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Mycotoxins in Poultry

Ayhan Filazi, Begum Yurdakok-Dikmen,
Ozgur Kuzukiran and Ufuk Tansel Sireli

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Abstract

Mycotoxins, the toxic secondary metabolites of fungi, particularly produced by many species of *Aspergillus*, *Fusarium* and *Penicillium*, have affected animal and human health for over thousand years, whereas little has been discovered so far about these complex substances in poultry, which are generally very sensitive. Even though it varies by species and sex, some common effects are reduced feed intake, weight gain, feed efficiency, growth performance, immunity and hatchability along with increased mortality, organ damages (mainly kidney and liver), carcinogenicity, teratogenicity and decreased egg production. Besides their adverse health effects and the decrease in production rate, concerns over their importance in public health is still under debate. Decontamination approaches to reduce mycotoxins in feed are technologically diverse and based on chemical, biological and physical strategies. Chemical remediation strategies involve the conversion of mycotoxins via chemical reactions. Biological strategies involve various substances such as plant ingredients, enzymes and microorganisms. Physical processes include sorting, milling, dehulling, cleaning, heating, irradiation or combinational approaches. New strategies for the prevention and treatment of mycotoxicosis, including beneficial microorganisms/products, along with alternative treatments, including plant extracts/essential oils, are current hot topics in the poultry industry.

Keywords: Control, mycotoxins, poultry, prevention

1. Introduction

Mycotoxins, the secondary metabolites of fungi, are a global concern. At aerobic conditions, fungal growth in various feed raw materials is inevitable. There are about 200 species of fungi that produce mycotoxins. Majority of the fungi that form mycotoxin belong to three genera: *Aspergillus*, *Penicillium* and *Fusarium*. Although more than 500 mycotoxins produced by these fungi are known, only some of these mycotoxins exert pathogenic characteristics.

The poisoning in humans and animals caused by feeds and foods contaminated with mycotoxins may range from a slight reaction to death [1–6].

Fungal growth and mycotoxin production initiate in the cropland, during transportation or storage, and are affected by the environmental conditions including seasons, location of grain cultivation, drought and time of harvest. Long-term analyses show that feed and feed-stuffs may be contaminated with mycotoxins, where these contaminated feed materials often include more than one mycotoxin [7]. Also, each of the cereals and oil seeds, available at the poultry feeds are vegetable substances obtained in different climatic conditions during vegetation in the cropland, transport and storage. For this reason, although generally only one mycotoxin is produced in raw feed materials, multiple types of mycotoxins might be found in mixed feeds. Such co-contamination examples in poultry feed are as follows: aflatoxin presence with ochratoxins, T-2 toxin or diacetoxyscirpenol; ochratoxins with T-2 toxin or citrinin and vomitoxin with fumaric acid in the poultry feeds [8].

According to the Food and Agriculture Organization Report, 25% of the world's growing crops are affected by mycotoxins each year, with annual losses of around 1 billion metric tons of food and food products [9]. Generally, there is yield loss or reduced crop value due to diseases induced by toxigenic fungi, and losses in animal productivity and animal or human health costs are due to mycotoxin contamination. Apart from these, the extra costs include the management of mycotoxin, such as prevention, control, sampling, mitigation, labor loss and research costs. Thus, the economic problems related to mycotoxins concern all sections of society [10].

The reasons for mycotoxins to receive this particular attention are their undesirable health effects, decrease in the production rate due to the spoilage of feed, and overall economic effects which are reflected in international trade of food and food products. Therefore, control of the fungal development and mycotoxin production are crucial for feed and animal producers [6].

Mycotoxins are metabolized in the alimentary canal, liver or kidneys of the poultry in accordance with their chemical properties. Their transfer to poultry meat and eggs leads to undesirable health effects in humans, leading to major concerns in public health. Contamination of the feeds with fungi both damages their organoleptic properties and increases poisoning risk by decreasing their nutritional value. Toxicity of the mycotoxins depends on the amount of absorption, number of the metabolites that are formed, exposure period and sensitivity of the animal [1].

Some mycotoxins like aflatoxins (AF), ochratoxin A (OTA), fumonisins (FUM), deoxynivalenol (DON) and T-2 toxin significantly affect the health and productivity of poultry species [11]. The aim of this review is to discuss in detail the important mycotoxins for poultry and their effects, along with the recent developments in prevention strategies.

2. Selected mycotoxins in poultry production

2.1. Aflatoxins

Aflatoxins, a group of harmful secondary metabolites characterized by polyketide-derived furanocoumarins, are produced mainly by *Aspergillus* fungi, such as *A. flavus* and

A. parasiticus. Aflatoxins B1 (AFB1), G1 (AFG1) and their dihydroxy derivatives B2 (AFB2) and G2 (AFG2) naturally contaminate feeds. The presence of Aflatoxin M1 and M2 (AFM1 and AFM2), the 4-hydroxy metabolites of AFB1 and B2 in biological fluids including milk and tissues, is related to the exposure of the contaminated feed. International Agency for Research in Cancer (IARC) classified these highly toxic compounds as highly carcinogenic to humans (Group 1) [12].

AFB1 was explored in the early 1960s as the main etiological agent of “Turkey X Disease” responsible for the death of young turkeys in England as a result of contaminated peanut-based feed [13]. It is a widespread dietary hepatotoxin and hepatocarcinogen, and a major public health concern throughout the world. There are substantial species-specific differences with regard to susceptibility to the toxic effects of AFB1, and domestic turkeys (*Meleagris gallopavo*) are among the most susceptible species known so far [14, 15].

Aflatoxins are usually found in feed ingredients used for poultry rations. Most extensive forms of AF include B1, B2, G1 and G2, with AFB1 being the most widespread and biologically active form [16]. In fact, AFB1 is a “pro-carcinogen” that is activated to a reactive form by the enzyme hepatic microsomal cytochrome P450 (CYP450), whereas electrophilic AFB1-8,9-epoxide (AFBO) is required for carcinogenic and toxic activity [13]. This compound forms AFB-N7-guanine adduct with DNA, which is not stable and is transformed into formamidopyrimidine. DNA adducts and repair activities through modulation are considered as important markers in carcinogenesis susceptibility. AFB-N7-guanine adduct in urine is also a potential biomarker of AFB1 exposure in animals and humans, and is vital for estimating exposure conditions and potential risk in individuals consuming AFB1 [12].

Major AFB1 detoxification route is via conjugation of the AFBO to endogenous glutathione (GSH) catalyzed by the classical detoxification enzymes glutathione S-transferases (GSTs) in mammals. Xenobiotics, including chemical carcinogens and environmental contaminants, are metabolized through detoxification processes in phase-II metabolism through these proteins [17]. Due to the expression of A3 subunit (mGSTA3), mice bioactivate AFB1 and are assumed as AFB1-resistant with great catalytic activity for AFBO. The present approach is that efficiency of GST conjugation is a major “rate-limiting” determinant for AFB1 action in individuals and species, irrespective of the efficiency of AFB1 bioactivation [14].

Aflatoxins cause a variety of effects in poultry, including decreased weight gain; poor feed efficiency; reduced egg production and egg weight; increased liver fat; changes in organ weights; reduction in serum protein levels; carcass bruising; poor pigmentation; liver damage; decreased activities of several enzymes involved in the digestion of starch, protein, lipids, and nucleic acids; and induction of immunosuppression. Evidence suggests that immunosuppression caused by AF results in many disease outbreaks, vaccination failures and poor antibody titers [9, 11]. At necropsy, livers are usually pale and enlarged, as a result of aflatoxicosis. Histologically, liver lesions include congestion of the hepatic sinusoids, focal hemorrhages, centrilobular fatty cytoplasmic vacuolation and/or necrosis, biliary hyperplasia, and nodular lymphoid infiltration. AF produces a malabsorption syndrome characterized by steatorrhea, hypocarotenoidemia, and decreased concentrations of bile salts and pancreatic lipase, trypsin, amylase and RNase at levels that do not affect growth [11].

Broiler chicken fed with 1.0 mg of AFB₁/kg of diet were found to show decreased hepatic gene expression of superoxide dismutase, GST, and epoxide hydrolase and increased gene expression of interleukin-6 and CYP1A1 and 2H1 at cellular level [18].

Ingestion of 2 ppm AFB₁ in male broiler chicks was found to alter various hepatic genes causing up-down regulation. For instance, enzymes having role in the production of energy and metabolism of fatty acids (carnitine palmitoyl transferase), development and growth (insulin-like growth factor 1), coagulation (coagulation factors IX and X), protection of immune system (interleukins), antioxidant protection (GST), detoxification (epoxide hydrolase) were found to be downregulated; while cell-proliferation enzymes (ornithine decarboxylase) were upregulated [19].

A study reported that wild turkeys are significantly more resistant to AFB₁ compared to domestic turkeys. Intensive breeding technologies and industrial alliance to produce modern domestic turkey led to the unintentional loss of AFB₁-protective GST alleles directing a relative resistance. Actually, it has been shown that similar breeding pressures have eventuated in a remarkable loss of rare alleles and genetic diversity of single-nucleotide polymorphisms in commercial breeds of chickens [14].

As mentioned previously, mycotoxins not only lead to the aforementioned economic and health problems in poultry, but also cause public health concerns due to their residues in food for human consumption. Major metabolites of AFB₁ formed in chicken liver are AFM₁ and AFB_{2a}. AFB₁ and B₂ are then degraded to cyclopentanol and aflatoxicol through NADP. Both AFB₁ and aflatoxicol are known to accumulate at the layer of the egg. While AFB₁ and AFM₁ are present in chicken muscle and blood, the levels are found much higher in turkeys; the aflatoxicol levels were found to be less prominent in these animals. As a comparison, 1/1200 of AFB₁ taken with feeds was found to accumulate in poultry meats, while 1/2200 of AFB₁ was found to accumulate in the eggs [20].

2.2. Ochratoxins

Ochratoxins are a family of structurally related metabolites that are produced by *Aspergillus* and *Penicillium* species, including *A. ochraceus*, *A. niger* and *P. verrucosum* [21]. The most prevalent form is ochratoxin A (OTA) followed by its non-chlorinated metabolite ochratoxin B (OTB) and the ethyl ester form ochratoxin C (OTC). OTA is the most frequent and relevant form of this family, while OTB and OTC are generally counted to be of lesser importance [22]. IARC classified ochratoxin A as a compound possibly carcinogenic to humans (Group 2B) [23].

Aspergillus species can generate OTA and OTB in parallel, and experiments with *A. ochraceus* have ascertained growth-associated production of OTA and OTB, in which the yield and the ratio were dependent on the current culture terms. Mostly, the amount of OTB generated was quite lower than that of OTA, but under some situations, the level of OTB production was comparable to that of OTA. The informed generation ratios (OTA:OTB) ranged from 2:1 to 34:1 [22]. Herein, it was reported that a complex interaction of various carbon sources, basal media and nitrogen sources seems to be considerable. High OTA production was related to an induction of OTA polyketide synthase expression, whereas OTB production is not connected

with transcription of the polyketide synthase gene. Laboratory fermentation experiments with *A. ochraceus* result in production of OTA at high yields (by 10 mg/g), OTB and temporarily also ochracin [24]. The intermediate metabolite OT β was determined to be biotransformed in an effective manner into both OTA and OTB (14% and 19%, respectively), whereas OT α was biotransformed only into OTA (4.9%). In addition, OTB is inadequately converted (1.5%) into OTA, whereas some OTB may be produced by dechlorination of OTA [22].

OTA is hepatotoxic, nephrotoxic, neurotoxic, teratogenic and immunotoxic as confirmed by *in vivo* experiments with different animal species and various *in vitro* methods; its adverse effects include renal toxicity and carcinogenesis. Molecular studies with OTA revealed a non-DNA-reactive genotoxic mechanism, which includes various epigenetic mechanisms principally connected to oxidative stress, compensatory cell proliferation and disruption of cell signaling and division [25]. However, a direct genotoxic mechanism including OTA bioactivation and DNA adduct formation was also suggested [23] and this mechanism was found to be in accordance with some *in vivo* gene expression results [25]. Overall, the mode of action of OTA for renal carcinogenesis is yet under discussion.

Ochratoxins cause significant health problems and economic losses in poultry [26] and cause mycotoxic porcine nephropathy (MPN) [27]. Ochratoxin-related diseases are characterized by severe kidney damage, which could be overtly related to the exposure to ochratoxins, sometimes in combination with different mycotoxins [27]. Likewise, a slow, progressive renal disease (endemic nephropathy, EN), characterized by cellular interstitial fibrosis, tubular atrophy, and karyomegaly predominately in proximal convoluted tubules was described in humans. The etiology of this disease is still unknown, but researchers agree that the causative agent is of natural origin. The most common causes of the multiethiologic disease, EN, were the aristolochic acid from the plant birthwort (*Aristolochia clematitis*) and mycotoxins (OTA and citrinin) [28].

OTA consists of an isocoumarin moiety linked through the 7-carboxy group to the amino acid L- β -phenylalanine. OTA interferes with DNA, RNA and protein synthesis by inhibiting the enzyme phenylalanine-tRNA synthetase at a cellular level. It also affects renal carbohydrate metabolism through the reduction of the renal mRNA coding for phosphoenolpyruvate carboxykinase, a key enzyme in gluconeogenesis [11]. The effects of OTA on DNA, RNA and protein synthesis are thought to be due to the phenylalanine moiety of the toxin competing with phenylalanine in the enzyme-catalyzed reaction. OTA also causes hypocarotenidemia which has more severe effects in broilers than AF [29].

Signs of OTA toxicity in poultry include weakness, anemia, decreased feed consumption, reduced growth rate and egg production, poor feathering and excessive mortality at high dietary concentrations [21]. Pathophysiological changes include decreased urine concentration and glomerular filtration rate, impairment of proximal tubular function, and degeneration and ultrastructural alterations in renal integrity [30]. Increases in the relative weights of liver, spleen, pancreas, proventriculus, gizzard and testes have also been reported in poultry fed OTA [21]. A study found that the expression of *Eimeria tenella* and its pathological effects were maximum in the presence of OTA compared to the incidence of coccidiosis alone in broiler chicks [31].

Comparative toxicity studies of OTA and OTB have shown that *in vivo* and *in vitro* effects are very different. OTB is overtly less toxic *in vivo* compared to OTA as indicated in different models. LD50 values, found in a comparative study using 1-day-old chicks, were 120 µg for OTA (about 3.5 mg/kg) and 1890 µg for OTB (54 mg/kg) [32]. OTB is more easily excreted and has a lower affinity for plasma proteins, which may partly elucidate its lower toxicity. Both OTA and OTB toxins induce acute cytotoxic effects *in vitro*, ensuring similar amounts are taken up and are intracellularly bound, while other complex molecular mechanisms were introduced for chronic cytotoxicity studies. Moreover, it can be supposed that the small structural difference, although not responsible for the toxicity, may be crucial for the differential uptake and binding in cells. Furthermore, OTC seems to be similarly acute toxic *in vivo* and *in vitro* compared to OTA; however, the mode of action of OTC and OTA remains to be explained. In a study, oral LD50 values were reported for OTC (216 mg animal⁻¹) and OTA (166 mg animal⁻¹) in day-old chicks. Other ochratoxin ethyl or methyl esters showed lower toxicity compared to OTA. In comparison to OTA, the methyl ester of OTA was less toxic than OTA in day-old chicks, while OTB methyl and ethyl esters were found to be non-lethal to orally exposed day-old ducklings [22]. OTα is much less toxic (approximately 100 fold) than OTA as indicated in different studies [33]. It is obvious that the isocoumarin moiety alone is not effective but must be bound to phenylalanine to show toxic effects. With the current knowledge, no clear general toxicity ranking can be drawn; after all, OTA seems to be overall the most toxic, followed by OTC, OTB and OTα [33].

2.3. Fumonisin

Fumonisin (FUM) are a group of mycotoxins that were first isolated from cultures of *Fusarium moniliforme* and chemically characterized in 1988 by Gelderblom and colleagues [34]. Six different FUM have been identified (A1, A2, B1, B2, B3, B4) and their structures elucidated. However, fumonisin B1 (FB1) has been reported to be the predominant form produced by *Fusarium moniliforme*. Several other *Fusarium* species and a species of *Alternaria* have also been found to produce FB1 [35]. Based on all these animal studies, FB1 is classified by IARC as possibly carcinogenic to humans (Group 2B) [36].

The metabolism of fumonisin is yet to be elucidated. FB1 is metabolized into partially hydrolyzed FB1 and then to the hydrolyzed form (HFB1) in both gastrointestinal tract and liver, where it persists at low concentration for few more days in pigs [37]. FB1 was found to be more toxic than HFB1 in piglets [38]. Even though N-acylation of FB1 and the formation of HFB1 are shown in human cell lines and in rats, the metabolism in the avian species still remains uncertain and yet it is not possible to generalize the metabolic pathways in all animal species [39].

The mechanism that causes toxicity of fumonisins in animals seems to be due to the disruption of sphingolipid metabolism. Present evidence shows that the FUM are specific inhibitors of ceramide synthase (sphinganine/sphingosine N-acyltransferase), a key enzyme needed for the synthesis of ceramide and more complex sphingolipids. Inhibition of this enzyme system causes an increase in tissue concentrations of the sphingolipids sphingosine (SO) and sphinganine (SA), and a change in the SA:SO ratio. An increase in the SA:SO ratio has been demonstrated in tissues of broilers, turkeys, and ducklings fed FB1 [40].

In comparison to horses and swine, two susceptible species, chicks and turkeys, are relatively resistant to the toxic effects of FB1. Mild to moderate toxicity was reported in chicks, ducks and turkeys fed rations containing 75–400 mg FB1/kg for 21 days. The primary changes in chicks, ducks and turkeys were decreased body weight gain and liver pathology [41–43]. Hepatic changes in chicks were multifocal hepatic necrosis and biliary hyperplasia. Hepatocellular hyperplasia and increased extramedullary hematopoiesis were also noted in one study [44]. The primary liver pathology observed in turkeys fed with 150–300 mg FB1/kg [43] and ducklings fed with 400 mg FB1/kg [41] were diffuse hepatocellular hyperplasia, with biliary hyperplasia (more evident in turkeys). In studies designed to evaluate the chronic effects of FB1, chick performance up to 7 weeks was not affected by up to 50 mg FB1/kg diet, whereas turkeys fed with 50 mg FB1/kg diet had lower feed intakes than birds fed 0 or 25 mg FB1/kg diet [45].

2.4. Trichothecenes

Trichothecene mycotoxins are a group of fungal metabolites with the same basic backbone structure and include T-2 toxin, HT-2 toxin, diacetoxyscirpenol (DAS), monoacetoxyscirpenol (MAS), neosolaniol, 8-acetoxyneosolaniol, 4-deacetylneosolaniol, nivalenol, 4-acetoxynivalenol (Fusarenone-X), DON (vomitoxin) and 3-acetyldeoxynivalenol. They are known as the most potent small molecule inhibitors of protein synthesis and the main toxic effect at the cellular level appears to be the primary inhibition of protein synthesis followed by a secondary disruption of DNA and RNA synthesis [11]. The overall conclusion by IARC was that toxins derived from *Fusarium sporotrichioides* are not classifiable as to their carcinogenicity to humans (Group 3) [46].

For livestock, the most important trichothecene mycotoxin is DON, which is commonly a contaminant of corn, wheat and other commodity grains. Lesser amounts of T-2 toxin and DAS are found sporadically in the same sources. Poultry and cattle are more tolerant of trichothecenes than are pigs. Compared to the related DON, T-2 toxin is less frequent in crops. Some reports indicate that trichothecenes such as DON, nivalenol and fusarenon X are more frequent (57%, 16% and 10% of tested grain samples) in European grain samples than other trichothecenes like T-2 toxin (20%), HT-2 toxin (14%), T-2 tetraol (6%), neosolaniol (1%), DAS (4%), MAS (1%) [47].

Trichothecene poisoning in poultry is acute or chronic. Acute poisoning has a characteristic clinical picture and can be readily diagnosed, while chronic poisoning shows unspecific clinical symptoms [48].

Toxic effects of trichothecenes include oral lesions, growth retardation, abnormal feathering, decreased egg production and egg shell quality, regression of the bursa of Fabricius, peroxidative changes in liver, abnormal blood coagulation, leucopenia and proteinemia, and immunosuppression [49]. Concentrations of T-2 that cause oral lesions are lower (0.4 mg/kg) than concentrations reported to decrease chick performance (3–4 mg/kg) [11]. In a comprehensive review, Danicke [49] concluded that broiler performance is affected at dietary concentrations of 3–4 mg/kg of T-2 toxin, whereas ducks were affected when the dietary concentration was as low as 0.4 mg/kg.

T2 toxin was found to decrease the immune response, represented by the decrease of lymphoid cells in the bone marrow, thymus and spleen causing resistance to infectious diseases including salmonellosis and *Escherichia coli* and cause resistance to treatments of these diseases in poultry [50]. In broilers, T-2 toxin may cause a decrease in body weight and relative weights of bursa of Fabricius, thymus, and spleen, enlarged liver, friable, and yellowish discoloration with distended gall bladder during *Mycoplasma gallisepticum* infections. Microscopical findings include vacuolar degeneration along with augmented hyperplasia in bile duct epithelia; Kupffer cell activity and infiltration of inflammatory cells in liver; vacuolar degeneration with pyknotic nuclei in kidney; lymphocytolysis and reduction of prominent reticuloepithelial cells in lymphoid organs; desquamation of villous-type epithelial cells and lymphoid intrusion in the submucosa of proventriculus; mild hemorrhage along with inflammatory cells in the heart; desquamation and erosion of the mucosa in trachea and the thickening of the air sacs along with edema and the presence of inflammatory cells in air sacs [51]. The toxic effect also manifests as reduced proliferation of lymphocytes stimulated by phytohemagglutinin and lipopolysaccharide in Pekin duck broilers [52].

DON was found to be less toxic than T-2 toxin, and the level of DON that affects chick performance is still disputed. Some researchers [53, 54] reported toxic effects at 16 mg/kg diet, whereas others [55] report no toxic effect until dietary concentrations exceeded 116 mg/kg of DON. A review paper summarizing results of 49 studies with DON concluded that a dietary concentration of 5 mg/kg had no negative effects on performance [56]. DON has also been demonstrated to have both immunosuppressive and immunomodulating effects in poultry [49]. Recent studies indicate that DON at concentrations ranging from 1 to 7 mg/kg diet significantly alters several key functions of the intestinal tract including decaying villus surface area available for absorption and altering the permeability of the alimentary canal [57].

3. Interactions among mycotoxins

In nature, co-occurrence of mycotoxins is generally observed. Meanwhile, for many years the research community focused on the occurrence of singular mycotoxins. Nowadays, scientific interest is shifted to studies involving multiple mycotoxins using various co-occurrence scenarios. One fungus may produce many different mycotoxins, and the same mycotoxin may be produced by several species. A paper conducted a meta-analysis of publications (> 100) describing toxicological interactions among mycotoxins. Results indicated that most of the studies showed a synergistic or additive interaction on animal performance. However, results with respect to other response variables indicated that there were many types of interactions ranging from synergistic to antagonistic for the same association [58]. They also observed from their review that a combination of mycotoxins, at concentrations that individually should not cause negative effects, may negatively affect animals.

The individual and combined effects of dietary AFB1 and FB1 on liver pathology, serum levels of aspartate amino-transferase (AST) and plasma total protein (TP) of broilers were quantified from 8 to 41 days of age with the dietary treatments of AFB1 (0, 50 and 200 µg AFB1/kg), and FB1 (0, 50 and 200 mg FB1/kg). Following treatment, AST levels were found to be higher in

all treatment groups (except 50 mg FB1) compared to controls at day 33. TP levels were found to be reduced at 6 days post feeding in AFB1-treated group (200 µg) and in FB1 combination group. At 33 days post feeding, the combination group (200 µg AFB1 and 200 mg FB1) were found to have higher plasma TP, proliferation of bile duct and trabecular disorders in liver tissue compared to control; while the changes in other groups were insignificant compared to controls. Overall AFB1 alone and in combination caused damage in liver at varying degrees and an increase of serum AST levels [59].

Aflatoxicosis causes a reduction in the production of egg and a decrease in egg weight in laying hens. Meanwhile, the antagonistic effects of AF and FB on egg production were reported in quails, where the decrease in egg production in FB-only treated group was much evident compared to AF+FB combination [60].

Few studies examined the combined effect of AF and FB on immunity. AF and FB co-contaminated feed was found to reduce lymphocyte proliferation by mitogenic stimulation as less than additive [61] or as additive [62] compared to single contamination. A study indicated a synergistic decrease of the antibody titers against Newcastle disease [59]. On the contrary, another study demonstrated an unexpected increase and an additive effect of the two toxins when looking at the hemagglutination titers against sheep red blood cells in turkey poults. However, the phytohemagglutinin delayed hypersensitivity response was not affected by dietary treatment. These results indicate that FB1 and AF, alone and in combination, can adversely affect poult performance and health [63].

As is known, both AF and OTA reduce egg production and hatchability. The combined effects of these two toxins were studied in laying hens [64, 65]. An additive interaction of AF and OTA was observed on egg production and on the feed efficiency (consumption for egg production) [64]; meanwhile this interaction was dependent on the concentration, resulting from synergistic to slightly lower than additive effect and also modulates the protein and energy usage [65].

AF- and OTA-contaminated feed resulted in microscopic lesions in the liver and kidneys, along with respective target organs in chicken, while contradictory results are presented in different studies. As such, OTA in the diet was found to prevent the hepatic fatty infiltration caused by AF in chicken [66]. Pigs fed the co-contaminated diet offer the same hepatic lesions as those fed with the diet contaminated with AF alone [67]. On the contrary, a study recorded more severe hepatic lesions in chickens taking the co-contaminated diet, with granular and vacuolar degenerative changes, necrosis of liver parenchyma and areas of hemorrhages [68]. The same conflict was realized for the histology of the kidney. In pigs, less severe renal lesions and lower creatinine and blood urea nitrogen concentrations were observed in animals fed the co-contaminated diet compared to animals fed the OTA-contaminated diet [67]. In contrast, a study observed that renal injuries appeared earlier and were more developed in chickens fed a multi-contaminated diet than in animals taking the mono-contaminated diets, which caused destruction of tubular epithelium, with detachment of tubular cells from basement membrane [68]. The species used may explain these conflicts. Apart from that, chronic DON exposure did not induce any effect on FB1 toxicokinetics in broilers [69].

The results on combination toxicity are yet quite limited and occasionally conflicting. Nowadays, very little is known about mycotoxin interactions although combined exposure is clearly more relevant to real-life conditions. It is known that the combined effects of mycotoxins are mostly additive or synergistic; whereas depending on the concentrations and the *in vitro* model employed, antagonistic interactions have also been determined. The results on multiple mixtures are still inadequate [22].

4. Prevention and control

Prevention of fungi production in the feeds may be achieved by always keeping the feeds fresh, keeping the humidity low and equipment clean and also adding fungistatic substances. Humidity exceeding 11% promotes fungal production in cereals and feed. Storage conditions that afford high relative humidity also significantly affect humidity content of the feed. Good ventilation of the storehouse removes humidity from the raw material of the feed and storehouse. Physically damaged cereals are more prone to fungus production compared to the healthy ones. Changing the raw materials at the places where they are stored at short intervals decreases mycotoxin formation [2, 3, 6].

Research efforts progressively increase to develop mitigation strategies based on risk monitoring, risk characterization, prevention, intervention, and remediation strategies for multiple mycotoxins, initiating from critical points along the production chain comprising field, storage, processing and transportation. However, monitoring and good agricultural, storage, and transportation practices along with an effective Hazard Analysis and Critical Control Point (HACCP) approach do not completely prevent mycotoxin presence in the food or feed chain. Decontamination strategies then offer a last resort to salvage contaminated batches along the production chain [70].

Considering the variation of mycotoxin structures, it could be inferred that there is no single method which can be used to deactivate mycotoxins in feed. Therefore, different strategies have to be combined in order to specifically target individual mycotoxins without affecting the quality of feed [11].

Decontamination strategies to reduce mycotoxins in food and feed commodities are technologically diverse and based on chemical, biological and physical approaches. Chemical remediation strategies involve the conversion of mycotoxins via chemical reactions. Ammoniation, alkaline hydrolysis, peroxidation, ozonation and the use of bisulphites are reported to be effective on one or more mycotoxins but a detailed insight into the toxicity of eventual end products or the impact on palatability and nutritive quality is questionable [71].

Biological approach in treatment strategies involves various substances (algae, plant ingredients, etc.) that protect critical organs such as the liver and strengthen the immune system of animals. Enzymatic or microbial detoxification, also referred to as “biotransformation” or “biodegradation”, uses microorganisms or purified enzymes thereof to catabolize the entire mycotoxin or transform or cleave it to less or non-toxic compounds [11]. Some microorganism such as *Rhodococcus erythropolis* [72], *Armiliariella tabescens* [73] and *Myxococcus fulvus* [16] have been suggested to have different AF-degrading ability. *Rhizopus oryzae* [74], *Bacillus*

licheniformis [75] and *Pseudomonas* sp [76] were searched for their abilities to degrade ZEA. Some studies found that *B. subtilis* had protective effects against aflatoxicosis in layers and broilers fed naturally AF-contaminated diets and also healed ZEA toxicosis in pre-pubertal gilts when fed diets including ZEA. Therefore, *B. subtilis*, as a new feed additive for biodegradation of AF and ZEA may have promising potential in feed industrial applications [77].

Some physical processes aim to remove highly contaminated fractions from bulk material through sorting, milling, dehulling, cleaning, heating, irradiation or combinational approaches [78, 79]. Another physical removal strategy is the use of inorganic or organic mycotoxin binders [80]. Due to low feed inclusion requirements and easy management of AF enterosorbents, the widespread acceptance of these products by the farm animal industry has led to the introduction of a variety of diverse materials and/or complex mixtures for AF binding. These have been labeled as mycotoxin enterosorbents, binders, sequestrants, interceptor molecules, trapping agents, adsorbents, toxin sorbents, and so on. These materials (and/or mixtures) are reported to contain smectite clays, zeolites, kaolinite, mica, silica, charcoal, sodium bentonite and various biological constituents including chlorophyllins, yeast products, lactic acid bacteria, plant extracts and algae. Some contain smectite or zeolite minerals that have been amended with natural or synthetic surfactants resulting in hydrophobic organoclays or organozeolites [81–84]. There is considerable evidence indicating that smectite clays are the most effective AF enterosorbents. Although these adsorbing binders have some promising features, some may have adverse nutritional effects due to binding of vitamins and minerals or reducing the efficacy pharmacokinetics of antibiotics [85]. Also, possible dioxin contamination might pose a risk for using natural clays in case of forest and trash fire near the sources [86]. Furthermore, the adsorption efficacy of binding agents is limited to only a few mycotoxins, such as AF, ergot alkaloids, and some other fungal toxins, while binders have been shown to be ineffective for trichothecenes [87]. Therefore, alternative approaches for efficient detoxification of mycotoxins are required.

Use of microorganisms and their specific products such as enzymes to detoxify specific mycotoxins not only work for non-adsorbable mycotoxins, but for all other toxins for which respective microbes can be isolated from nature. This approach has been known for a long time, even longer than the binder concept. Within few years after the discovery of AF, the first report on a bacterium capable of detoxifying AF by catabolization was published [88]. Since then, many microorganisms were isolated from different habitats such as the gastrointestinal tract (GIT) of animals, soil, mycotoxin-contaminated materials (e.g., grains) and insects feeding on such materials. The ability of various bacteria, yeast, fungi and enzymes in detoxifying mycotoxins by transformation, cleavage and catabolization has been recently reviewed [89]. However, only a few of these organisms were useful or further investigated for practical applications in animal nutrition. Such microorganisms or enzymes need to fulfill many different requirements before they can be used for gastrointestinal detoxification of mycotoxin in animals, such as:

- The microorganism and its reaction products need to be non-toxic and safe.
- High detoxification reactivity.
- Good technological properties (fermentation, downstream processing, stabilization).

- High stability in feed and during feed processing.
- No negative impact on feed (ingredients).
- Compatibility and stability in the GIT.
- Detoxification reaction in the GIT needs to be fast and as complete as possible.

One of the microorganisms which has been further developed for practical application is *Trichosporon mycotoxinivorans*, a yeast strain capable of detoxifying OTA and ZEN [90]. Application of this yeast in poultry diets has been proven to detoxify OTA. Another organism is an anaerobic rumen bacterium BBSH 797 (Genus Novus of family Coriobacteriaceae, formerly Eubacterium) which was isolated and developed as a trichothecene-detoxifying feed additive [91]. BBSH 797 detoxifies trichothecenes by cleavage of the 12, 13 epoxide ring resulting in deep-oxy trichothecenes. Several microorganisms, mainly aerobic bacteria and also yeasts, with FUM degradation properties were also explored and isolated in order to detoxify FUM. However, for various reasons, none of these microorganisms were found to be useful as a mycotoxin-deactivating feed additive. Therefore, the catabolic pathway of FUM degradation was investigated and the gene coding for the key enzyme of FUM detoxification (FUMzyme) was identified, cloned and expressed in a yeast strain [92]. FUMzyme (carboxyl-esterase) was further developed and tested in swine for gastrointestinal detoxification of FUM by cleaving the tricarballic side chains of FUM leading to the non-toxic metabolite hydrolyzed FUM (HFB1) [93].

One of the common approaches to overcome mycotoxicosis in poultry is using herbal products including essential oils as plant-based fumigants in feed storage [94]. Essential oils are complex compounds, and their chemical composition and concentrations of various compounds are variable. Essential oils basically consist of two classes of compounds, the terpenes and phenylpropenes, depending on the number of 5-carbon building blocks. For example, 500 ppm of the ethanolic extract of *Thymus vulgaris* could partially restore the negative impact of AFB1 (600 ppb) in commercial broilers [9]. They suggested that this herb can be used as natural non-antibiotic feed additive on broilers in the prevention of aflatoxicosis. As a result of the change in diet (change in nutrients, phytochemicals, contamination, xenobiotics), the levels of the drug-metabolizing enzymes (phase-I and phase-II) are expected to change, which would eventually lead to a change in AFB1 adducts. On the other hand, as phenolic phytochemicals have antioxidant effects at varying degrees due to their various chemical structures, they are assumed to have a protective role in the cellular components against free radical-induced damage caused by aflatoxicosis [95]. Apart from that, a herbal mycotoxin binder comprising of a combination of minerals (extra purified clay containing diatomaceous earth minerals), antioxidants (curcuminoids extracted from turmeric) and enzymes (Epoxidases and Esterases) in proportions of 15, 10 and 75%, respectively, partially restored feed consumption and egg production, alleviating some side effects of AFB1 (500 ppb in feed) in broiler breeders [96].

5. Conclusion

Understanding the occurrence and prevalence of mycotoxins and their individual as well as additive negative effects on poultry has become imperative. New insights on actual microbial

detoxification routes are needed in the field, which could be based on the biodegradation metabolisms of non-mycotoxins found in diverse microbial communities. Indeed, many hazardous, undesirable, deleterious or recalcitrant molecules in other research fields share structural analogies with diverse mycotoxins and are reported to be successfully degraded by microorganisms. These unexplored worlds may serve as resource for cutting-edge research in the field of mycotoxin remediation or in the field of metagenomics screening surveys in search for new microbial degraders of mycotoxins. The usage of latest analytical techniques such as liquid chromatography tandem-mass spectrometry will increase the precision in determination of the concentrations of multiple mycotoxins present in agricultural commodities, at once. Latest enzymatic deactivation technologies help to eliminate the mycotoxins that cannot be bound using binder products. Overall, mycotoxins still impose a great risk for the poultry sector and alternative approaches for the prevention are still being sought by researches around the world.

Author details

Ayhan Filazi^{1*}, Begum Yurdakok-Dikmen¹, Ozgur Kuzukiran² and Ufuk Tansel Sireli³

*Address all correspondence to: filazi@veterinary.ankara.edu.tr

1 Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

2 Veterinary Control Central Research Institute, Ankara, Turkey

3 Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey

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