Chapter 7

Aflatoxins: Their Toxic Effect on Poultry and Recent Advances in Their Treatment

Yasir Allah Ditta, Saima Mahad and Umar Bacha

Additional information is available at the end of the chapter

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Abstract

About 25% of total agriculture products are contaminated with aflatoxins (AFs) and other mycotoxins in the world especially in Africa, Asia and Latin America, completely losing about 2–3% of food values and thus causing economic losses to farmers. The mycotoxin contaminations of food supply chain impact on human and animal health primarily, whereas production is the second major concern especially in developing countries. Aflatoxins (colorless to pale yellow colored crystals) are the most studied (>5000 research articles) group of mycotoxins. AFs impose major problems regarding health, growth, FCR (feed conversion ratio), etc. in the subtropical zone. In the agricultural commodities, the prevention of fungal contamination during plant growth, harvesting and storage seems to be the most effective and rational precautionary measures to avoid mycotoxins. Activated charcoal; aluminosilicates; polymers, such as polyvinyl pyrrolidones and cholestyramine; and yeast, yeast-based products, and humic acid have been studied extensively with promising but variable results. A live yeast, named Saccharomyces cerevisiae (*S. cerevisiae*), has also been observed to lighten the adverse effects of aflatoxicosis in poultry. These beneficial effects were later attributed to glucomannan, being derived from the cell wall of *S. cerevisiae*.

Keywords: aflatoxins, poultry, toxin binders

1. Background

Mycotoxins are known to affect human and animal health since 1370s BC. Ergotism or St. Anthony's fire is one of the oldest known mycotoxins. The mysterious deaths of archeologists are also considered due to the prevalence of ochratoxin A (OTA) in certain Egyptian tombs [1]. In 1673, the disease was linked to consumption of grains infected with ergot (sclerotia of

Claviceps purpurea) in France. An epidemic resulted in first ergotism control measures in 1770. In 1952, an outbreak of "moldy corn toxicosis" was caused by the consumption of mold contaminated corn-based feed for swine in southern USA [2]. In the early 1960s, over 100,000 turkey poults and 20,000 ducklings, pheasants and partridges poults in England died with clinical signs of liver necrosis and biliary hyperplasia. This incidence brought together world renowned scientists under the umbrella to resolve the puzzle related to turkey "X" disease [3, 4]. Brazilian peanuts used in formulating feeds for these domesticated animals were found to be heavily infected with aflatoxin B_1 (AFB_1) were found to be the main reason for this huge fatality after a series of analyses in England [3] and was named after *Aspergillus flavus* in 1962. A year later (in 1963), its complete structure was characterized by Prof. Buchi's team [1] and subsequently, aflatoxins (AFs) were further categorized as AFB and AFG because of blue and green fluorescence under UV light, respectively [3]. The most extensively publicized case came under the spotlight with an outbreak in humans in western India in October 1974 [5]. Unseasonal rainfall resulted in extensive mold production of extremely high AFs (6.3–15.6 mg/kg) in corn crops [6]. In 2004, several hundreds of Kenyans became severely ill and almost 125 casualties were reported during an acute aflatoxicosis outbreak [7]. Since the identification of Aflatoxins (AFs) in 1965, the momentum of scientific paper publication toward mycotoxin is an increasing trend where 16,821 papers are recorded in Scopus and is an indicative of its importance [8].

2. Mycotoxins

Mycotoxins (MW $\sim$ 700 Da) are secondary metabolites produced by mycelial filamentous structures, specifically called molds [4, 9, 10]. Aspergillus, Penicillium and Fusarium species are responsible for the production of most prevalent mycotoxins, i.e. AFs, ochratoxin, zearalenone, deoxynivalenole, trichothecene-2, etc. [11]. Cereals are more prone to mycotoxins contamination by fungal growth on plants in fields or fungi growing saprophytically during storage. Not all fungal growth results in mycotoxins production (e.g. penicillin, is widely used an antibiotic) or the detection of fungi implies necessarily the presence of mycotoxins [12, 13]. All the secondary metabolites from molds do not impose toxic effects [4].

In response to the environment, five different mechanisms are involved in the production of mycotoxins viz. secondary fungal metabolism, bioconversion of plant compounds (dicoumarol), defense mechanism of plants to fungal aggression and plant-fungus associations [9]. Among the environmental conditions, agronomic practices including harvesting technology as well as the health status of the plant are the most approachable factors for fungal contamination in plants and ultimately mycotoxin production. Humans and animals can be exposed to mycotoxins by various routes like ingestion, aerosol and placental routes [14], which may lead to different fatal consequences as these toxins can be carcinogenic, neurotoxic and immunotoxic, mutagenic, teratogenic, esterogenic and/or hepatotoxic [15]. The severity of health effects posed by mycotoxins depends on species, sex, age, nutritional status, etc. [16]. AFs, OTA and possibly fumonisin B_1 (FB_1) have been classified as being carcinogenic [9]. All

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countries with mycotoxins regulations should have at least regulatory limits for AFB_1 or the sum of AFB_1, AFB_2, AFG_1, and AFG_2 in foods and/or feeds [11]. Mycotoxins exposure includes both pure mycotoxins and also masked mycotoxins which are formed when plants protect themselves by conjugating mycotoxins to biopolymers [8].

3. Factors affecting mycotoxin production

Cereals and their products are susceptible to fungal invasion that may be accompanied by mycotoxin production [17]. Approximately 25–40% of cereals produced worldwide are directly or indirectly contaminated with mycotoxins especially AFs with annual losses of around 1 billion MT of food products [9, 18]. *A. flavus* and *A. parasiticus* are responsible for producing AF during storage particularly in hot and humid countries in the tropics as compared to those in the temperate regions of the world [9, 19].

A. flavus is commonly found in energy rich concentrates (corn, rice etc.) and protein rich concentrates (peanuts, cottonseed etc.) but are not commonly found in tree nuts. *A. parasiticus* occurrence in South East Asia is rare and has the same hosts as those of *A. flavus* [20]. *A. flavus* is generally responsible for AFB_1 and AFB_2 production, whereas *A. parasiticus* produces AFB_1, B_2, G_1 and G_2 [3]. AFB_1 ranges 77% of total AFs as major contaminant in cereals [21]. In the grains, the germ is the main site for *Aspergillus* sp. development which leads to greater potential of AF accumulation [22].

The on-going global warming is going to be an alarming condition for the aflatoxins contamination [8]. Williams et al. [23] observed that improperly dried stored food is commonly invaded by fungus (Aspergillus sp.) in areas within latitude 40°N and 40°S of the equator with temperatures that range between 24 and 35°C and moisture content >7% (10% with ventilation). About 4.5 billion people are chronically exposed to AFs in developing countries. Tropical and sub-tropical regions have favorable environment for AFs production as compared to temperate region [19, 24].

4. Mycotoxin occurrence

Binder et al. [11] found low concentrations of Deoxynivalenol, T-2 toxin and Zearalenone as major contaminants in European (temperate areas) feed samples while AFs, DON, FUM and ZON tended to be dominant in Asia and Pacific (tropical areas) significantly. Elzupir et al. [25] found a total of 64.29% animal feed (130.63 μg/kg) and 87.50% manufactured animal rations (54.41–579.87 μg/kg) followed by 69.32% groundnut samples (4.07–79.85 μg/kg) contaminated with AFs in Khartoum State of Sudan. Summer was found to be the most favorable for AFs growth (78.95% samples) followed by autumn (66.67% samples) and winter season (43.37% samples). AFB_1 was found the most common contaminant followed by AFG_1, AFB_2 and AFG_2.

Shareef [26] found AFs to be most prevalent mycotoxins group (91.1%) with average concentration of 179.1 μg/kg followed by ochratoxins (127 μg/kg) during a two-year survey (2005–2007) on different poultry feed samples in Pakistan. Anjum et al. [27] found AFB_2 (10.80 ± 2.16 to 39.20 ± 3.67 μg/kg) in layer and broiler starter rations from ten different commercial feed mills in Punjab, Pakistan. Among them, 40% of samples were contained AFB_2 at levels above 20 μg/kg (maximum tolerable levels for poultry). Bokhari [29] found 26.1% samples (seeds, oilseeds, spices, milk and milk products) contaminated with AFs principally poultry feed, cereal grains and oil seeds with AFB_1 found as the most frequent contaminant especially in corn grains.

Luttfullah and Hussain [29] found maximum incidence rate of AFs in walnuts with shell (40%), walnuts without shell (70%) and in peanuts with shell (40%) during a survey in Khyber Pakhtun and northern areas of Pakistan. Lutfullah and Arshad [30] found highest AFs incidence rate in corn (40%), sorghum (30%) and rice (25%) from different retail shops and local markets of different location in Pakistan. In Pakistan, *A. flavus* contamination occurs at the highest incidence rate, being responsible for the production of AFB_1 in the corn in Swat valley [31].

Borutova et al. [32] found a positive correlation between AFB_1 and AFB_2 prevalence on different feedstuffs i.e. corn, wheat, soybean meal, corn gluten meal, dried distiller grains, etc. in Asian-Oceania region in 2010 and concluded that the occurrence of single mycotoxins in any of the feedstuffs is rare. Mardani et al. [33] did not find via High Performance Liquid Chromatography (HPLC) any of the AFs at detectable levels in food samples from Kaskinen in Iran except for one sample that contained AFB_1 (0.64 μg/kg). Basaran and Ozcan (2009) concluded AFB_1 to be the most abundant in concentration (0.2–36.81 μg/kg) followed by four samples containing AFG_1 (0.6–20.2 μg/kg) among 217 samples of hazelnuts, pistachio nuts and peanuts in the Turkey. About 87.09% of total samples were very low in AFB_1.

5. Chemical nature and structural illustration

Due to recent advances in technology, modern methods and budding interests, more than 300–500 mycotoxins have been discovered and characterized. Mycotoxins have very special chemical configurations [11, 18, 34]. However, only a relatively small number of toxins are of relevance in feed milling [11]. The AFs are difurocoumaro-lactones (difurocoumarin derivatives) in structure. These chemical structures comprise of a difuran ring with complex coumarin nucleus with a pentenone ring (in AFB and AFM)/a six membered lactone ring (AFG). The four compounds viz. AFB_1, B_2, G_1 and G_2 (**Figure 1**) can be differentiated by fluorescence under ultraviolet illumination (B = blue, G = green) [3]. AFs are indistinctly soluble in H_2O and hydrocarbons, soluble in methanol, acetone and chloroform and insoluble in non-polar solvents. They appear to be unstable in air and light. These toxins are decomposed at their respective melting points which range between 237°C (G) and 299°C (M_1) but not destroyed under normal cooking conditions. Rather these can be completely denatured by autoclaving in the presence of NH_3 or by treatment with bleach [35].

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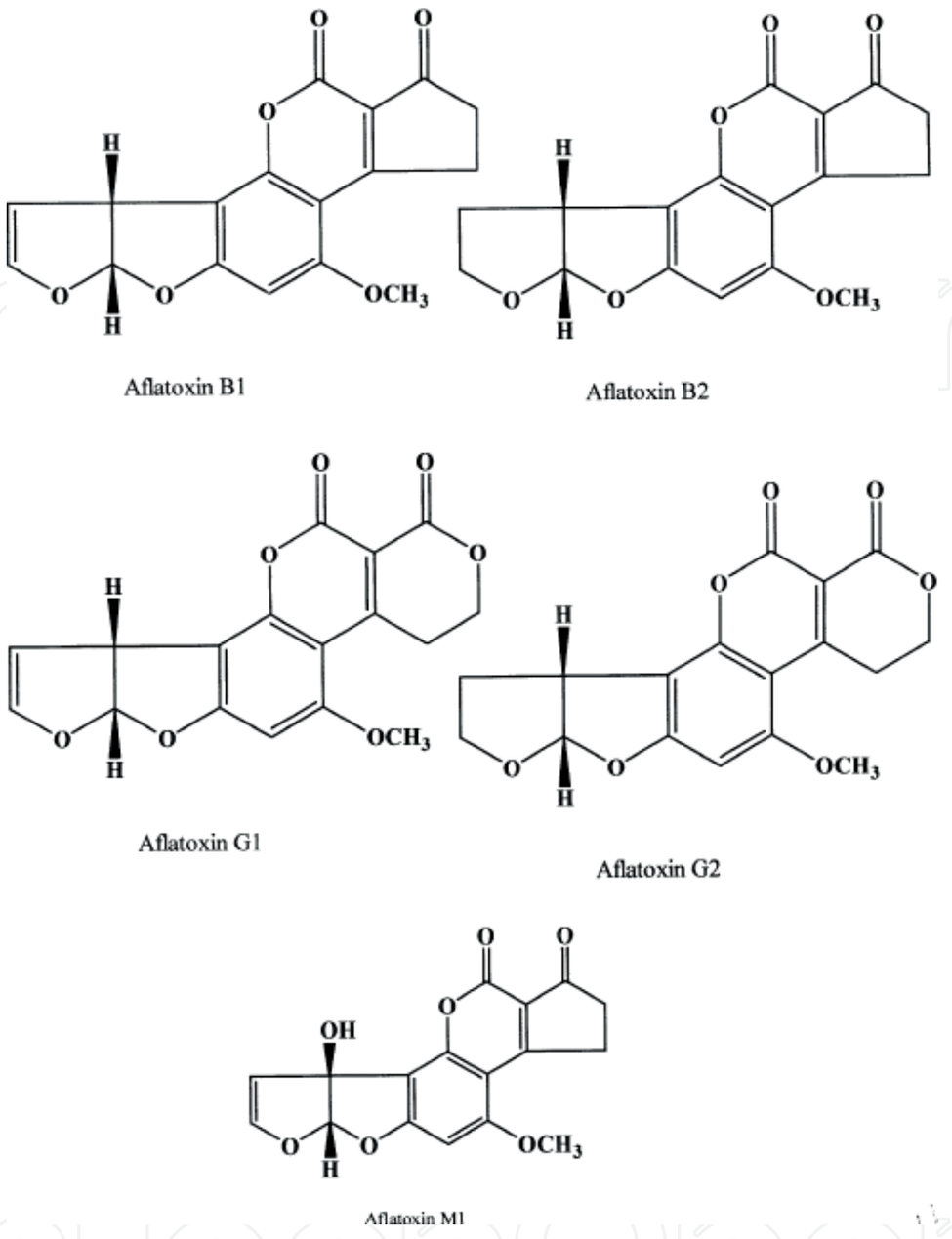

Figure 1. The chemical structure of aflatoxins [19].

6. Levels of toxin production

According to Wayne [43], the amount of toxins produced depends on different factors that can be physical, chemical or biological. Physical factors include moisture, relative humidity, temperature and mechanical damage, while chemical factors include CO_2, O_2, substrate composition, pesticide and fungicide. Plant variety, stress (harsh weather), insects, and spore concentration collectively are biological factors that may affect toxin production.

Temperature, water activity (a_w), oxygen and pH [1, 27, 36–39] play vital role in the production of mycotoxins by fungi. The a_w range should be between 0.61 and 0.91 as most of storage fungi grow at aw <0.75. The ideal temperature for AFs production by *A. flavus* and *A. parasiticus* ranges between 12 and 41°C with optimum production occurring at 25–32°C. But the AF synthesis increases by temperature >27°C, humidity >62% and moisture >14% [3]. Relative to AFG_1, AFB_1 production is stimulated by higher temperature [40]. Optimal production of AFB_1 occurs between 24 and 28°C, whereas 23°C is optimal for AFG_1 production. Low temperature (8–10°C) induces the production of equal amounts of AFB and AFG. However, total AFs production is suppressed with more time is required [3]. At higher a_w, fungi compete with bacteria as food spoilers [17]. Moreover, *Aspergillus* can tolerate lower aw than *Fusarium* [41]. Initially, fungal growth in grains produces adequate metabolic water for further expansion and mycotoxins production [42]. Oxygen is an essential factor for the fungal growth and its growth is restricted at less than 1% oxygen [17].

The broken grains (by insects and birds) are often more susceptible to mycotoxins production. The grains with "musty" odor should be suspected and analyzed for mycotoxins [42]. Aflatoxins contamination is directly influenced by insects' attack to plants and is probably dominated by drought and high temperature [43]. These predisposing conditions allow "hot spots" to occur in stored grains. In severely affected crop of corn, the individual kernel may contain AFs as high as 400,000 μg/kg AFs [42].

The accrual of mycotoxins in the grains before and after harvest largely reflects the prevailing climatic conditions. For example, *Fusarium* toxins are produced in cereals with high moisture content during harvest, whereas pre-harvest AF contamination of crops like peanuts and maize is linked with high temperatures, insect damage and prolonged drought conditions [43].

Fungal geneticists have unraveled the pathways and genes for the synthesis and regulation of mycotoxins production, especially AFs and trichothecenes [37, 44], which assist in the breeding of plants resistant to toxin accumulation [45]. The transgenic Bt corn contains a gene isolated from the soil bacterium *Bacillus thuringiensis*, which encodes for a protein, being toxic to common lepidopteran corn pests. These hybrids offer a new tool for mycotoxins management as insect damage is often a major factor in facilitating toxigenic fungal infection of crops [46].

7. Toxicity of aflatoxins

AF (AFB_1, G_1, B_2 and G_2) concentration, duration of dietary exposure, species, sex, breed, age and health status of animals are different factors that affect toxicity [42, 47]. Young animals are less resistant than older one presumably due to the lack of well-developed hepatic enzymatic systems required to degrade the toxins depending upon the specie [48]. Guinea-pig, duckling and rabbit represent a "fast metabolizing group" actually capable of handling LD50 dose in <12 minutes. Sheep, pig, mouse and chick fall into "intermediate group" metabolizing LD50 dose in few hours [49]. Currently, rat is the only example of a "slow metabolizing group" in which LD50 dose would probably disappear from the liver over a period of days (Hu et al.,

2011). AFB_1 is classified by IARC [35], as a highly toxic compound (LD50, 1–50 mg/kg body weight) among most species, although it is extremely toxic (LD50 < 1 mg/kg) for some species such as cats, ducklings and rainbow trouts [3].

Ducklings followed by turkey poults, broilers and laying hens are the most sensitive species to AFs as these showed 100% mortality at 1 mg/kg AFB_1. Moreover, 0.11–0.2 mg/kg AFB_1 decreased 230 and 163 g/bird feed intake and weight, approximately from 0 to 14 days of age, respectively [50]. Goslings, quails and pheasants are ranked at intermediate position regarding sensitivity while chickens appear to be the highly resistant. Ducklings are 5–15 times more sensitive than laying hens, but among layers, certain strains may be as much as 3 times more sensitive than others [38]. Broilers are more susceptible to AF than layers [36, 51]. Aflatoxin-contaminated feed affect almost all systems in the body are affected, i.e. interference in bone metabolism resulting decreased bone strength, reduction in bone diameter, decrease in dressed weight and breast yield etc. [52].

8. Mode of action of aflatoxins

AFs are toxic to poultry at <1 mg/kg with liver as main target organ as the relative liver weight is altered by low levels of AFs [53, 54]. Respiratory exposure to AFB_1 contaminated dust has been allied with increased incidence levels of tumor along the respiratory tract of animals and humans [3]. The AFs molecules are subjected through complex metabolic processes of different cytochrome P450 dependent pathways (bio-activation or detoxification processes) [55].

The carcinogenic and mutagenic effects of AFB_1 [4], AFG_1 and AFM_1 occur after metabolic activation by microsomal mixed function oxidase system [3, 56]. AFs bind to both RNA and DNA and blocks transcription [17]. In the liver, cytochrome P450 activates AFB_1 (pro-carcinogens) to form AFB_1–8, 9-exo-epoxide (catalyzed by CYP3A4 leading to the formation of AFQ_1) and endo-epoxide (catalyzed by CYP1A2) at 8, 9 position of the terminal furan ring and its subsequent covalent binding to nucleic acid but only exo-epoxide that is highly unstable binds with DNA resulting in the formation of 8,9-dihydro-8-(N7-guanyl)-9-hydro-AFB_1 (AFB_1-N7-Gua) adduct [18, 56, 57]. Toxin interaction with DNA and some enzymes to alter *p53* gene results in GC to TA transversion, which results in mutagenic properties. This transversion is capable of binding to lysine in serum albumin [58] and also inhibits different activities on biological molecules e.g. synthesis of DNA adducts and conjugation with glutathione, and blocks of ribosomal translocase and RNA polymerase (inhibiting protein synthesis) and essential enzymes [59]. The RNA and DNA syntheses were inhibited in rats fed feed contaminated with 5 mg/kg AFs of over six weeks period [4]. AFB1-epoxide can covalently bind to different proteins which in turn, may affect structural and enzymatic protein function [3]. The structure of interaction between base pairs in DNA helix is determined by binding of exo-epoxide with guanine [60, 61]. The metabolites (AFQ_1, AFM_1 and AFP_1) of AFB_1 and other naturally occurring AFs such as AFG_1, B_2 and G_2, are weaker for epoxide formation, thus they have less carcinogenic and toxic properties than AFB_1.

In liver cells, cytoplasmic reductase and microsomal mixed-function oxidase system metabolize AFB_1 to aflatoxicol and aflatoxins M_1, Q_1, P_1 and B_1-epoxide (the most toxic and carcinogenic derivative), which are less toxic than AFB1. These are further conjugate with other molecules and rapidly eliminated from the body [3]. The metabolites (AFQ_1, AFM_1 and AFP_1) being formed from AFB_1 and other naturally occurring AFs e.g. G_1, B_2 and G_2 are weaker for epoxidation, thus possess less carcinogenic and toxic properties than AFB_1. The AFM_1, AFQ_1 and AFP_1 are secreted as metabolites of AFB_1 in the urine and can be used as biomarkers [62].

9. Absorption of aflatoxins in small intestine

Aflatoxins are liposoluble compounds that are readily absorbed at the site of exposure (usually gastrointestinal tract) into the blood stream to liver where they are metabolized in the microsomal system to active or detoxified metabolites [63]. AFB_1 may occur as free or unconjugated forms of primary metabolites. Water soluble conjugate metabolites bound covalently with cellular macromolecules and degradation/metabolic products of AFB_1 adducts. These conjugates of AFB_1 metabolites are excreted in the bile and consequently eliminated through feces. Water soluble conjugates and degradation or metabolic products of AFB_1 macromolecule adducts and unconjugated AFB_1 metabolites are excreted into general circulatory blood system. This results the systemic distribution of AFB_1 to eggs or milk and body tissues [3].

AFs are known to alter the synthesis, absorption, and transport of lipids to extra-hepatic tissues. Liver fatty acid composition is drastically altered among birds with aflatoxicosis [43]. AFB1-8, 9-epoxide (formed by action of cytochrome P450 on AFB_1) may cause significant increase in hepatic lipid peroxide level. Lipid peroxidation initiates to affect membrane integrity negatively; membrane bound enzyme activities which lead to cell lysis. The oxidative damage of cell/tissue occurs when the concentration of reactive oxygen species ($O_2\cdot$, H_2O_2, and $OH\cdot$) predominates the antioxidant capability of cells. This may be the consequence of significant decrease in non-enzymatic antioxidants (e.g. glutathione, vitamin E, and vitamin C) and enzymatic antioxidants (e.g. catalase, glutathione peroxidase, superoxide dismutase). Superoxide dismutase shields cells from oxidative damage by metabolizing free radical superoxide ($O_2\cdot$) to H_2O_2 and $O_2\cdot$. The metabolically produced H_2O_2 can then be decomposed enzymatically with glutathione peroxidase (GSH-Px) and catalase. Glutathione peroxidase not only decomposes H_2O_2 but also can interact with lipid peroxidation. Reduced protein biosynthesis may be responsible for the decline in enzyme activities. Significantly lower glutathione peroxidase levels further intensify the toxic effects of AFs [24]. AFs promote free radical formation thus causing liver peroxidation which in turn results in antioxidant depletion, oxidative stress and apoptosis. All of these contribute to the development of malabsorption [64].

The metabolites such as AFB_1-N_7-Gua, AFM_1, AFB_1-mercapturic acid and serum AFs-albumin are also considered as AF biomarkers [65]. AFs show specific selection for guanine bases with a guanine or cytosine at the 5′ base causing G → T transversion [66]. Puisieux et al. [67] showed that the guanine at the third position of codon 249 of the *p53* gene (a known mutational hotspot in HCC (hepatocellular carcinoma) was the site of modification by AFB_1 (in human

hepatocytes, about three folds mutations at the third base of codon 249) but neighboring guanines (247, 248 and 250) were also modified. About 20% of total AFB_1 ingested remain in the body after a period of one week with a half-life in the plasma of 36.5 minutes, whereas M_1 is almost excreted via urine within 48 hours [68]. Because there is a half-life of 20 days in serum albumin, the AFB_1-albumin adduct can be used as an AF biomarker to check the chronic exposure within 1–2 months and is considered as an independent factor for advanced liver diseases in HCV-infected patients. The adduction levels of AFs with albumin by covalent bonding in the peripheral blood reflect AF exposure 2–3 months earlier depending on albumin half-life [66].

10. Effect of aflatoxins on enzymes

A marked decrease in digestive enzymes (pancreatic ribonuclease, amylase, trypsin and lipase), hypocarotenoidaemia, steatorrhea and bile salts can be observed during aflatoxicosis in poultry. Protein requirements for growth were increased during aflatoxicosis which can be alleviated by dietary methionine fortification [43]. Fernandez et al. [69] conducted trials to investigate the hematological and serological changes on broilers from 21 to 42 days of age with oral administration of 2500 μg/kg AFB_1. It was found that hematological (red blood cell, hemoglobin, leucocytes, eosinophils and basophils) and serological (serum protein, aspartate aminotransferase, alanine aminotransferase, urea, creatinine) parameters remained unchanged but caused hepatic and renal lesions which matches the findings of Bianchi et al. [39]. AFs are known to reduce protein synthesis that may lead to decreased blood protein levels. The AFs intoxications have been reported to decrease total protein, cholesterol, triglyceride and glucose levels significantly [70].

11. "Carry-over" of aflatoxins

Mycotoxins including Aflatoxins are metabolized in the gastrointestinal tract, liver or kidneys according to their chemical structure. Their transfer to poultry meat and eggs leads to undesirable effects on human health [18]. Agag [3] examined the *"carry-over"* of AFB_1 from layer feed to eggs was examined in laying hens at dietary levels of 100–400 μg/kg AFB1. This resulted in 0.2 to 3.3 μg/kg in eggs, and AFs ratios in feeds and tissues found to be are very low ranging from 500:1 to 14,000:1 excluding the liver, particularly when compared with milk (70:1). On the other hand, Zaghini et al. [55] showed no measurable residual AFB_1 or its metabolites in eggs. These contrasting findings may be ascribed to mannan oligosaccharides in naturally AFs contaminated feeds at different levels of toxicity [55].

In broilers and layer birds, the AFB_1 residues have been reported to vary from no detection to 3.0 μg/kg in liver in birds fed 250–3310 μg/kg AFB_1 over certain periods [71]. Fowler et al. [72] found no significant increase in AFs residues in liver until the 1800 μg/kg AF contaminated feed was fortified with AF at a concentration of 1200 μg/kg with no clay used as a binding agent. Younger birds were found to have significant increase in liver residues than those in

non-exposed birds. Moreover, birds 3rd weeks of age that received 1800 μg/kg AFs were found to have detectable levels of AFB_1 in the liver.

12. Immunosuppression

Aflatoxins intoxications suppress immunoglobulins (IgM, IgG and IgA) and enhance susceptibility of birds to parasitic, viral and bacterial infections. At 0.5 to 1 mg/kg Aflatoxins, these interfere with B and T-lymphocytes functioning [73], apparent alteration of splenic functioning, atrophy of bursa of Fabricius [74], suppresses cell mediated immune response, phagocytosis, and complement system as well as interferon production. Moreover, hematopoietic suppression and anemia have been observed by decrease in RBCs, packed cell volume and hemoglobin [75–78].

AFs decrease total serum proteins due to a reduction in α, β and γ globulins, with IgG being more sensitive than IgM [79] which may cause substantial suppression of acquired immunity from vaccination programs in some disease models. The Low levels of AFB_1 appears to affect the vaccinal immunity negatively and may enhance the occurrence of diseases such as Marek's disease, IBD virus, congenitally acquired salmonellosis and duodenal and cecal coccidiosis, etc. even in properly vaccinated flocks [80]. The failure of vaccines is correlated to the immunotoxic effect of toxins which compromise for immune function of birds by decreasing cell-mediated immunity and inducing an inflammatory response [81]. Decrease chemotactic ability of leucocytes, impaired heterophils phagocytosis [3] and cellular and serum factors required for optimal phagocytosis can be observed in aflatoxicated chickens. Although dietary AFs depress thrombocyte counts, no effect on their phagocytic activity has been observed [82].

13. Safe level of aflatoxins and detoxification

Due to synergistic effect of Aflatoxin B_1 and hepatitis B exposure, there are no specific safe levels for aflatoxin regarding resistance/tolerance to AFs. Ideally, there should be zero level for AFs in feed [83]. The Food and Drug Administration and European Union have established 20 μg/kg and 10 μg/kg AFs as maximum level for poultry, respectively. Based on feeds available, AF contaminated feeds should be fed at lowest possible level and for the shortest period of time [84]. The production of AFs can be controlled by maintaining physical integrity of cereal grains, drying and use of anti-fungal especially propionic acid to inhibits molds growth by decreasing pH and ATP formation through electron transport pathway. UV, X-rays or microwave irradiation and dilution of contaminated feed with AF free feed is also one of the methods to dilute the concentration of AFs [9]. However, AFB_1 contamination of feed is practically unavoidable universally [85]. Mycotoxins decontamination refers to methods by which these metabolites are removed or neutralized in contaminated feed, while mycotoxins detoxification refers to methods by which the toxic properties of the mycotoxins are eliminated [86]. Since early 1990s, studies on mycotoxin adsorbents have yielded success but high

inclusion rates and potential interactions with dietary nutrients are causes for concern [87]. Numerous strategies for the detoxification and inactivation of mycotoxins in feed have been tested but most of these are ineffective or impractical [22]. Dietary fortification with methionine, selenium, vitamins, plant and herbal formulations, etc. may detoxify the adverse effects of AFs by glutathione systems which contain cysteine (derivatives of methionine) in broilers [43, 86]. Approaches to detoxify contaminated grain and finished feed can be physical, chemical and biological treatments [88].

14. Physical and chemical methods

Thermal inactivation, cleaning of the kernel surface, and hence the removal of highly contaminated particulate matter, have proven effective in reducing moderate mycotoxins contamination of feed [43, 89]. However, it seems quite laborious to remove highly contaminated feedstuffs. On the other hand, a lot of chemicals e.g. acids (sulfuric acid, hydrochloric acid, phosphoric acid, benzoic acid, citric acid, acetic acid), alkaline compounds (ammonia, sodium bicarbonate, sodium hydroxide, potassium hydroxide, calcium hydroxide, caustic soda), salts (acetate ammonium, sodium bisulfite, sodium hydrosulfite, sodium chloride, sodium sulfate), oxidants (H_2O_2, sodium hypochlorite, ozone), reducing agents (bisulfites), chlorinated agents and formaldehyde, etc., are being used for the degradation of mycotoxins in feed [90]. These methods are inefficient but comparatively expensive. Ammoniation has been demonstrated to reduce AFs levels but not accepted in the United States [91].

High level dosages of methyl bromide, ethylene dibromide, propane/propene ethylene oxide, sulfur dioxide, phosphine propionic, acetic and isobutyric acids show fungicidal activity. However, these chemicals lower nutritional quality and are corrosive on human and animal tissues [92]. Therefore, the use of these chemicals is discouraged. Several related patents involving the use of ozone in agricultural products decontamination are found. This decontamination method involves placing the agricultural products in a treatment chamber, generating ozone in the vicinity of chamber, supplying ozone to the product through continuous flow and exposing the agricultural product to ozone, which then reacts with the toxins and/or microorganisms.

There are different types of adsorbents, which can be used for the detoxification of AFs in the feed. The use of activated carbon for the detoxification of mycotoxins can also be another option but different activated charcoals have less/no effect against mycotoxins, which show their unspecified adsorbent nature. Moreover, certain essential nutrients are also adsorbed when at higher concentration in as compared to mycotoxins [93].

The most applied method for protecting animals against mycotoxicoses is the utilization of adsorbents in the feed, aimed at binding mycotoxins efficiently in the gastrointestinal tract, thus limiting or at best preventing the toxins from being absorbed by the body thereby, preventing their toxic effects and "carry over" of the toxins to animal products [89]. Selected adsorbents added to AFs-contaminated feeds as feed additives can sequester AFs during the digestive process, allowing the mycotoxins to pass harmlessly through the gastrointestinal tract of animal [94]. This is one of the more effective and practical approaches to address the problem of AFs.

The degree of adsorption capacities may vary (0–87%) among various mineral clay materials [95], and very few are actually used commercially. These considered as good absorbents include bentonites, zeolites and aluminosilicates. Studies have shown that sodium aluminosilicates, HSCAS (hydrated sodium calcium aluminosilicates) and sodium bentonites adsorb AFs [96] with adsorption potential of bentonites varying from 17 to 36%. A major advantage of these adsorbents is that they are relatively inexpensive and safe and can be easily incorporated in animal feeds [97].

Mineral adsorbents based on zeolites, silicates and phyllosilicates show different abilities to bind AFs. These possess active sites within interlayer channels at the basal planes on the surfaces or within pores, and at the edges of particles [98]. Bentonites are white, light weight and originate from volcanic ash comprising mainly of montmorillonite, the main constituent of bentonites. These are composed mostly of salts of Na, K, Ca of hydrated aluminosilicates and occasionally Fe, Mg, Zn, Ni, etc. but the composition varies from one deposit to another because of interchangeable mono and divalent ions e.g. Na^{+}, K^{+}, Ca^{+2}, and Mg^{+2}. So they can be classified as Ca, Mg, K or Na bentonites [86]. They have a layered microstructure, which allows AFs to bind at multiple sites including edges and basal surfaces especially at the interlayer region for adsorption [99, 100].

Zeolites possess strong colloidal properties to absorb water rapidly resulting in swelling and manifold increase in volume, giving rise to a thixotropic gelatinous substance [101, 102]. Hydration of the exchangeable cations creates a hydrophilic environment in the interlayer of montmorillonite, which influence the adsorption of different organic molecules, including mycotoxins on zeolite and montmorillonite particles [103]. The surfaces of zeolites derived HSCAS, attract polar functional groups of AFs, thus inhibit their absorption [93, 104] but is less effective against other mycotoxins. Zeolites selectively retain or release calcium during its passage through digestive system. Zeolites can absorb nitrogen of some amino acids and reduce the energy required for meat production. Zeolites suppress phosphorus utilization by forming indigestible compound with phosphorus through its aluminosilicate component [105]. Supplementation of HSCAS at the rate of 1.0% seems to diminish significantly, the adverse effects of AFs in young animals [93] as these have a high negative charge and are balanced by cations of such metals as magnesium, potassium and sodium located in the cavities, and therefore do not react with food/feed ingredients and act as inert material due to their neutral pH or slightly alkaline nature [106].

Aluminosilicates are also used at a level up to 2% as "anti-caking" agents but a several disadvantages have been observed including the impairment of minerals utilization and having a narrow range of binding efficacy [93]. Bentonites minerals can influence Ca-metabolism and bind nitrogenous cations such as NH_4^{+}. These are found to be effective for the adsorption of AFB_1 and T-2 toxin but not for zearalenone. Kececi et al. [107] determined decrease in calcium and phosphorus levels by AFs (2.5 mg/kg) for 21 days. Southern et al. [108] did not find any adverse effect on the growth and tibial mineral concentrations in chicks fed nutrient-deficient diets. Mineral clays reduce utilization of minerals including manganese, zinc, magnesium [109], chloride [95], copper and sodium [110]. Solís-Cruz et al. [111] conducted an *in vitro* study to evaluate the adsorption capacity of Chitosan (CHI), and three cellulosic polymers (Hydroxy propyl methyl cellulose, Sodium Carboxy methyl cellulose, and Microcrystalline Cellulose), on six mycotoxins (AFB_1; FUB_1; OTA; T-2; DON; and, ZEA) for poultry. All four cellulosic polymers

showed significant ($p < 0.05$) binding activity against mycotoxins as compared to control with non-treated group. However Hydroxy propyl methyl cellulose, Sodium Carboxy methyl cellulose, and Microcrystalline Cellulose showed better adsorbent capacity for all mycotoxins when compared with Cholistan.

15. Biological methods

Various bacterial, yeast and fungal species are able to degrade/remove mycotoxins and also can restrict fungal growth. This includes the use of *Bacillus subtilis*, NK-330 and NK-C-3 that effectively inhibit the fungus growth and AFs production [92]. The application of micro-organisms e.g. *Corynebacterium rubrum* for bio-transformation of mycotoxins into less toxic metabolites is another option [9]. These micro-organisms act in intestinal tract of animals prior to absorption of mycotoxins but the concerned toxicity of products by enzymatic degradation and undesired effects of fermentation with non-native micro-organisms on food quality is yet to be investigated completely.

Saccharomyces cerevisiae and lactic acid bacteria (LAB) i.e. propionibacteria, bifidobacteria and *lactobacillus rhamnosus* strongly bind to their cell wall constituents mycotoxins without deleterious effects on animal health [9, 85, 93, 112]. Most yeast strains bind more than 15% (w/w) AFB_1, which is highly strain specific by *S. cerevisiae* [112] and LAB for mycotoxins detoxification [113]. Generally, *S. cerevisiae* shows very low adhesion to the intestines [114], as opposed to LAB that show considerable adhesion to intestinal cells [115]. Coallier-Ascah and Idziak [116] and Thyagaraja and Hosono [117] found LAB to be inefficient binders of AFB_1 due to the strains used, which may also depend on initial concentration of AFs [118]. Haskard et al. [119] showed that cell wall of *L. rhamnosus* has the ability to bind AFs predominantly to carbohydrates and to some extent, protein components that which is unaffected by pH of GI tract. The outer part of cell wall (26–32%) of *S. cerevisiae* contains a structure called glucomannan, which binds against mycotoxins [9]. The yeast cell wall comprises of 30–60% polysaccharides (β-glucan and mannan sugar polymers), 15–30% protein, 5–20% lipids and a small amount of chitin. Mainly, it contains 15–30% β-glucan and 15–30% MOS. Lahtinen et al. [120] found that peptidoglycans might be the most likely carbohydrate involved in the AFB_1 binding process [121]. Kusumaningtyas et al. [122] used *S. cerevisiae*, *Rhizopus oligosporus* and their combination for detoxifying AFB_1 in the chicken feed.

The supplementation of whole yeast and only yeast cell wall rather [53, 112, 123] have shown a reduction in mycotoxins toxicities, indicating possible stability of the yeast-mycotoxins complex along the gastrointestinal tract. The cell wall represents about 30% of total weight of yeast cell [112]. Glucomannan is a bi-layered structure that consists of a network of β-1,3 glucan with β-1,6 glucan side chains. This network is in turn attached to highly glycosylated mannoproteins. The proteins and glucans provide numerous easily accessible binding sites with different binding mechanisms such as Van Der Waals bonds, hydrogen bonding, ionic or hydrophobic interactions [93, 112, 124, 125]. Yeast glucomannan showed markedly high binding ability with AFs *in vitro* (75–90%) and *in vivo* [126, 127]. The carbohydrate fractions of cell wall may represent 90% of mannoproteins. MOS constitute approximately 50% of total carbohydrates [112]. The effect of 500 g of glucomannan is comparable with that of 8 Kg of clay for mycotoxins bindings [9].

16. Conclusion

Feed contamination by fungi can be a predicament for feed security. Under the current condition of temperature, humidity and global warming, the occurrence of mycotoxins including aflatoxins has become overbearing. There is a need for more research on multiple effects of mycotoxins, their trans-conversions and masked mycotoxins. New insights on the development of mycotoxins resistant seed varieties are need which could decrease the damage to grains in fields and during storage and thus could decrease the health risks and financial losses. The advances in Activated charcoal, aluminosilicates; polymers, such as polyvinyl pyrrolidones and cholestyramine, yeast, yeast based products and enzymatic deactivation have been quite successful to decrease the harmful effects of mycotoxins.

Author details

Yasir Allah Ditta[1]*, Saima Mahad[1] and Umar Bacha[2]

*Address all correspondence to: yasirad@uvas.edu.pk

1 Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore, Pakistan

2 University of Management and Technology, Lahore, Pakistan

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