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Review Paper

# Occurrence, Prevention and Limitation of Mycotoxins in Feeds

**M.F. Abdallah[1], G. Girgin and T. Baydar***

Department of Toxicology, Faculty of Pharmacy
Hacettepe University, 90-06100, Ankara, Turkey

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## ABSTRACT

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There has been a significant concern regarding the potential health risks for humans and animals via foods and feeds that are contaminated with different agents. Particularly, mycotoxin contamination is of great importance as it is widespread and unpreventable. In both foods and feeds, molds produce secondary metabolites called mycotoxins; these are produced generally after the fungi reach their maturity. Depending on the definition used, hundreds of fungal compounds are recognized as mycotoxins. However, the attention is mainly focused on aflatoxins, ochratoxins, fumunisins, and zearalenone which are considered the most important threats for human and animal health. Mycotoxin contamination causes a fundamental problem all over the world including developed countries. Additionally, the economic impact of mycotoxins is another global concern on the agricultural markets. These concerns are based on toxicological data, which show that naturally occurring levels of mycotoxins have adverse effects in farm and laboratory animals as well as humans. The diversity of mycotoxin structures induces various toxic effects. Owing to the significant health risks and economic impacts, considerable investigations are being performed to diminish their harmful effects and to prevent their formation. In order to limit their levels, much research has been focused on detecting the mycotoxins in contaminated food and feedstuffs. This review will focus on information about primary mycotoxins, their occurrence, related regulations, prevention and methods of detection within the light of the current literature.

**Key words:** Aflatoxin, Fumonisins, Mycotoxins, Ochratoxin, Zearalenone.

## INTRODUCTION

Mycotoxins are secondary metabolites produced naturally by about 200 recognized filamentous fungi growing under a wide range of climatic conditions on different

*Corresponding author: tbaydar@hacettepe.edu.tr, terkenbaydar@gmail.com
[1]Department of Toxicology, Faculty of Veterinary Medicine, Assiut University, Egypt.

Table 1. Toxic effects of mycotoxins in different animals

| Mycotoxin | IARC† classification | Major effects | Clinical and pathological signs on most susceptible animals |
|---|---|---|---|
| Aflatoxins | 1 | Carcinogenic, hepatotoxic and | Reduced productivity; inferior egg shell and carcass quality; increased |
| Aflatoxin M1 | 2B | impaired immune system | susceptibility to infectious disease. |
| Ochratoxin A | 2B | Carcinogenic, nephrotoxic, hepatotoxic, neurotoxic and teratogenic | Kidneys are grossly enlarged and pale due to nephrotoxicity; fatty livers in poultry; shell decalcification/thinning. |
| Deoxynivalenol | 3 | Immunotoxic and ATA (alimentary toxic leukopenia) | Decreased feed intake and weight gain in pigs; feed refusal and vomiting at very high concentrations. |
| Other trichoth-ecenes (T-2 toxin) | 3 | Immuno-depressants, gastrointestinal haemorrhaging and hematotoxicity | Reduced feed intake; vomiting, skin, and gastrointestinal irritation; neurotoxicity; abnormal offspring; increased sensitivity to infection; bleeding. |
| Zearalenone | 3 | Fertility and reproduction (estrogenic activity) and disrupts endocrinesystem | Swollen, reddened vulva, vulvovaginitis, anestrus vaginal prolapse and sometimes rectal prolapse in pigs; feminization and suppression of libido; suckling piglets may show enlargement of vulvae; fertility problems. |
| Fumonisins | 2B | Carcinogenic,hepatotoxic,central nervous system damage and immuno-depressants | Equine leucoencephalomalacia (ELEM), porcine pulmonary edema, liver damage in poultry. |

†International Agency for Research on Cancer. 1: carcinogenic to humans AFs; 2A: probably carcinogenic to humans; 2B: possibly carcinogenic to humans; 3: not classifiable as to its carcinogenicity to humans; 4: probably not carcinogenic to humans.

agricultural stuffs. A number of fungal genera, mainly *Aspergillus*, *Penicillium*, *Alternaria*, *Fusarium*, and *Claviceps* produce mycotoxins (Zollner and Mayer-Helm, 2006; Marin *et al.*, 2013). Some fungi have the ability of producing more than one mycotoxin and some mycotoxins can be produced by more than one mold species (Hussein and Brasel, 2001). Mycotoxin contamination in animal feed and the potential transfer into animal products to be consumed by humans still remains a major problem alerting the entire world (Cheli *et al.*, 2014). Several outbreaks have been reported in humans and animals after the consumption of mycotoxin-contaminated food and feed. Mycotoxin production and/or contamination in agricultural products can take place at different stages in food and feed chain: pre-harvest, harvest and post-harvest (Binder *et al.*, 2007). Mycotoxins are commonly present in nuts, dried fruits, coffee, cocoa, spices, oil seed, dried beans, corn, wheat, and several other cereals. Not only foods, but also animal feeds and products such as milk, cheese and meat are important exposure sources (Imperato *et al.*, 2011; Da Rocha *et al.*, 2014). As a result of mycotoxin-contaminated animal feed consumption, decreased feed intake, feed refusal in some

cases, poor feed utilization, reduced body weight gain, increased disease susceptibility, and reduced reproductive abilities are commonly observed; moreover deaths can occur which leads to serious economic losses (Binder *et al.*, 2007).

Mycotoxicoses are diseases caused due to mycotoxin exposure. Mycotoxins have different toxicological effects in humans and animals. Table 1 shows toxic effects of some mycotoxins in different domestic animals. Some of them have the same characteristic clinical and pathological signs with differences in severity (Duarte *et al.*, 2011). The clinical signs may vary according to the species in addition to the exposure dose and period (Cheli *et al.*, 2014). Nausea, vomiting, abdominal colic, and diarrhea are the general signs that occur as a result of contaminated food/feed stuffs ingestion(Fung and Clark, 2004). In animals, the first observed symptoms are decreased feed intake and growth retardation. After that, immunosuppression signs like ineffective vaccine response and decreased drug efficacy against infectious diseases can also be observed (Bryden, 2011). Many internal factors play a role in mycotoxin impact such as health state of affected living organism, and also, sex, age and body weight (Steyn, 1995).

Human exposure is possible either through contaminated foods of plant origin, mostly cereal grains, or foods of animal origin such as contaminated milk, meat, and eggs. Another rare way is also possible through inhalation of polluted air and dust (Bryden, 2011). There are hundreds of mycotoxins that have been isolated and chemically described. Up to now, it has been documented that approximately 400 secondary metabolites with toxicity potential are produced by more than 100 moulds. Most of the researches have focused on those forms causing significant injuries to humans and farm animals. Rate of occurrence and severity of the diseases are the detrimental factors to identify which mycotoxins are important. Some mycotoxins with harmful effects on animals and human health are aflatoxins (AFs), ochratoxin A (OTA), trichothecenes (deoxynivalenol (DON) and T-2 toxin), zearalenone (ZEN) and fumonisins (FBs) (Trucksess and Diaz-Amigo, 2011; Afsah-Hejri *et al.*, 2013). In this review, mycotoxins and their occurrence in foods and feeds, exposure and outcomes will be reviewed in light of the recent literature.

**Aflatoxins**

Aflatoxins are difuranocoumarins mainly produced by two *Aspergillus* species, *A. flavus* and *A. parasiticus* (Reddy *et al.*, 2010). Concerning their chemical structures, there are two main categories for AFs; the first category is difurocoumarocyclopentenone group and includes aflatoxins B1, B2 (AFB1, AFB2) and aflatoxins M1, M2 (AFM1, AFM2). The second category is difurocoumarolactone group involving aflatoxins G1 (AFG1) and G2 (AFG2). The four major naturally occurring aflatoxins B1, B2, G1 and G2 are ubiquitous in animal feed stuffs. The nomenclature of AFs B1 and B2 derives from the blue fluorescent color produced and visualized under UV light while AFs G1 and G2 produce green fluorescent color (Gupta, 2011; Womack *et al.*, 2014). AFsM1 and M2 are the main metabolites of aflatoxins in which ‘‘M’’ refers to milk

of mammals consuming aflatoxin-contaminated feeds (Kara and Ince, 2014). In liver, cytochrome P450 (CYP)-associated enzymes convert AFB1 to AFM1, the major monohydroxylated derivative. In humans, the main CYP enzymes involved in aflatoxins metabolism are CYP3A4, 3A5, 3A7, and 1A2. However, liver is the primary target organ, under certain conditions, lung, kidney, and colon may also be affected (Marin *et al.*, 2013).

Among all discovered mycotoxins, aflatoxins are the most intensively researched group, because of their potent acute toxicological and chronic hepatocarcinogenic effects in various susceptible animals. Consumption of aflatoxins contaminated agricultural stuffs is the main route of exposure. Adverse effects of aflatoxins are anorexia, decreased feed intake, immune system suppression in both animals and humans. Immunosuppressive, hepatotoxic, carcinogenic, mutagenic, and teratogenic effects can be observed according to animal species, sex, age and aflatoxintype, exposure dose and period (Arslan and Essiz, 2009; Afsah-Hejri *et al.*, 2013). It has been detected that median lethal dose (LD50) of AFB1 is estimated to be between 0.3 and 18 mg/kg according to the administration route, animal species, age and health condition. Poultry are more sensitive to aflatoxins than mammals. Within poultry, ducks are the most susceptible species then turkey poults and then chicken. Within domestic animals, the order is canine, swine, calves, cattle and sheep. Young animals are more susceptible to AFs than matures. Nutritional deficiencies, especially protein and vitamin E increase the susceptibility to AFs (Bryden, 2011).

In animals, aflatoxicosis can also cause reduction in feed efficiency, immunosuppression, vaccination failure, and reduced reproduction efficacy or reduced fertility which clinically appears as decreased weight gain, rough hair coat, lowered meat, wool, and milk yield. Similarly in poultry, severe hepatic damage, stunning in growth, and egg production, hemorrhagic syndrome as a result of increased blood capillary fragility are observed (Hussein and Brasel, 2001; Zain, 2011).

In humans, the plasma half-life of AFB1 is short and it is estimated that after absorption, about 65% is removed from the blood within one and half hours (Fung and Clark, 2004). Of the known AFs, AFB1 is a potent human carcinogen. The International Agency of Research on Cancer (IARC) has classified AFB1 as a human carcinogen, Group 1 while AFM1 is categorized as possible human carcinogen; Group 2B as its carcinogenicity is 10 times less than the parent compound (Arslan and Essiz, 2009; Kara and Ince, 2014). Carcinogenic and mutagenic effects result from the highly electrophilic intermediate AFB1-8,9-epoxide, which is produced by CYP mediated biotransformation of AFB1. Somatic mutation and carcinogenesis probably occur due to depurination process of DNA molecules. Covalent bonds at the N-7 guanine residue are formed as a result of a reaction between AFB1-epoxide and DNA leading to carcinogenesis (Fung and Clark, 2004; Bryden, 2011).

Acute toxicity in humans is rare and generally, it includes a wide variable clinical signs range from sudden death without signs to general unspecific symptoms

like nausea, vomiting, abdominal cramp, anorexia, diarrhea, ataxia, edema in lung, fatty liver which is manifested by jaundice, anemia, depression, and photosensitivity. Chronic aflatoxicosis leads to moderate to severe icterus, hepatic cirrhosis, benign hepatoma, cholangiocarcinoma, or hepatocellular carcinoma. Chronically, aflatoxins are implicated in hepatocellular carcinoma synergistically with hepatitis B or C virus. The highest percentage of hepatocellular carcinoma incidences occur in parts with frequent exposure to contaminated foods and high rate of infection with hepatitis as Eastern and South-Eastern Asia and Middle and Western Africa. AFs act as immune modulators, causing suppression of resistance to secondary infections. They can also affect testes and sperm quality which leads to infertility (Reddy *et al.*, 2010; Trucksess and Diaz-Amigo, 2011; Marin *et al.*, 2013).

**Ochratoxin A**

Ochratoxin A is the most commonly encountered and toxic member of ochratoxins group. Two genera of fungi produce OTA, *Aspergillus* and *Penicillium*. The main OTA producing species are *A. ochraceus*, *A. carbonarius*, *A. melleus*, and *A. sclerotiorum*, *P. verrucosum*, and *P. nordicum*. OTA nomenclature is derived from *A. ochraceus*, the first fungus that was discovered to produce the toxin (Reddy *et al.*, 2010; Zain, 2011).

Chemically, OTA is similar to phenylalanine(Phe), the toxin involves Phe linked by a peptide bond to an isocoumarin molecule. Because of the structural similarity, OTA inhibits all biological processes involving Phe, particularly, Phe-tRNA synthetase and thus, inhibits protein synthesis as well as DNA and RNA. OTA also interferes with lipid peroxidation through impairing the endoplasmic reticulum membrane and causes oxidative stress and mitochondrial damage, triggering cytotoxicity (Steyn, 1995; Fung and Clark, 2004; Abrunhosa *et al.*, 2010).

Ochratoxin A has been detected in a wide range of different animal feed ingredients such as cereal and cereal-based products (barley, corn, wheat soy) and also foods, including various cereal products, coffee, spices, beans and other products of animal origin including milk (Imperato *et al.*, 2011; Martins *et al.*, 2012). OTA is carcinogenic, genotoxic, immunotoxic and nephrotoxic agent. The main target organ in OTA toxicity is kidneys. In Balkan countries, Croatia, Bulgaria, and Romania, OTA causes high incidence of urinary tract carcinoma which is called Balkan Endemic Nephropathy (BEN) and also, causes urothelial tumors. The main pathological feature is tubular damage in proximal convoluted tubules. The common signs include anemia, proteinuria, icterus, and uremia. As stated by IARC, OTA has sufficient evidence of carcinogenicity in experimental animals but inadequate in humans, classified as possibly carcinogenic to humans, Group 2B. At high concentrations, OTA affects many organs including liver and brain which is manifested by multifocal hemorrhages and may involve intravascular coagulation of heart (Richard, 2007; Duarte *et al.*, 2011; Afsah-Hejri *et al.*, 2013; Marin *et al.*, 2013).

Similar to humans, in domestic animals, chronic renal failure is the main effect accompanied by OTA toxicity. OTA exposure leads to testicular carcinoma. Immunologically, OTA causes obvious immunosuppression with atrophy of immune organs. Mono gastric animals such as dogs and pigs are more susceptible to ochratoxicoses then chickens while ruminants are more resistant (Yiannikouris *et al.*, 2002; Gupta, 2011; Zain, 2011; Martins *et al.*, 2012).

**Fumonisins**

Fumonisins are a group of non-fluorescent mycotoxins, mainly produced by *Fusarium moniliforme*, *F. proliferatum*. *F. napiforme*, *F. dlamini* and *F. nygamai* (Lino *et al.*, 2007; Marin *et al.*, 2013). Corn is mostly infected with fumonisin producing moulds, particularly when corn is imported from humid climates (Abbas *et al.*, 2006; Seo *et al.*, 2013; Scussel *et al.*, 2014). Structurally, fumonisins contain a long hydroxylated hydrocarbon chain having a methyl and either acetyl amino or amino groups. Fumonisins B1, B2, and B3 are the most significant toxins among more than 12 fumonisin analogues. Fumonisins are cancer-inducing toxins due to its similarity with sphinganine and sphingosine, the main constituent of sphingolipids (Hussein and Brasel, 2001; Yiannikouris *et al.*, 2002). Disruption of sphingomyelin through inhibition of sphingolipid formation is considered as the main pathway of fumonisin toxicity (Fung and Clark, 2004). In mammals, equines and swines are the most susceptible species among domestic animals then ruminants. Poultry are more resistant than mammals. In horses, fatal neurological disease, equine leukoencephalomalacia (ELEM) is the prominent toxic effect. Massive softening and liquefaction of the white matter of brain is the prominent post mortem lesion. ELEM is characterized by nervous signs including ataxia, aimless moving, facial paralysis, blindness, coma, and death. In swine, FB1 cause cardiotoxicity and acute fatal porcine pulmonary edema (PPE). PPE is characterized pathologically by presence of pale yellow colored proteinaceous fluid in lungs and interlobular pulmonary edema with severe respiratory distress and cyanosis. In addition to neurotoxicity, pulmoner toxicity and cardiotoxicity, FB1 may also exert hepatotoxicity and nephrotoxicity (Steyn, 1995; Yiannikouris *et al.*, 2002; Oruc *et al.*, 2006; Voss *et al.*, 2007; Scussel *et al.*, 2014).

Till now, there are no specified impacts of FBs on human health. It's suggested that some types of esophageal and hepatic tumors and cardiac toxicity in humans are associated with fumonisin exposure through consumption of contaminated maize (Waskiewicz *et al.*, 2012; Marroquin-Cardona *et al.*, 2014). FB1, the most important member of fumonisins, is a tumor promoter, but has no genotoxic effects. Regarding IARC, FB1 is categorized as possible carcinogen to humans, Group 2B (Richard, 2007; Voss *et al.*, 2007).

**Zearalenone**

Zearalenone is a mycotoxin with hyperestrogenic effects in animals produced by *Fusaria*, mainly by *F. graminearum*, *F. culmorum* and *F. sporotrichioides*. Maize, wheat, oats, barley and rye are mostly infected with ZEN producing moulds. (Saeger

*et al.*, 2003; Trucksessand Diaz-Amigo, 2011). Furthermore, milk contamination by zearalenone and its metabolites has been reported (Signorini *et al.*, 2012; Huang *et al.*, 2014). Structurally, ZEN is similar to 17β-estradiol and it is classified as non-steroidal estrogen (Hussein and Brasel, 2001; Da Rocha *et al.*, 2014). Zearalenone is metabolized into two diastereoisomeric zearalanols, α and β-zearalanol. All ZENs have the same estrogenic properties but their potential differs possibly due to variations in binding affinity to estrogen receptors. It has been found that α-zearalanol is three times more estrogenic than zearalenone itself. Reproductive problems in domestic farm animals are the frequent disorders occurring with ZEN exposure. Although it is not common, in humans hyperoestrogenic syndromes can be observed (Zinedine *et al.*, 2007; Marin *et al.*, 2013).

In animals, ZEN is a weak estrogen and it inhibits follicle stimulating hormone (FSH) and therefore delays the maturation of preovulatory follicle in ovaries thus exerts reproductive toxicity. Animal susceptibility shows a variation according to species, sex, age, and reproductive state. Swine are the most susceptible farm animals to reproductive effects of ZEN. Prepubertal gilts are more sensitive than mature ones. Sows in estrous cycle are more sensitive than both pregnant and non-cycling pigs. Prepubertal gilts show a hyperestrogenism, enlargement of the mammary glands while mature sows exhibit nymphomania and pseudopregnancy. Castrated males may develop enlargement of the prepuce and nipples and immature boars demonstrate reduced or loss of libido and testicular atrophy. Ruminants may exhibit some adverse effects, reduced fertility and repeated breeding but generally are of low clinical incidence. ZEN can be excreted into milk of pigs and cows as a result of exposure to high doses in feed. Poultry shows some resistance to ZEN, only highly contaminated feed stuff can lead to infertility and reduced spermatogenesis (Fung and Clark, 2004; Richard, 2007; Zain, 2011).

In humans, toxicity is mainly chronic while acute form after oral administration is rare. ZEN and its metabolites can effectively stimulate mammary gland cells growth. Thus, it was suggested that ZEN may be implicated in breast cancer. There are some reported cases of precocious puberty in adolescent girls with ZEN exposure (Zinedine *et al.*, 2007; Marroquin-Cardona *et al.*, 2014). ZEN is included in non-carcinogenic agents to humans, Group 3, according to the IARC (Afsah-Hejri *et al.*, 2013).

**Trichothecenes**

Different *Fusarium* species such as *F. culmorum*, *F. sporotrichioides*, *F. tricinctum*, *F. roseumF. graminearum*, *F. nivale* and *F. sambucinum*, and some members of *Myrothecium* are able to produce trichothecenes. Corn, barley, wheat, oats, rye, soybeans, and fruits as well as animal feeds are mostly attacked by fusarium. Fungal infection appears as a red-pink colored area, mostly at the tip of the crop (Berthiller *et al.*, 2005; Marques *et al.*, 2008; Kim *et al.*, 2014). During the last 40 years, more than 180 trichothecene mycotoxins have been discovered. Structurally, trichothecenes

have been classified according to the difference in the functional group, hydroxyl and acetoxy side, into four groups. Type A involves HT-2, T-2, diacetoxyscirpenol (DAS) and neosolaniol; type B is represented by Deoxynivalenol (DON), 3-acetyl-DON, 15-acetyl-DON, and nivalenol (NIV); type C including crotocin; and type D or macrocyclics. Despite so many forms, a few numbers of trichothecenes have a toxicologic potency. The most important are DON, HT-2, and T-2. Mechanism of toxicity is conducted through inhibition of protein synthesis by interaction with the 60S ribosomal subunit and the peptidyltransferase (Sweeney and Dobson, 1998; Chrevatidis, 2003; Zou *et al.*, 2012). Disruption of DNA and RNA is occurred through peptidyltransferase enzyme inhibition. It affects the actively mitotic cells such as intestinal epithelial cells, dermal, lymphoid and erythroid cells. Trichothecenes affect mitochondrial functions, enhance lipid peroxidation, and stimulate cell death. They also stimulate type four hypersensitivity reactions concurrently with inhibition of suppressor T-cells. At low doses, they can interrupt glucose and calcium ions transfer. Acute toxicity in humans shows abdominal cramps, nausea, vomiting and bloody diarrhea. Alimentary Toxic Aleukia (ATA) is a disease caused due to long term exposure totrichothecenes, manifested by gastrointestinal problems followed by gastroenteritis, fever, immunosuppression, and lastly bronchial pneumonia and death. According to IARC, both DON and T2 are classified as non-carcinogenic toxins to human, Group 3 (Reddy *et al.*, 2010; Mostrom and Raisbeck, 2012; Da Rocha *et al.*, 2014; Marroquin-Cardona *et al.*, 2014).

Among animal species, large ruminant, small ruminant, equine, poultry and swine are susceptible. The most sensitive are swine while cattle and birds are more resistant. Oral exposure to trichothecenes leads to feed refusal as a primary sign even after adding flavoring agents. Moreover, weight loss, anemia, and weakness may occur as consequences. In poultry, decreased egg production, abnormal feathering especially in broilers and some neurological signs are observed. Some studies suggest that trichothecenes are able to cause limb and tail deformities (Fung and Clark, 2004; Richard, 2007; Mostrom and Raisbeck, 2012).

**Occurrence of mycotoxins in plant and animal products**

Factors affecting the production and occurrence of mycotoxins in crops and consequently, the extent of contamination in feed and food involve climate conditions such as temperature, relative humidity; and agricultural practices such as fungicide usage and techniques used in agriculture. Other factors may include drying, processing, handling, packaging, storage and transport conditions. Insects play an important role through physical damage of the grains and mechanical transmission of the microorganisms (Chrevatidis, 2003; Abbas *et al.*, 2006; Richard, 2007; Marroquin-Cardona *et al.*, 2014). Most of cereal grains, oil seeds, tree nuts, and fruits (especially dried ones) are susceptible to fungal attack and mycotoxin formation. Agricultural products like cereal grains and forages can be contaminated during pre-harvest (field period), harvest, and post-harvest (storage and transportation period). Corn and other

grains used in animal feed could also be contaminated by pathogenic moulds, thereby mycotoxins, even they may be destroyed at different rates during industrial processing (Griessler *et al.*, 2010; Reddy *et al.*, 2010; Bryden, 2011; Kocasari *et al.*, 2013).

Distribution of mycotoxins varies according to fungus nature. Depending on the geographical and climate conditions, different fungal species can invade foods and feedstuffs. For example, aflatoxins are mostly expected in tropical areas where climate conditions and storage practices are favorable to fungal growth and toxin production, while ochratoxin A is frequently detected in moderate and subtropical regions; fumonisins in subtropical and tropical locations; zearalenone and trichothecenes are worldwide mycotoxins (Sweeney and Dobson, 1998; Afsah-Hejri *et al.*, 2013). In general, the crops are susceptible to contamination by the most dangerous mycotoxins in tropical and subtropical areas with high humidity and temperature. AFs are ubiquitous in corn-based animal feed and mostly present in groundnut meal and cottonseed. OTA may be present in most of cereals, corn, hay, oats, and wheat, and also in oilseed products, particularly if the products were not dried well after harvest. Corn is a major source for ZEN particularly if it is not harvested on time. Interestingly, ZEN has been detected in damp hay and straw. Furthermore, corn, wheat, barley, oats, rye and other crops have been reported to contain T-2 toxin and DON (Chrevatidis, 2003).

Direct contamination of dairy products is through colonization of mycotoxigenic fungi especially in cheese. Mould contamination can occur either from unhygienic manufacturing medium or fungal starter cultures used for the production of specific dairy products. Another route for mycotoxins and their metabolites is excretion through milk. In dairy cows, AFM1 reaches the maximum concentration in milk two days after ingestion of AFB1 containing feed and can disappear after 4 days from removing the contaminated feeds. The amount of AFM1 in milk represents approximately 3-5% of AFB1 found in the animal feed stuff. AFM1 has been commonly found in many of food stuffs including infant formulas, dried milk, cheese and yoghurt. Not only dairy products, but also meat of swine or eggs of laying birds can be contaminated if the animals consumed considerable amounts in their feed. It is possible that fungi may spread from one country to another with increases in global grain trade. In many European regions, OTA has been revealed in swine viscera, muscle tissue, and blood (Steyn, 1995; Sweeney and Dobson, 1998; Yiannikouris *et al.*, 2002; Arslan and Essiz, 2009; Marin *et al.*, 2013). A lot of investigations have reported the contamination of mycotoxins and summarized in Table 2.

**Limitation in food and feed**

Beside their obvious health impacts, mycotoxins also affect the agricultural trade among countries through decreasing livestock and crop yield production. Total elimination of moulds and their toxins from agricultural products seems to be impossible or impractical. The possible risk caused by mycotoxins to human and animal health has presupposed the urgent need to control their levels. According to the geographical

Table 2. Levels of different mycotoxins reported in some food and feed commodities.

| Commodity | Mycotoxins | Reported range | Detection method(s)† | References |
|---|---|---|---|---|
| Corn | Total aflatoxins | 21-699 μg /kg | LC-MS/MS | Abbas *et al.*, 2006 |
| | Aflatoxins B1, B2 | 2- 20 μg /kg | HPLC-FLD | Irama *et al.*, 2014 |
| | Ochratoxin A | 3-5 μg /kg | HPLC-FLD | Majeed *et al.* 2013 |
| | Deoxynivalenol | 96- 1,790 μg /kg | HPLC-FLD | Marques *et al.*, 2008 |
| | T-2, HT-2 | 0.8-18.3 μg /kg | LC-MS/MS | Berthiller *et al.*, 2005 |
| | Zearalenone | 40- 64 μg /kg | HPLC-FLD | Almeida-Ferreira *et al.*, 2013 |
| Milk | Fumonisins | 66-7832 μg /kg | HPLC-FLD | Scussel *et al.*, 2014 |
| | Aflatoxin M1 | 0.008 -0.05 ng/l | LC-MS/MS | Kara and Ince, 2014 |
| | Aflatoxin M1 | 55-116 ng/l | ELISA | Kamkara *et al.*, 2014 |
| | Ochratoxin A | 1-50 ng/l | HPLC-FLD | Imperato *et al.*, 2011 |
| | Zearalenone | 0.003-45.8 ng/l | UHPLC-MS/MS | Huang *et al.*, 2014 |
| | α-Zearalenol | 0.009-73.5 ng/l | UHPLC-MS/MS | Huang *et al.*, 2014 |
| | Zearalenone | 0.016-0.469 ppb | ELISA | Signorini *et al.*, 2012 |
| | Deoxynivalenol | 0.049-1.396 ppb | ELISA | Signorini *et al.*, 2012 |
| Cereal flour | Total aflatoxins | 0.03-3.16 μg/kg | HPLC-FLD | Baydar *et al.*, 2005 |
| | Ochratoxin A | 0.025-10.5 μg/kg | ELISA | Aydin *et al.*, 2008 |
| | Deoxynivalenol | 0.0-23.0 μg/kg | ELISA | Manthey *et al.*, 2004 |
| | Zearalenone | 40- 64 μg/kg | HPLC-FLD | Almeida-Ferreira *et al.*, 2013 |
| | Fumonisins | 142-550 μg/kg | HPLC-FLD | Lino *et al.*, 2007 |
| Animal feed | Total Aflatoxins | 0.17 -0.92 μg/kg | HPLC-FLD | Beheshti and Asadi, 2014 |
| | Ochratoxin A | 2-130 μg/kg | HPLC-FLD | Martins *et al.*, 2012 |
| | Zearalenone | 0.009-0.405 μg/kg | HPLC-FLD | Kim *et al.*, 2014 |
| | α-Zearalenol | 25-600 μg/kg | HPLC-FLD | Saeger *et al.*, 2003 |
| | Fumonisin B1 | 30-14.600 ng/g | LC-MS/MS | Seo *et al.*, 2013 |
| | Fumonisin B2 | 35-2.280 ng/g | LC-MS/MS | Seo *et al.*, 2013 |
| | Fumonisins | 104-2999 μg/kg | LC-MS/MS | Njobeh *et al.*, 2012 |
| | Deoxynivalenol | 18.5 -500 μg/kg | ELISA | Kocasari *et al.*, 2013 |
| | T-2, HT-2 | 35-40μg/kg | HPLC-FLD | Griessler *et al.*, 2010 |

†UHPLC-MS/MS: Ultra high performance liquid chromatography combined with electrospray ionisation triple quadrupole tandem mass spectrometry.

LC-MS/MS: Liquid chromatography tandem mass spectrometry.

HPLC-FLD: High performance liquid chromatography with fluorescence detector.

ELISA: Enzyme linked immunosorbent assay.

and climatic variations, different limits are being set to monitor and control mycotoxin levels (Afsah-Hejri *et al.*, 2013; Da Rocha *et al.*, 2014).

In this regard, establishment of strict limitation and tolerance levels of mycotoxins is held by national and international authorities such as the European Commission (EC), US Food and Drug Administration (FDA) and World Health Organization (WHO) as shown in Table 3. FDA has established the maximum acceptable limits in food for sum of AFs (B1, B2, G1, and G2) at 20 μg/kg and AFM1 in milk at 0.5 μg/kg while the total AFs residue limit in feeds for mature and immature animals is 100 μg/kg and 20 μg/kg, respectively (Richard, 2007; Womack

Table 3. Permitted limits for mycotoxins in various species

| Mycotoxins | Feed stuff(s) | Limit (ppb) | Country / Authority |
|---|---|---|---|
| Aflatoxin B1 | Maize | 5 | Turkey, Russia, Egypt |
| | Maize | 10 | China, Korea, Japan |
| | Animal feed | 10 | Egypt |
| | Animal feed | 50 | Turkey |
| | All cereals except rice and maize | 2 | EU |
| | Unprocessed maize and rice | 5 | EU |
| | Animal feed ingredients | 20 | EU |
| | Feed stuffs for immature animal | 20 | FDA |
| Aflatoxin B1& G1 | Maize | 30 | Brazil |
| Aflatoxin M1 | Milk | 0.5 | U.S.A, Russia, Egypt |
| | | 0.05 | Turkey |
| | Milk and milk products | 0.05 | EU |
| | Milk | 0.5 | FDA |
| Deoxynivalenol | Unprocessed cereals other than wheat, oats and maize | 1250 | EU |
| | Unprocessed wheat and oats, maize | 1750 | EU |
| | Cereal products | 500 | EU |
| | Cereals and cereal products for feed | 8000 | EU |
| | Maize by-products for feed | 12000 | EU |
| | Animal feed | 100 | FDA |
| Fumonisin B1, B2 | Animal feeds except Equines | 50 | EU |
| | Animal feeds for Equines | 5 | EU |
| Fumonisin B1, | Animal feeds except Equines | 30 | FDA |
| B2, B3 | Animal feeds for Equines | 5 | FDA |
| Fumonisins | Unprocessed maize | 2000 | EU |
| | Maize products for human | 1000 | EU |
| Ochratoxin A | Unprocessed cereals | 5 | EU |
| | Cereals and cereal products for feed | 250 | EU |
| | Cereal products for food | 3 | EU |
| T-2 and HT-2 | All cereals grains | 100 | EU |
| Total aflatoxin | Animal feed ingredients | 50 | EU |
| | Animal feed | 20 | Canada, Egypt, Iran |
| | | 50 | Brazil |
| | Maize | 10 | Turkey, Egypt |
| | | 30 | India |
| | Cereals feedstuffs | 200 | Mexico |
| | Feedstuff (ingredient)s | 20 | Japan, U.S.A, Korea |
| | All cereals except rice and maize | 4 | EU |
| | Maize and rice | 10 | EU |
| | Feed stuffs for mature animal | 100 | FDA |
| Zearalenone | Unprocessed cereals other than maize | 100 | EU |

EU: Europena Union. FDA: Food and Drug Administration.

*et al.*, 2014). According to the EC, maximum permitted level for total AFs in feed stuffs used for animal and poultry is 50 μg/kg while this limitation in Turkey is 100 μg/kg in feeds for ruminant, swine. For immature animal and poultry feeds, the limit is 50 μg/kg (EC, 2006; Oruc *et al.*, 2006). Limit of AFB1 for mature animal feed materials is 20 μg/kg, the same value as EC (EC, 2006; Arslan and Essiz, 2009). Up to date, the major source of food and feed all over the world is cereal grains. As a result of their health implications and increasing knowledge of health hazards, regulations for major mycotoxins in commodities exist in at least 100 countries. Limitation of some authorities are summarized in Table 3 (Egmond and Jonker, 2003; EC, 2006; EC, 2007; Cheli *et al.*, 2014).

**Detection of mycotoxins in food and feed**

The growing concern over food and feed safety has led to development of several methodologies for mycotoxins detection. Toxicity of mycotoxins may occur at very low concentrations, therefore sensitive and reliable methods for their detection are required. Proper sampling, homogenization, extraction, and concentration of samples are generally the most common steps in many analytical procedures. Detection methods can be categorized into qualitative and quantitative (Berthiller *et al.*, 2005; Trucksess and Diaz-Amigo, 2011). Thin layer chromatography (TLC) methods can be used as a preliminary test for AFs, ZEN, and ochratoxin, but for fumonisins and some member of the trichothecenes it is not a useful method (Zinedine *et al.*, 2007; Bryden, 2011). Recently, for a rapid specific screening determination of mycotoxin type, immunological methods such as enzyme-linked immunoassay (ELISA) and radioimmunoassay (RIA) are the best approaches because they depend on specific antibodies beside their relatively low cost, easy application and their results could be comparable with those obtained by other conventional methods such as TLC and high-performance liquid chromatography (HPLC) (Fung and Clark, 2004; Zheng *et al.*, 2006; Berthiller *et al.*, 2007).

There is no doubt that correct detection needs a correct extraction and clean-up methods, these include liquid-liquid extraction, supercritical fluid extraction, and solid phase extraction (Turner *et al.*, 2009). Several chromatographic techniques are used as quantitative methods for a massive number of samples; high-performance liquid chromatography (HPLC), gas chromatography mass spectrometry (GC-MS) or liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS-MS), capillary electrophoresis, and fluorometric assay (Zheng *et al.*, 2006; Berthiller *et al.*, 2007; Turner *et al.*, 2009). The most appropriate analytical method differs according to the nature of detected mycotoxin, e.g., for AFs, ZEN, OTA, HPLC-fluorescence andLC-MS/MS are commonly used, while for trichothecenes, GC-MS is mainly preferred (Saeger *et al.*, 2003; Berthiller *et al.*, 2005; Zollner and Mayer-Helm, 2006; Njobeh *et al.*, 2012; Waskiewicz *et al.*, 2012; Seo *et al.*, 2013).

**Prevention**

Elimination of mycotoxins is the main goal of food and feed safety programs. Unfortunately, mycotoxins are very stable compounds and their detoxification during food and feed processing is difficult. Therefore, their contents in food and feedstuffs must be controlled to a minimum level. Strict regulations are important to decrease the risk of mycotoxin contamination which can be achieved by rigorous monitoring including strategies to decrease the mycotoxin production in feed stuffs before and after harvest, and systematic protocols to decrease their exposure and modulate the metabolism of the toxins to reduce toxicity. This requires a better understanding of the ecology of mycotoxin producing organisms, animal production regimes, and feed chain systems (Jarday *et al.*, 2011; Trucksess and Diaz-Amigo, 2011; McCormick, 2013; Womack *et al.*, 2014).

Pre-harvest precautions include efficient agricultural practice which involves; the wise use of fungicides and insecticides to prevent fungal and insect invasion; irrigation to avoid moisture stress; harvesting of plants in maturity when moisture content is lowest. Improvement of plant genes to resist fungal attack is based on genetic engineering, effective breeding programs, and using of biocompetitive fungi (Bryden, 2011; Jarday *et al.*, 2011; Trucksess and Diaz-Amigo, 2011).

A novel way has been used, in which non-toxigenic fungi are cultivated in the field to substitute naturally occurring toxic fungi. This approach gives considerable results for aflatoxins in some agricultural products, for instance, peanuts and maize. Ultimately, a combination of strategies using biocompetitive fungi and enhancement of host-plant resistance may be needed to adequately prevent mycotoxin contamination in the field. There are two important parameters in controlling the fungal activity in stored agricultural stuffs, temperature and moisture which are affected by geographical location and other circumstances such as drying, aerating, turning of the grains and transport. A direct link between mycotoxin contamination and improper post-harvest storage conditions has long been recognized. Therefore, it's important to keep storage equipment and transporters free of insect and other vector activities, water condensation, and water leakages to prevent fungal invasion (Cleveland *et al.*, 2003; Trucksess and Diaz-Amigo, 2011; Womack *et al.*, 2014).

A variety of chemical, biological, and physical approaches have been developed to control mycotoxin contamination as shown in Table 4. Moreover, many studies have been carried out on adsorbent materials, organic and inorganic binders. These compounds are added to the feed to bind the toxin during digestion process resulting in reduction of toxin bioavailability. Examples for inorganic adsorbents are bentonites, zeolites, diatomaceous earth, clays, modified clays, and activated charcoal. For organic adsorbents different substances have been examined such as fibers from plant sources like alfalfa, oat fibers, extracted cell wall fraction of *Saccharomyces cerevisiae* and recently, beta-D-glucan fraction of yeast cell wall. These dietary additives offer one of the greatest potentials for preventing toxicity in a stable digestive tract where

Table 4. Detoxification or degradation of some mycotoxins.

| Process | Effect | References |
|---|---|---|
| *Chemical process* | | |
| Ozone | Total degradation of ZEA. No detected ZEA orZEA-like products. | McKenzie *et al.*, 1997 |
| | Reductions of 80% and 93% AFB1 | Inan *et al.*, 2007 |
| Ammonium hydroxide | Decrease fumonisin B1 content by 30-45% | Norred *et al.*, 1991 |
| Bisulfite solutions | At 80°C for 18h can convert 85% of DON into a DON-sulfonate conjugate | Young *et al.*, 1987 |
| Ammonium persulfate | Reduction of AFB1 by 53-87% | Burgos-Hernandez *et al.*, 2002 |
| Ammonia | At 40-50°C for 48 h decrease AFB1 from 1280 ppb to 10 ppb | Chen *et al.*, 2013 |
| $NaNO_2$ | Deamination of fumonisin B1 | Lemke *et al.*, 2001b |
| HCl | Reduce AFB1 levels by 19.3% within 24 h (pH 2.0) | Doyle *et al.*, 1982 |
| *Physical process* | | |
| Activated carbon | Binding 100% ZEA (pH 3 and 7.3) | Bueno *et al.*,2005 |
| Activated carbon | Reduction of AFM1 (76%) | Rao and Chopra, 2001 |
| Sodium bentonite | Reduction of AFM1 (67%) | |
| Bentonite, diatomite and zeolite | Binding of 95% AFB1 (pH 3.0 and 6.9)<br>Binding of 25% DON in case of diatomite and 50% DON for the others (pH 3.0)<br>Binding of 12.2% to 37% ZEA (pH 3.0 and 6.9)<br>Binding of 16.7% to 33.33% T-2 toxin (pH 3.0 and 6.9) | Bocarov-Stancic *et al.*, 2011 |
| Diatomite | Binding of 66.67% OTA (pH 3.0) | |
| Sorting and/or washing | Efficient with respect to Fusarium sp. | Fandohan *et al.*, 2005 |
| Thermal treatment | Fumonisin B1 and B2 losses exceed 70% in maize after heating at 190°C for 60 min and reached 100% when heated at 220°C for 25 min | Scott & Lawrence, 1994 |
| Dehulling | Induced a 48% reduction of DON and ZEN levels | Fandohan *et al.*, 2005 |
| Modified clays | Effective in sorbing the estrogenic ZEN | Lemke *et al.*,2001a |
| UV | Reduction of total aflatoxins by 25% | Isman and Biyik, 2009 |
| Gamma irradiation | A dose 5 kGray inactivates the growth of Fusarium & mycotoxin formation in seeds | Aziz and Moussa, 2004 |
| | Reduction of total AFs by 34-40% and AFB1 by 33-43% | Herzallah *et al.*, 2008 |
| Microwave | Reduction of total AFs by 21-33% and AFB1 by 23-32% | |

Table 4. Contd. …

Table 4. Detoxification or degradation of some mycotoxins. Contd. …

| Process | Effect | References |
|---|---|---|
| *Biological process* | | |
| Mixed bacterial culture | Total degradation of ZEA. No detected ZEA orZEA-like products. | Megharaj *et al.*, 1997 |
| Trichosporon mycotoxin ivorans | Degradation of ZEA to non-toxic metabolites | Molnar *et al.*, 2004 |
| *Lactobacillus rhamnosus* strains (GG and LC-705) | 80% AFB1 removed | El-Nezami *et al.*, 1998 |
| *Lactobacillus plantarum* strain-102 | Reduction of DON and T-2 toxins by physical binding and biotransformation | Zou *et al.*, 2012 |
| F420-dependent reductases (FDR-A and FDR-B) | Reduction of total AFs through biotrans-formation of *Mycobacterium smegmatis* | Lapalikar *et al.*, 2012 |
| Aspergillusniger | Degradation of OTA by lipase | Abrunhosa *et al.*, 2010 |
| Gram negative bacterium ATCC 55552 | Deamination of fumonisin B by aminotransferase | Heinl *et al.*, 2011 |

the bounded toxins can be excreted via urine or feces. Additionally, other physical approaches such as washing with water or sodium carbonate, dehulling, sorting of contaminated grains, heating at high temperature, milling, and irradiation treatment (UV, X-rays or microwave irradiation) have been employed to reduce mycotoxin contamination post-harvest (Young *et al.*, 1987; Fandohan *et al.*, 2005; Jouany, 2007; Isman and Biyik, 2009; Bocarov-Stancic *et al.,* 2011).

Various chemicals (acids, bases, oxidizing agents, different gases) have been examined for detoxification of mycotoxins, but most of them cause reduction in the nutritive value and palatability of the feed. Generally, they are very expensive and time consuming due to their need for additional cleaning treatments. Furthermore, toxic by-products may be produced. Many organic acid compounds, especially propionic acid, inhibit fungal growth and form the core stone of many commercial antifungal agents used in animal feed industry. Extensive research has been done to consider ozonation as a practical method for decontamination of mycotoxins, especially aflatoxins (Norred *et al.*, 1991; McKenzie *et al.*, 1997; Burgos-Hernandez *et al.*, 2002; Inan *et al.*, 2007; Jouany, 2007; Womack *et al.*, 2014).

Biological approach for mycotoxins decontamination by using microorganisms such as Eubacterium and certain types of isolated yeasts have been used successfully for the management of mycotoxins in particular aflatoxins and ochratoxin A in food as well as animal feeds. Recent studies indicate that molecular approaches may offer insight into the interactions of mycotoxin-producing fungi and other organisms including mycotoxin-degrading microbes (El-Nezami *et al.*, 1998; Molnar *et al.*, 2004; Abrunhosa *et al.*, 2010; Zou *et al.*, 2012; McCormick, 2013). However, none of the approaches

individually fulfills the required efficacy, safety and cost needed for the removal of mycotoxins from contaminated agricultural products. Under all circumstances, detoxification processes should eliminate or inactivate mycotoxins, generate no toxic products, guarantee the nutritional value of the feed and induce no modification to the technological properties of the product.

## CONCLUSION

Despite all the efforts to prevent mycotoxin contamination and related outcomes, outbreaks of mycotoxicosis or untoward effects due to mycotoxin exposure are still possible. Not only human but animal exposure should also be considered by authorities. Awareness of mycotoxin properties, limiting their presence in the environment, preventing exposure above toxic levels will help to maintain both human and animal welfare. Countries should have their own national policies and limits to save public health from toxic outcomes.

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