CHAPTER

Mycotoxins

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Introduction

Mycotoxins are fungal metabolites which when ingested, inhaled, or absorbed through the skin can cause disease or death in humans and domestic animals, including birds. By general agreement this definition excludes the toxins produced by macrofungi (the mushrooms), and compounds that cause disease only in plants or lower animals such as insects. Fungi produce a large number of metabolites, but only a few are classified as mycotoxins, i.e., they have been demonstrated to cause illness. Specific mycotoxins are produced only by specific fungi, usually by only a few species

Despite a few excellent studies of disease caused by fungi in feeds and foods in the first half of the twentieth century, the significance of mycotoxins in human and animal disease came only more recently. The term "mycotoxicosis" was first used in 1952, in a study of animal disease [1]. However the discovery of aflatoxins, following the deaths of 100,000 young turkeys in the UK in 1960, was the start of modern mycotoxin research. Over the next few years, laboratory and field experiments showed that many common fungi that cause both food spoilage and plant disease are able to produce a vast array of more or less toxic metabolites. Molecular structures of mycotoxins vary widely (Figure 30.1), so their effects on human and animal health also vary widely. The most commonly induced diseases include liver cancer, kidney failure, and effects on the brain or nervous system. Perhaps the most important point is that acute toxicity is rare: toxicity due to mycotoxins is almost always insidious, without any overt indication of effects on health in the short term. For this reason, the health effects of mycotoxins are among the most neglected areas of medical science.

It is generally agreed that the most important mycotoxins are: aflatoxins, ochratoxin A, fumonisins, deoxynivalenol, and zearalenone [2]. Each of these will be dealt with below.

Aflatoxins

Health effects

Aflatoxins are the most important mycotoxins, both because they are of common occurrence and because aflatoxin B_1 is the most powerful liver carcinogen known.

Foodborne Infections and Intoxications.

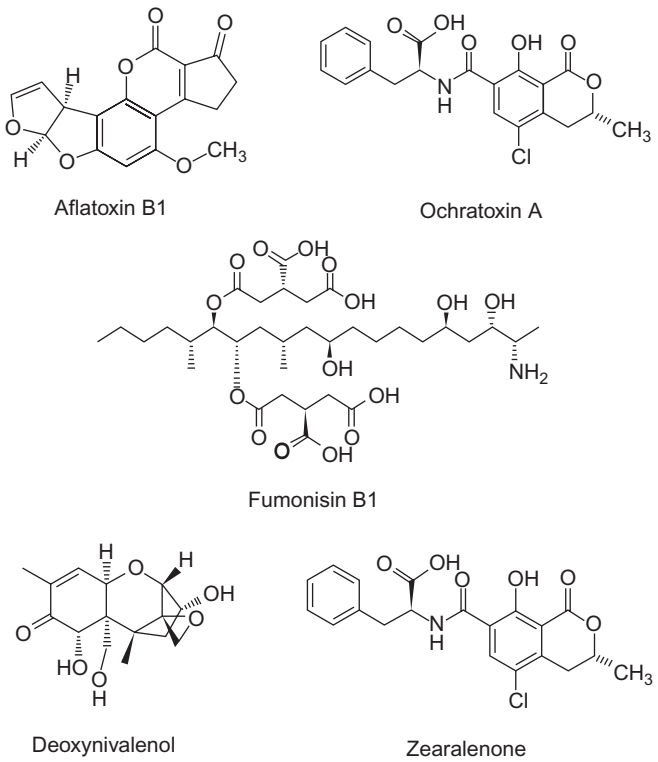

FIGURE 30.1

Structures of some major mycotoxins: aflatoxin B_1, ochratoxin A, fumonisin B_1, deoxynivalenol, and zearalenone.

The International Agency for Research on Cancer lists aflatoxin B_1 and naturally occurring mixtures of aflatoxins as Class 1 carcinogens, i.e., they are recognized as carcinogenic to humans. Hepatitis B virus also causes human liver cancer. In its risk assessment of aflatoxins, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has derived two potency factors for cancer formation by aflatoxins: for aflatoxin alone, 0.01 cases per 100,000 people per annum per ng kg^{-1} body weight per day, and for individuals carrying hepatitis B infection, 0.30 cases. Thus the two agents together are synergistic, indeed about 30 times as potent as aflatoxin alone [3]. Estimates of deaths due to aflatoxin ingestion range up to 100,000 or more per annum worldwide [4].

Aflatoxins are toxic in other ways as well. The acute disease (aflatoxicosis) is rare, with the most recent cases being from Kenya where several hundred deaths were reported in 2004 [5]. Evidence now exists that aflatoxin exposure before birth and in early childhood is associated with stunted growth—defined by WHO as height for age being more than two standard deviations below average height for

age in a given population. Aflatoxins have also been shown to suppress the cell-mediated immune response in both cell lines and domestic animals. Few studies have been reported in humans, but it is apparent that if the effects in humans mirror those in animals even approximately then the immunosuppressive effects of aflatoxins also have very wide implications for human health [6].

Fungal species producing aflatoxins

The major species producing aflatoxins are *Aspergillus flavus, A. parasiticus*, and *A. nomius*. *A. flavus* produces only B aflatoxins (B_1 and B_2), and some isolates also produce the lesser mycotoxin cyclopiazonic acid (CPA). About 40% of *A. flavus* isolates from nature produce aflatoxins. As well as B aflatoxins, *A. parasiticus* produces G aflatoxins (G_1 and G_2), but not CPA, and almost all isolates are toxigenic. *A. nomius* is morphologically similar to *A. flavus* but, like *A. parasiticus,* produces B and G aflatoxins, without CPA.

At least 10 other *Aspergillus* species are known to produce aflatoxins but have little or no significance in food crops, except *A. minisclerotigenes*. This species produces both B and G aflatoxins and also CPA but otherwise is distinguished from *A. flavus* with difficulty.

A. flavus is of universal occurrence in food crops in the tropical and warm temperate zones of the world but is especially associated with peanuts, maize, and cottonseed. Under conditions of drought stress, slow drying, or inadequate storage, aflatoxins are often produced at dangerously high levels in these commodities. *A. flavus* also infects tree nuts, usually as the result of insect damage or inadequate drying and storage, and less commonly rice, oilseeds, and other food commodities [7].

A. parasiticus has a more limited geographical range than *A. flavus*, being rare in Southeast Asia at least. It is associated with peanuts and is relatively uncommon in other food commodities [7]. *A. nomius* is not commonly found in foods but has been shown to be a major source of aflatoxin in Brazil nuts [8]. *A. minisclerotigenes* is common in peanuts in the Southern Hemisphere.

Aflatoxin formation in crops and its control

Unlike other mycotoxins discussed here, aflatoxins can be produced in nuts or kernels of susceptible crops throughout production—pre-harvest, during drying, and in transport and storage [7]. In peanuts, maize, and cottonseed, *A. flavus* is commensal, i.e., it grows in the plant and developing nut or kernel without any apparent damage to the plant.

Good agricultural practice (i.e., adequate moisture, weed control, and crop rotation) is usually considered to be the best method of aflatoxin control. Irrigation is effective; however peanuts are a drought-resistant crop and so are mostly grown under dry culture. Harvesting early can reduce contamination, while rapid mechanical drying of peanuts has a major effect in reducing the levels of aflatoxins. Insects are often involved in infection in maize, so the use of Bt maize cultivars is beneficial.

Entry of *A. flavus* into pistachio nuts depends when hull splitting occurs. Nuts in which hull splitting occurs early are more susceptible to *A. flavus* invasion. Some cultivars are more prone to early splitting than others, and this is especially important where nuts are harvested from the ground.

Figs are sometimes infected by *A. flavus* because of their unique structure developed for insect fertilization and because in some countries figs are harvested from the ground. Immature figs are not colonized by *A. flavus*, but once they are ripe, infection occurs readily and fungal growth continues during drying.

Biocontrol

Biocontrol of the type called competitive inhibition has performed well in cottonseed and peanuts in the USA, and is being extended to use in maize. In this technique, high numbers of spores of a non-toxigenic strain of *A. flavus* are introduced into fields where a susceptible crop is being grown, where they compete against the naturally occurring toxigenic spores for invasion sites on seeds, nuts, or kernels.

Control of aflatoxins in stored commodities

The prime consideration for storage of food commodities is the maintenance of sufficiently low water availability, i.e., water activity below 0.65, to prevent fungal growth during storage. That corresponds to 8% moisture for peanuts and other nuts, 12% for grains, and 22% for raisins, which contain a higher level of soluble carbohydrate [9].

Reducing aflatoxin by processing

Sorting is the main method used to reduce aflatoxins in most crops. Removal of discolored or damaged grains or nuts, mechanically or by hand, will decrease toxin content in the remaining sound commodity. In peanuts, color sorting was developed originally to reject discolored nuts, so as fungal growth is a cause of discoloration the process is an effective, non-destructive method for reducing aflatoxin levels. Blanching to remove skins and roasting to increase discoloration improves color sorting.

Maize samples are sorted by ultraviolet light, but the technique requires cracking of the grain, so it cannot be used for sorting individual kernels. Figs are sorted individually by UV light. Sieving of contaminated maize reduces both aflatoxin and fumonisin. No effective, non-chemical testing techniques exist for cottonseed or pistachios and, as with other commodities, non-destructive chemical assays are not available.

Aflatoxins are destroyed to some extent during heat processing. Destruction varies from less than 25% in boiling water, during extrusion or autoclaving, to up to 80% in dry roasting [10]. The alkali process used to produce refined table oil completely removes aflatoxin.

Ochratoxin A

Health effects

Ochratoxin A (OTA) is a chronic nephrotoxin, affecting kidney function. OTA has a long half-life in the bloodstream, so that in areas where OTA is common in

foods, the blood of healthy humans regularly contains detectable amounts of this toxin. OTA also has carcinogenic properties, but the mechanism of carcinogenicity remains unknown. The carcinogenic effects in animals are considered to be of less importance than the nephrotoxicity. Although OTA is demonstrably toxic to animals of all kinds, its effects in humans remain unclear and the subject of debate. Both genotoxic and non-genotoxic modes of action have been proposed [3]. The International Agency for Research on Cancer has classified OTA as a possible human carcinogen (Group 2B), based on sufficient evidence of carcinogenicity in experimental animal studies and inadequate evidence in humans.

Fungal species producing OTA

The ecology of OTA formation in foods and feeds is more complex than that of aflatoxin, because OTA is formed by both *Aspergillus* and *Penicillium* species, with differing—and quite specific—ecological niches. The major commodities susceptible to OTA production can be divided into warm and cool temperate crops. In warm climates, the foods most likely to contain OTA are coffee, dried vine fruits, and wines, while in cool climates cereals are the main crop affected.

In cool temperate climates, ranging across Northern and Central Europe, Canada, and Northern Asia, OTA is produced in cereal crops by *Penicillium verrucosum* [11]. As a consequence, in these regions OTA is found in products such as bread and flour-based foods, and in the meat of animals which are fed cereal grains. It should be noted that *P. verrucosum* is unable to grow above 30 °C and is not found in warmer climates, so small grains from tropical and subtropical areas rarely contain OTA [7].

One group of *Aspergillus* species producing OTA is centered on *A. ochraceus.* This species grows at low water activities and is quite common in stored food commodities. Until 2004, it was considered to be the major source of OTA in coffee [12]. Advances in molecular and chemical techniques resulted in the splitting of *A. ochraceus* into three species [13], and *A. westerdijkiae* is now recognized as the main OTA producer in coffee.

The second group of *Aspergillus* species making OTA centers on *A. carbonarius.* This species grows at quite high temperatures and has dark hyphae and spores, so it is resistant to UV light and sunlight. These characteristics provide a competitive advantage in vineyards and grape drying yards. Discovered to be a source of OTA only a decade ago, it is now recognized as the primary source of OTA contamination in grapes and grape products [14]. Grapes are dried in the sun without preservatives, so dried grapes (raisins, sultanas) may contain unacceptable levels of OTA [15]. *A. carbonarius* also produces OTA in coffee from some producing regions [12].

A low percentage of isolates of *A. niger*, a species closely related to *A. carbonarius*, are also able to produce OTA. However, in addition, Frisvad et al. [16] reported that *A. niger* can also produce fumonisins, previously regarded as produced exclusively by *Fusarium* species. Fumonisins produced by *A. niger* have been found in raisins and coffee.

Control of OTA formation in crops

OTA formation in foods is usually a post-harvest problem, associated with slow drying. However, *A. carbonarius* infects grapes before harvest as the result of damage by pathogenic fungi or rain [17]. OTA occurs in wines throughout the world, but levels are usually low, as the fermentation process positively stops growth of the fungus. Populations of *A. carbonarius* in vineyards can be reduced by irrigation and good agricultural practices [17].

Both *A. westerdijkiae* and *A. carbonarius* can infect coffee cherries if drying is too slow—a common problem in the misty upland areas where coffee is grown. Good sun drying or a combination of sun drying and mechanical dehydration provide effective control of OTA in coffee [12].

Reducing OTA by processing

OTA is largely removed during the winemaking process as it is bound to solid fractions, sediment, and some fining agents. The carryover from grapes into finished wine is between 1 and 8% [18].

OTA is partially destroyed during coffee roasting, instant coffee production, and decaffeination [19].

Fumonisins

All *Fusarium* species grow only at high water activities, above about 0.90 [7]. Production of *Fusarium* mycotoxins occurs only during growth of the fungus in the living plant and seed, or during early stages of drying.

Health effects

Fumonisins are remarkable for the wide range of effects they cause. Fumonisins inhibit the enzyme ceramide synthase, which causes accumulation of intermediates in the sphingolipid metabolism pathway and depletion of complex sphingolipids. These effects interfere with the binding of folate and some other proteins in cell membranes. The most dramatic effect occurs in horses, in which the disease called equine leucoencephalomalacia occurs. This is a rapidly progressing disease that causes equine brains to liquefy. In pigs, fumonisins cause pulmonary oedema, due to left ventricle heart failure, while in rats the primary effect is to cause liver cancer; programmed cell death (apoptosis) also occurs [20].

In humans, fumonisins produced by *F. verticillioides* cause none of these animal syndromes but are associated with esophageal cancer. Extensive studies in areas of low and high maize consumption in South Africa have suggested this connection. This disease is also prevalent in areas of China and occurs at significantly high levels in parts of Iran, northern Italy, Kenya, and a small area of the southern USA. In all of those areas consumption of maize and maize products is very high [20].

There is also some evidence that high intakes of fumonisins from maize are associated with neural tube defects such as spina bifida in areas of Guatemala, South Africa, and China and in a population along the Texas–Mexico border [20].

Fungal species producing fumonisins

Fumonisins are produced by *F. verticillioides* and the closely related species *F. proliferatum*. These species are endemic in maize worldwide and are endophytic in the plants [21]. Under conditions of water or insect stress, the symptomless endophytic relationship may convert to a disease and/or mycotoxin-producing interaction [22]. Fumonisins produced by these species are found only in maize and sorghum, as these species rarely infect other crops.

Control of fumonisin formation in crops

Good agricultural practice, the control of insects, development of resistance to ear diseases, and development of cultivars adapted to drought and temperature tolerance are all important in reducing the risk of fumonisin and other *Fusarium* toxin accumulation in maize. Some progress has been made in breeding cultivars resistant to ear rot [21,22].

Reducing fumonisins by processing

Sorting and cleaning reduce fumonisins in maize by removal of broken and damaged kernels. Milling does not destroy fumonisins, but they are concentrated in bran and germ rather than flour. Significant reductions of fumonisin levels occur during processes at temperatures >150 °C, as in maize meal production, frying chips, and extrusion processing, which is used extensively in the production of breakfast cereal and snack and textured foods [23].

Nixtamalization is a centuries-old process used in Central America, in which maize is soaked and then cooked with ash or lime high in alkali. It removes almost all fumonisins, resulting in tortillas and other maize-based foods being substantially free of these mycotoxins.

Deoxynivalenol (DON)

Health effects

The main risk from DON for humans is gastrointestinal poisoning. In one episode in India, DON levels in wheat ranged from 0.4 to 8.4 mg/kg, while in China, poisoning was linked to wheat contaminated with 0.3–100 mg/kg DON. Consequently, it has been suggested that acute toxicity may occur from exposures in the low mg/kg range. No other risks to human health from DON in foods have been identified.

Fungal species producing DON

Fusarium graminearum (often reported as *Gibberella zeae*—its sexual stage) and *F. culmorum* produce the trichothecene toxins deoxynivalenol and nivalenol and also the estrogenic mycotoxin zearalenone. These species are plant pathogens, invading maize, wheat, and barley plants, and causing diseases, known as Gibberella ear rot and Fusarium head blight, in developing grain. These diseases are prevalent in north temperate climates, especially in wet years, and are much less common in the tropics [21].

Control of DON formation in crops

Some success has been achieved in controlling DON formation in wheat by the use of azole fungicides at anthesis. Forecasting systems to advise farmers of the likelihood of DON formation have been developed in Canada and Europe [24]. Otherwise, control relies on reducing levels of *Fusarium* species in the field by good management and crop rotation.

Reducing trichothecenes in processing

Sorting and cleaning will reduce levels of trichothecenes in grains.

Zearalenone

Health effects

Zearalenone has a low acute toxicity, with oral 50% lethal doses in rats and chickens exceeding 2 g/kg body weight. However, much lower levels of zearalenone and its metabolites possess estrogenic activity in experimental and farm animals. The most obvious problems are seen in pigs: doses as low as 1–5 mg/kg can induce vulvovaginitis and vaginal and rectal prolapse in young female pigs. Sheep and cattle are more resistant, and it seems likely that rumen microorganisms are responsible for metabolizing zearalenone to compounds of lower toxicity. Chickens are also comparatively resistant, tolerating up to 30 mg/kg of zearalenone in feed.

Fungal species producing zearalenone and control

Zearalenone is produced by the same fungi that produce DON, in the same crops, and usually at the same time. The ecology of zearalenone production mirrors that of DON, at least in general terms. In consequence, occurrence and control are similar.

Methodology in mycotoxin detection

Mycotoxins constitute a very heterogeneous group of substances, so extraction and detection methods vary widely.

Mycotoxins are usually very unevenly distributed in commodities. For that reason, sampling is often the largest source of error in mycotoxin assays. Sampling plans have been developed for many commodities, especially for aflatoxins: for continuous lines, for 10 ton lots, and for bag stacks. Relevant papers should be consulted for more information. For example, the Codex Alimentarius Commission has developed sampling plans for various toxins and commodities.

It cannot be overemphasized that sample sizes should be as large as possible, because of the uneven distribution of toxins. For example, for aflatoxin in peanuts, entire samples of 8 kg or more should be comminuted in a vertical chopper or similar mill. Subsamples should then be further processed.

Extraction of mycotoxins from subsamples employs a variety of mixed polar and non-polar solvents, depending on the food matrix being analyzed. Methanol:water (80:20) is now the most commonly recommended, as it is non-toxic and does not interfere with immunological assays. For analysis, thin layer chromatography (TLC) is the oldest and most versatile method. It has been superseded in industrial countries by high performance liquid chromatography (with ultraviolet, mass spectrometry, or fluorescence detection), gas chromatography (with electron capture or flame ionization detection), or mass spectrometry and liquid chromatography tandem mass spectrometry [25]. However, TLC remains a cheap and versatile technique of great value in less industrialized nations.

Enzyme-linked immunoassay (ELISA) technology has found widespread application for the detection of mycotoxins in foods. ELISA has advantages of rapidity, accuracy, and sensitivity, and requires no complex equipment. However, immunoassays require antibody preparations, which are relatively unstable and usually require refrigeration.

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