kernels for direct human consumption	8	10
	Hazelnuts and Brazil nuts for direct human consumption	5	10
	All cereals except maize and rice	2	4
	Maize and rice for processing	5	10
Aflatoxin B1	**USA: Action and guidance levels** All food crops	20	
Aflatoxins	**Canada: Maximum and guidance levels** Nuts	15	

Aflatoxins	**Australia: Maximum levels** Peanuts, tree nuts	15	
Aflatoxin B1	**Japan: Maximum and provisional maximum levels** All food crops	10	
Aflatoxin B1	**China: Maximum and guidance levels** Maize Rice (brown rice) Wheat, barley, other cereals Peanuts	 10 10 5 20	

Conclusion

AFs are toxic to humans and animals and cause different diseases. AFs, their metabolites, the AFB1-8,9-epoxide and the generated ROS cacauseeleterious effects on the various body organs and body systems including the development of cancers especially the liver cancer mainly due to AFB1 exposure. AFs are also responsible for the suppression of both humoral and cell-mediated immunity and thus making individuals susceptible to infectious diseases. AFs are also responsible for malabsorption of various nutrients and thus lead to impaired immune function, malnutrition and growth retardation.

There are two main ways are usually exposed to AF. The first is when someone takes in a high amount of AFs in a very short time. This can cause liver damage, cancer, mental impairment, abdominal pain and death. The other way suffers AF poisoning is by taking in small amounts of AFs at a time but over a long period. It may cause growth and development impairment, liver cancer, DNA and RNA mutation.

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