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Occurrence and Decontamination of Mycotoxins in Swine Feed

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ABSTRACT : Contamination of agricultural crops by mycotoxins results in significant economic losses for grain producers and, when consumed, it can cause reduced growth and health in a wide range of animal species. Hundreds of mycotoxin producing molds exist, however each has a different frequency and pattern of occurrence, as well as differences in the severity of the diseases (mycotoxicoses) they cause. Among the mycotoxins considered to be major contaminates are aflatoxin, deoxynivalenol, fumonisin, ochratoxin, and zearalenone. Although a multitude of species can be harmed by consumption of these mycotoxins, swine appear to be the most commonly affected commodity species. The swine industry can thus experience great losses due to the presence of mycotoxin contamination in feeds. Subsequently, recognition and prevention of mycotoxicoses is extremely important and dependent on adequate grain sampling and analysis methods pre-harvest, as well as effective strategies post-harvest to reduce consumption by animals. The aim of this review is to provide an overview of the major mycotoxin contaminants in grains, to describe methods of analysis and prevention to reduce mycotoxicoses in swine and other animals, and finally to discuss how mycotoxins directly affect swine production. (**Key Words :** Aflatoxin, Deoxynivalenol, Feed Additives, Mycotoxins, Swine)

INTRODUCTION

Mycotoxins are secondary metabolites of fungi that have toxic properties and are commonly found in cereal grains (Binder et al., 2007). These toxins appear to have no biochemical significance on the growth or development of the mold, and their production is ubiquitous throughout the United States and around the world (Hussein and Brasel, 2001). Growth of the fungus can occur under a variety of environmental, temperature, and moisture conditions both pre- and post-harvest (Binder et al., 2007). Ingestion of mycotoxins can result in decreased growth and productivity, organ damage, and immune suppression (CAST, 2003). Many are considered carcinogenic, and high exposure is fatal. Though there are more than 300 known mycotoxins, only a small number are relevant in the feed industry (Binder et al., 2007). However, these few are some of the most common and harmful, and are likely to occur together to cause multiple exposure. These major mycotoxins include aflatoxin (AF), deoxynivalenol (DON), fumonisin (FUM), ochratoxin (OCH), and zearalenone (ZEA) (Huwig at al., 2001; Richard, 2007; Marasas et al., 2008).

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Mycotoxin contamination greatly affects the health and economic stability of many farm industries, including swine production. The United States Food and Drug Administration (FDA) has estimated that the annual cost of crop losses due to mycotoxins can total $932 million, with research and monitoring adding another $500 million to a total of $1.5 billion (CAST, 2003; Marasas et al., 2008). Production losses for swine farmers can also be severe, as losses can soar well over $100 million annually (Hussein and Brasel, 2001). Mycotoxin contamination of feed is important to consider in swine production, both for economic reasons and for maintaining the health and productivity of pigs. Thus the aim of this review is to provide a description of the major mycotoxin contaminants in grains, to describe methods of analysis and prevention that can reduce mycotoxicoses, and to discuss how mycotoxins relate directly to the swine industry.

MAJOR CLASSES OF MYCOTOXINS

Aflatoxin

Aflatoxin (AF) is a common mycotoxin which can infect a wide range of crops and subsequently impact numerous animals. This toxin is produced by several different species of the fungus *Aspergillus*, including *A. parasiticus*, *A. nomius*, *A. pseudotamarii*, and the most

common form, *A. flavus* (CAST, 2003; Dersjant-Li et al., 2003). *A. flavus* was reported to cause moldy corn as early as 1920, but it was not until the 1960s that it was noted to be a dangerous fungus after being linked to Turkey X Disease (Diener et al., 1987; CAST, 2003; Agag, 2004; Richard, 2007). This disease caused immense losses of poultry in England after consumption of AF contaminated grains. Thereafter, the toxin produced by the fungus *A. flavus* was named aflatoxin, and the four different toxin components were identified, including B1, B2, G1, and G2. These toxins are differentiated based on their blue (B1 and B2) or green (G1 or G2) fluorescence on grains under ultraviolet light (Agag, 2004). Two other metabolites, M1 and M2, can be produced in animal tissues and fluids (such as milk) after they are hydroxylated from AF B1 and AF B2 (CAST, 2003; Dersjant-Li et al., 2003; Richard, 2007). Of these 6 forms, AF B1 (Figure 1) is considered to be the most toxic after both acute and chronic ingestion.

Normally, the fugal strains that produce AF reside in soil as saprobes, but can be transmitted to plant tissues when conditions are favorable (Gourama and Bullerman, 1995). Growth, which is highly dependent on environmental conditions, can occur in two distinct phases: infection of the developing crop and contamination after maturation and harvest (Cotty and Jaime-Garcia, 2007). The infection process by *A. flavus* is best described in corn, where the fungi is first carried from the soil it originally inhabits to the plant by wind, insects, or birds (Payne, 1998). Naturally occurring *A. flavus* first infects the corn silk and then moves onto the kernels from the top of the cob to the base (Diener et al., 1987). Wounding of the plant tissue by insects or birds will interfere with the kernel seed coat and increase entry of the fungal organism (CAST, 2003; Agag, 2004; Cotty and Jaime-Garcia, 2007; Richard, 2007). Heat or drought stress to the host plant will also increase infection rates, as well as when the kernel moisture is between 15 and 32% (CAST, 2003).

After kernel maturation and harvest, there is a second chance for contamination with AF. Fungal growth is enhanced when grains are stored in warm (20 to 30°C) and damp (>14% moisture) conditions (CAST, 2003; Dersjant-Li et al., 2003; Richard, 2007). Under high humidity, grains that were initially dry can develop suitable water content for *Aspergillus*. Contamination by this method can be most severe if the crop is caught in rain just prior to harvest (Cotty and Jaime-Garcia, 2007).

Aflatoxin contamination occurs most often in the Southern United States, but can also occur throughout the world on many major crops such as corn, cotton, peanuts, seeds, and tree nuts (CAST, 2003; Richard, 2007). Different species of *Aspergillus* may grow in different climates and infect varying hosts. For example *A. parasiticus* thrives in warmer subtropical and tropical regions and commonly contaminates peanut crops, while *A. flavus* is most common in temperate latitudes of 25° to 35° north and south of the equator infecting corn in many of the high cereal producing states of the Southern and Midwestern United States (Gourama and Bullerman, 1995; CAST, 2003). Naturally, AF concentrations in grains can vary greatly. Although individual kernels may contain as high as 400 mg/kg, contamination usually peaks at 1 mg/kg (Dersjant-Li et al., 2003; Richard, 2007).

Absorption of AF occurs through the lining of the intestinal tract, where it then moves into the blood stream and is carried to the liver. During this process, the formation of a reactive epoxide at the 8, 9-position of the terminal furan ring occurs (Agag, 2004). After this bioactivation, AF can bind covalently to nucleic acids, to alter structure and function of proteins, block RNA polymerase and ribosomal translocase. Liver metabolism of AF can also result in the production of the M1 and M2 metabolites which can be incorporated into milk, meat products, and eggs (Agag,

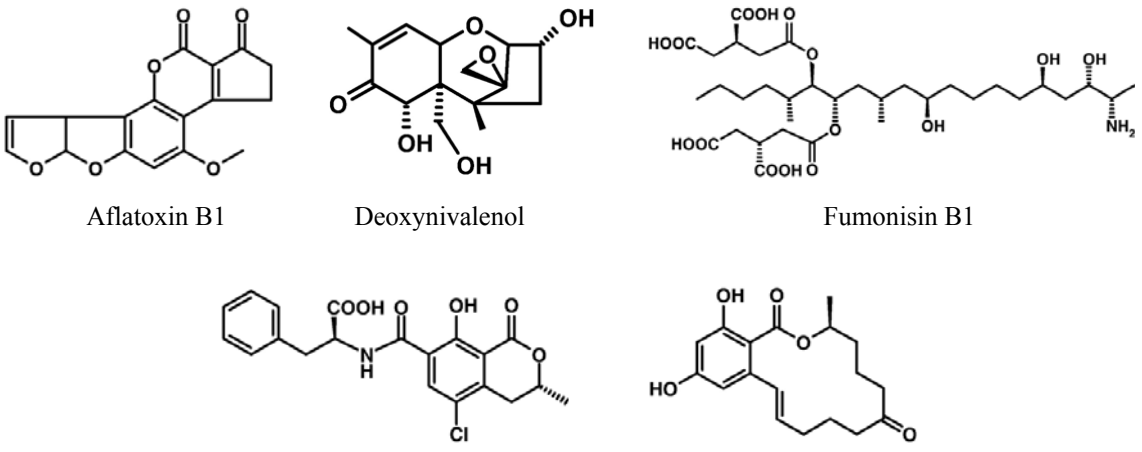

Figure 1. Structures of the major mycotoxins (adapted from Richard, 2007).

2004; Richard, 2007). The AF M1 form excreted in milk is often associated with the milk protein, casein (Agag, 2004). For this reason, cheese products often have 3 to 5 times higher AF M1 than the original milk due to the casein being highly concentrated. Consumption of the M1 metabolite can result in similar growth reduction and tissue damage to the other forms of AF.

Aflatoxin ingestion by animals can result in many problems, including decreased growth rates, liver damage and immune suppression. Decreased immunity is problematic because it can result in a heightened susceptibility to infectious diseases such as coccidiosis, salmonellosis, and candidiasis (CAST, 2003). Aflatoxin is also considered carcinogenic, and can result in death when consumed in high concentrations. Although AF can affect any species at any age, problems are often greater in younger animals and certain species. Pigs are the most susceptible commodity species to AF, but the toxin can also greatly affect rodents, companion animals, poultry, cattle, horses, humans, and some species of fish (Hussein and Brasel, 2001; Agag, 2004; Richard, 2007; Meissonnier et al., 2008; Thieu et al., 2008). Ruminants are generally less sensitive to AF due to the buffering effect of the rumen (Hussein and Brasel, 2001). However, some rumen microorganisms are sensitive to AF, so animal growth, reproduction, and production of meat, wool, and milk can be altered when ruminants consume high concentrations of AF for extended periods of time.

To protect humans and animals from the harmful effects of mycotoxin ingestion, the FDA is responsible for regulating mycotoxin levels in feed ingredients and animal feeds. FDA standards for mycotoxin contamination are determined based on the availability and capability of sampling procedures, analytical methodology, crop health, genetic biotechnology, and available expertise to minimize human and animal exposure. Standards also consider the unavoidability of the mycotoxin (CAST, 2003). To determine the need for regulatory control, extensive data for exposure effects must also be obtained. For AF, action levels are set for the amount of this toxin allowed in grains used for food and feeds (Table 1). Action levels refer to precise levels of toxin allowed before the FDA can take enforcement actions (CAST, 2003). Acceptable levels of toxin in feed can differ across species and between age

Table 1. United States Food and Drug Administration regulations for the major mycotoxins (adapted from Richard, 2007)

Mycotoxin	Commodity and species	Limit[1] (μg/kg)
Aflatoxins (total)	Cottonseed meal as a feed ingredient	300
	Corn and peanut products for finishing beef cattle	300
	Corn and peanut products for finishing swine	200
	Corn and peanut products for breeding swine and beef cattle, as well as mature poultry	100
	Corn for immature animals and dairy cattle	20
	All other feedstuffs	20
	All products designated for humans, except milk	20
	Milk	0.5
Deoxynivalenol	Grain and grain byproducts designated for swine and other animals except cattle and chickens, not to exceed 20% of diet for swine (<40% for other species)	5,000
	Grain and grain byproducts for beef and feedlot cattle older than 4 months, <50% of the diet	10,000
	Finished wheat products for human consumption	1,000
Fumonisins (total)	Corn and corn byproducts for animals	
	Horses and rabbits (<20% of the diet)	5,000
	Swine and catfish (<50% of the diet)	20,000
	Breeding ruminants, poultry, mink, dairy cattle, and laying hens (<50% of the diet)	30,000
	Ruminants less than 3 months before slaughter and mink for pelts (<50% of the diet)	60,000
	Poultry for slaughter (<50% of the diet)	100,000
	Livestock and pet animal species (<50% of the diet)	10,000
	Human food consumption	
	Degermed dry milled corn products	2,000
	Whole or partially degermed dry milled corn	4,000
	Dry milled corn bran	4,000
	Cleaned corn intended for mass production	4,000
	Cleaned corn intended for popcorn	3,000
Ochratoxin	None	
Zearalenone	None	

[1] Limits: action levels for AF; advisory levels for DON; guidance levels for FUM.

groups. Concentrations of AF are allowed in corn at up to 20 μg/kg for ingestion by dairy cattle and immature animals, and 100 μg/kg for breeding animals and mature poultry. Finishing swine feed is allowed to contain up to 200 μg/kg, and beef cattle diets may contain up to 300 μg/kg. The FDA also has an action level of 20 μg/kg for all products designated for human consumption (FDA, 1994; FAO, 2003).

Deoxynivalenol

Deoxynivalenol (DON), the most common member of a group of mycotoxins called the trichothecenes, is produced by strains of fungi in the *Fusarium* family including *F. graminearum* and *F. culmorum* (Hussein and Brasel, 2001). Other trichothecenes include T-2 toxin, diacetoxyscirpenol, and nivalenol (Hussein and Brasel, 2001; Richard, 2007). Although all are slightly different, these 4 trichothecenes are grouped together based on their similar structures containing sesquiterpene rings characterized by a 12,13-epoxy-trichothec-9-ene nucleus (Figure 1). Although each of these toxins are problematic, DON is of significant importance since it commonly contaminates corn, wheat, oats, and barley (Darjant-Li et al., 2003). It is one of the most prevalent mycotoxins in temperate regions of the world such as Europe and North America (Wu, 2007; Meissonnier et al., 2008; Thieu et al., 2008). Deoxynivalenol may commonly co-contaminate with several mycotoxins produced by strains of *Fusarium* fungi, such as other trichothecenes and zearalenone, which form under similar environmental conditions.

Infection of grains by *F. graminearum* can occur when the organism survives on residues left on fields (CAST, 2003; Richard, 2007). When blown by wind or transferred by insects and birds, the previous year's fungus can infect a new crop. Environmental conditions favoring fungi development in the field include cool temperatures and high humidity (Dersjant-Li et al., 2003; Richard, 2007). Formation can also occur after harvest if grains are improperly stored under high moisture conditions. When infection of grains occurs, *F. graminearum* causes the diseases known as ear rot in corn or head blight in small grains such as wheat and barley (Richard, 2007). Deoxynivalenol contamination can be observed when corn kernels ripen prematurely and unevenly, as well as have a blanched appearance. At harvest, kernels may also have a pink coloring. The natural occurrence of DON in grains used for animal feeds is normally between 0 and 5 mg/kg, although concentrations can be higher (Dersjant-Li et al., 2003).

The absorption of DON in the intestinal tract is damaging to the epithelial cells by altering their barrier function (Pinton et al., 2010). As a consequence of DON exposure, an extracellular kinase p44/42 ERK is phosphorylated to its active form. This action results in decreased expression of the tight junction protein claudin-4, which in turn reduces the barrier function of the intestine (Pinton et al., 2010). Once through the intestinal epithelium, DON can enter into cells to effectively inhibit DNA, RNA, and protein synthesis (Hussein and Brasel, 2001; CAST, 2003). When DON binds to polysomes and ribosomes within animal cells, it causes peptide chains to be interrupted due to altered initiation and termination sequences. This will result in altered ribosomal function causing phosphorylation of mitogen-actived protein kinases (MAPKs), which then influence transcription factors in the nucleus. Transcription factor activation can cause increased immune and inflammatory responses in response to immunoglobulin and cytokine stimulation (Pestka, 2007). However, DON can also cause immune suppression when it results in apoptosis (programmed cell death) in tissues such as the thymus, bone marrow, and lymph nodes (CAST, 2003; Pestka, 2007). The result is that this toxin is found to increase an animal's susceptibility toward bacterial and viral contaminants (Fink-Gremmels, 2006).

Deoxynivalenol is shown to alter brain functioning by increasing uptake of the amino acid tryptophan, which will increase concentrations of the neurotransmitter serotonin (Swamy et al., 2002; Cheng et al., 2006). High levels of serotonin may be the link to decreased feed intake and decreased weight gain seen in animals consuming DON. The most commonly known reaction to ingesting grains highly contaminated with DON is vomiting, which is why DON is often referred to as vomitoxin (Richard, 2007). Kidney damage is also a frequent symptom of toxicity by DON (Richard, 2007; Sabater-Vilar et al., 2007; Chen et al., 2008). Despite its many damaging effects, DON is not significantly incorporated into body tissues or fluids when consumed by animals, and thus will not be transferred to humans consuming animal products. Deoxynivalenol that is excreted from the body into the urine or feces is primarily in the form of de-epoxy-deoxynivalenol (DOM-1), which is a nontoxic metabolite due to an altered ring structure (Schatzmayr et al., 2006; Pestka, 2007).

Though many species can be affected by DON toxicity, swine are typically the most sensitive because they exhibit the strongest symptoms after ingestion (Goyarts et al., 2005; Pestka, 2007; Richard, 2007; Wu, 2007). Deoxynivalenol is also of concern for companion animals where it commonly contaminates pet foods. Though not as severe, negative effects of ingesting DON can be seen in other monogastric species such as poultry, rodents, and humans (Hussein and Brasel, 2001). Research has shown that ruminants are not as greatly affected by DON contamination of grains since rumen microorganisms can

transform DON into nontoxic metabolites.

The FDA currently has only advisory levels set for DON, meaning these levels provide guidance for industries and a public health risk is not anticipated below the set concentrations (Table 1). Enforcement of advisory levels is rare, but the FDA reserves the right to take action if the situation warrants enforcement (CAST, 2003). The advisory level is currently set at 10 mg/kg for grains used for beef and feedlot cattle, and at 5 mg/kg for grains used in swine diets (FDA, 1994; FAO, 2003). Though these limits are important for regulation, they may not represent the lower concentration of toxin commonly found in grains which could still affect the health of animals.

Fumonisin

Fumonisins (FUM) are a group of mycotoxins produced by the fungi *Fusarium verticillioides* and *F. poliferatum* (Richard, 2007). Three forms of the toxin that are known to exist include FUM B1, B2, and B3. Fumonisin B1 (Figure 1) is the most common and harmful, with toxicity linked to the long hydrocarbon unit in its structure (Hussein and Brasel, 2001). For the most part, FUM contaminates corn, but it can also be found in other grains such as sorghum and rice (CAST, 2003; Dersjant-Li et al., 2003).

The fungi producing FUM was not discovered until the late 1980s and research has not yet shown the exact conditions needed for disease occurrence (FAO, 2003). It is known that *F. verticillioides* and *F. poliferatum* grow well when crops are subjected to drought stress followed later by warm, wet weather (Richard, 2007). Detection of *Fusarium* is difficult as some grains may show physical damage and others may not. However, on corn, FUM contaminants may appear as a white or pink discoloration of the kernels. Further contamination during storage is not a problem if grains are kept in a low moisture environment. Natural concentrations of FUM in corn can vary greatly, with infection levels often rising to 10 mg/kg (Dersjant-Li et al., 2003).

Fumonisin is involved in diseases of organ systems such as the brain, lungs, kidneys, and liver (CAST, 2003). The primary mechanism of toxicity includes interference of the enzyme N-acyltransferase, involved with sphingolipid metabolism (Hussein and Brasel, 2001; Taranu et al., 2005; Richard, 2007). Sphingolipids, together with phospholipids, are the main lipid components of cell membranes (Murray et al., 2009). The enzyme N-acyltransferace is involved in the formation of ceramide, which is subsequently converted into sphingolipids. Disruption of this pathway will result in damage to important cellular structures and biochemical processes involved in liver functioning (Richard, 2007). The FUM toxin can also affect other biological functions such as protein metabolism and the urea cycle (Hussein and Brasel, 2001). It is a major mycotoxin affecting horses, often causing equine leukoencephalomalacia, a disease which results in softening of white tissue in the brain (Richard, 2007). Fumonisin is also linked to causing cancer in rats, as well as porcine pulmonary edema, a lung disease in swine (Hussein and Brasel, 2001; CAST, 2003; Richard, 2007). Despite its strong effects on internal organs, FUM is not carried over into milk and does not appear to be metabolized into edible tissues.

Fumonisin is regulated in the United States based on guidance levels issued by the FDA (Table 1). Guidance indicates that there are no action levels or enforceable limits of toxin contamination, but rather maximum concentrations that are adequate for protecting health (FDA, 1994; CAST, 2003). The FDA has maximum FUM contamination of corn for human consumption set at 2 to 4 mg/kg depending on the grains used. For corn designated for animal consumption, guidance levels are set at 5 mg/kg for horses and 20 mg/kg for swine (FDA, 1994; FAO, 2003). Ruminants and laying poultry have recommended maximum diet contamination levels set at 30 mg/kg, while feed for meat birds should not exceed 100 mg/kg of FUM.

Ochratoxin

Another significant mycotoxin is ochratoxin (OCH). One of the major toxin compounds in this group is OCH A (Figure 1), which is produced primarily by *Aspergillus ochraceaus* and *Penicillium verrucosum* (CAST, 2003). Ochratoxins are chemically described as containing an amide bond linked to their amino group of L-β-phenylalanine (Hussein and Brasel, 2001). This mycotoxin can infect a wide range of commodities, including grapes, coffee, soy products, and barley (Richard, 2007). Contamination of these particular crops makes OCH an important factor in human health. Another aspect which makes it important for human health is that it can be found in house dust and other airborne particles.

Most crops infected with OCH become contaminated during storage when there is high humidity and warm temperatures, although field contamination is occasionally seen on some crops such as grapes (Richard, 2007). The presence of *Aspergillus ochraceaus* and *Penicillium verrucosum* is difficult to detect since there is no common appearance. Visible mold may be seen on some crops, whereas there is no fungal growth on others (Richard, 2007). A moldy odor may also coincide with contamination of OCH.

Ochratoxin is described as a nephrotoxic mycotoxin, causing kidney damage in exposed individuals (Hussein and Brasel, 2001; CAST, 2003). Swine research on ingestion of this mycotoxin has shown that changes in renal function are caused by impairment of the proximal tubular functions and

altered urine excretion and glucose metabolism (Krogh, 1977). Ochratoxin is also considered a carcinogen in rats, mice, and humans since it is often linked to kidney tumors (Richard, 2007). Balkan endemic nephropathy is one such kidney disease in humans that is caused by OCH (Pfohl-Leszkowicz et al., 2002). Other effects of consuming contaminated feed include decreased growth and feed efficiency, reduced egg production in laying hens and at high concentrations OCH can cause liver damage and mortality (CAST, 2003). Organ damage and accumulation of OCH in tissues is due to its slow excretion rates after entering tissues and body fluids. Despite the wide occurrence of OCH in food and air, there are no regulations for this mycotoxin in the United States since risk assessments have deemed them not necessary.

Zearalenone

Zearalenone (ZEA) is another mycotoxin of practical relevance for the swine industry. This mycotoxin may commonly co-exist with the previously described DON since it is formed by the same fungal organism of *Fusarium graminearum* and *F. culmorum* (CAST, 2003). The chemical structure of ZEA (Figure 1) contains a phenolic resorcyclic acid lactone (Hussein and Brasel, 2001; Richard, 2007). This structure plays a role in ZEA's effects on estrogen signaling within the body.

Zearalenone most commonly contaminates corn but can also be found on wheat, barley, sorghum, and rye (CAST, 2003). Contamination can occur worldwide, with incidence varying by year, crop, and geographical region. When infected, the grain often develops a pink coloring that the fungus produces simultaneously with the toxin (Richard, 2007). Contamination by *F. graminearum* or *F. culmorum* occurs in damp, cool environmental conditions similar to that of the *Fusarium* species that produce DON. Zearalenone more commonly occurs in the field, but can also be a postharvest mycotoxin.

Swine are the most affected animals by ZEA, although poultry, cattle, and rodents can also show signs of toxicity after ingesting contaminated grains (CAST, 2003; Richard, 2007). This mycotoxin is notable for its effect on the reproductive and urinary systems. When consumed by animals, ZEA causes estrogenic effects when it mimics the action of estradiol-17β by binding to estrogen receptors that influence estrogen dependent transcription in the nucleus. The binding of ZEA can disrupt reproductive processes in pre-pubertal, cycling, and pregnant animals (Cheng et al., 2006; Chen et al., 2008). Gilts exhibit puberty at a younger age but will have unchanged conception and ovulation rates. Embryonic death, smaller litter sizes, and weak piglets are common for sows ingesting ZEA during gestation (Cheng et al., 2006; Richard, 2007). This mycotoxin can also cause feminization of young boars which will alter sperm formation and decrease libido (Hussein and Brasel, 2001; CAST, 2003; Richard, 2007). Recently, ZEA has been linked to stimulating growth of breast cancer cells in humans which have estrogen response receptors (Hussein and Brasel, 2001; Fink-Gremmels, 2006).

Although the mycotoxin ZEA is harmful to reproductive systems, animal mortality due to consumption of ZEA is not an issue. In the United States there are currently no regulations for the occurrence and contamination of ZEA in feeds as risk assessments have indicated that regulatory standards are not needed (CAST, 2003). In the European Union however, ZEA regulatory levels exist with a maximum of 200 μg/kg for grain products designated for human consumption (Richard, 2007).

Minor classes of mycotoxins

Several mycotoxins exist which may be harmful when consumed, but are considered minor mycotoxins since they infrequently contaminate crops (CAST, 2003). The bulk of these toxins are not regulated by the FDA since exposure data have indicated that regulations are not currently warranted (CAST, 2003). Some of these minor mycotoxins include T-2 toxin, fusaric acid, cyclopiazeaic acid, patulin, gliotoxin, citrinin, and ergot alkaloids. T-2 toxin is one of the trichothecenes, and is produced by the fungi *Fusarium sporotrichioides* (Richard, 2007). This mycotoxin can cause low level infection of many crops including corn, wheat, barley, rice, rye, and oats. Like DON, ingestion of T-2 toxin is shown to disrupt protein and nucleic acid synthesis (Richard, 2007). Weight loss, immune suppression, and skin lesions are also commonly observed after consumption of T-2 toxin.

Fusaric acid, produced by several *Fusarium* species, is a mycotoxin of low to moderate toxicity that can alter brain neurotransmitters and metabolites (Bacon et al., 1996). Although commonly found only at low concentrations on corn, fusaric acid is of concern due to the fact that it may have synergistic relationships with other *Fusarium* produced mycotoxins to increase overall toxicity. Cyclopiazeaic acid is another minor mycotoxin produced by *Aspergillus flavus*, *A. versicolor*, *A. tamarii*, and several *Penicillium* species (CAST, 2003). It is known to occasionally contaminate agricultural products including corn, as well as fermented human food products such as Camembert cheese and soy sauce. Cyclopiazeaic acid is most often associated with AF production by *A. flavus*, and like AF, clinical signs of ingestion include reduced feed intake, weight loss, tissue damage, and death.

Patulin is produced by species of *Penicillium* and *Aspergillus* (CAST, 2003). This toxin is also involved in human health, as it contaminates apples and subsequently apple juice and applesauce. Unlike other minor mycotoxins, the FDA does have action levels of 50 μg/kg patulin in

apple juice and apple juice products since these are the primary sources of patulin in the human diet. A mycotoxin which can have minor effects on agriculture commodities is gliotoxin. This toxin is produced by many species of fungi and is found to primarily cause immune suppression (CAST, 2003). Gliotoxin may be involved in respiratory diseases of turkeys, as well as human yeast infections caused by *Candida albicans*. Another minor mycotoxin is citrinin, which is produced by several *Penicillium* and *Aspergillus* species of fungi. It functions similar to OCH, causing kidney damage. Citrinin is thought to interact synergistically with OCH A in pigs to cause swine nephropathy.

Ergot alkaloids are toxins produced by species of *Claviceps* which have a clavine or ergoline ring system (CAST, 2003). Ergotism, a human disease caused by ergot alkaloids, is one of the oldest known mycotoxicoses. The fungi which produce this mycotoxin thrives in several grasses, most commonly tall fescue, but can also occur in cereal grains (Hussein and Brasel, 2001). Tall fescue is a perennial grass grown on about 40 million acres in the United States (CAST, 2003). These grasses are used most commonly for grazing and hay production for ruminant consumption, making the risk to these animals greatest. In cattle, the disease termed fescue toxicosis can cause reduced growth performance, increased body temperature, decreased conception rate, and gangrenous necrosis of tissue of the feet, tail, and ears. A combination of these problems can cause economic losses of about $800 million yearly (CAST, 2003). Ergot alkaloid contamination can also similarly affect horses and swine (Richard, 2007). Despite the resulting health problems and economic damage, there are no regulations for ergot in grain. However, certain methods such as feeding infected fescue in the winter can be implemented to reduce symptoms of fescue toxicosis.

DETECTION METHODS FOR MYCOTOXINS

Accurate methods of analysis are important for determining mycotoxin contamination. The first step of detection involves sampling of grains, which poses the largest source of variation. After sampling, analytical testing methods determine toxin levels following a process which usually involves toxin extraction via an adequate extraction solvent, a clean-up step to remove extract interference, and finally detection of the toxin using analytical instruments (Pascale and Visconti, 2008). Various chromatographic methods used include high performance liquid chromatography (HPLC), thin layer chromatography (TLC), liquid chromatography coupled with mass spectrometry (LCMS), and gas chromatography (GC). Enzyme linked immunosorbent assays (ELISA) are also frequently used.

Sampling

Representative sampling is the most important step in determination of mycotoxin contamination. Usually, the mycotoxin concentration in a bulk lot of feedstuffs is estimated by measuring the toxin concentration in a small sample of the total lot. If the sample taken does not accurately represent the whole, a lot may be misclassified. To minimize error, sampling plans can be implemented to reduce uncertainty (Whitaker et al., 2005). Previous research has shown that some commodities, such as milk, are uniformly contaminated and easy to analyze, whereas mycotoxins in grains are often in highly concentrated "hot-spots" compared to the surrounding particles (Krska and Welzig, 2006).

When analyzing grains, a random sample should be taken so that every particle has an equal chance of being chosen. This process may be completed by collecting several incremental portions from many locations throughout the lot (CAST, 2003; Whitaker et al., 2005). Following sample collection, whole grains are ground into smaller particles in order to achieve a more uniform mixture. Depending on particle size, a subsample of 25 to 1,000 grams is taken for toxin extraction (Whitaker et al., 2005).

Analytical testing methods

Once a subsample is obtained, mycotoxins are extracted from the solid matrices by blending with polar solvents such as water, methanol, or acetonitrile (Pascale and Visconti, 2008). Generally, 5 ml of extraction solvent should be used per gram of sample over a period of 3 to 120 minutes depending on the extraction technique (Krska and Welzig, 2006). After extraction, clean-up methods remove extraction solvents from the toxin which cause interference during toxin detection; however, some detection methods such as ELISA do not require a clean-up process (CAST, 2003).

One of the most widely and frequently used mycotoxin detection method is HPLC (CAST, 2003; Pascale and Visconti, 2008). This procedure is highly sensitive and selective, and is easily repeatable. High performance liquid chromatography separates compounds present in an extract sample by determining their affinity for a stationary column and a mobile solvent (CAST, 2003). The compounds from the column then pass through a detector, usually UV or fluorescence, which will then quantify the specific compound in the original sample. Aflatoxin, DON, OCH, ZEA, and patulin are often detected using HPLC (Krska and Welzig, 2006; Pascale and Visconti, 2008). Although HPLC is a valuable technique, it may not detect mycotoxin metabolites or conjugates which can also be dangerous

when consumed.

Another technique for mycotoxin detection is TLC, which is a simpler and more cost effective screening method than HPLC but does not permit critical quantification like HPLC (CAST, 2003). Thin layer chromatography is often used when low detection limits are not required (Pascale and Visconti, 2008). This method can be used without cleaning up the extract, although extract clean-up can increase the sensitivity of this analysis. The process of TLC involves plating extracts and reference standards at one end of a glass plate coated with a thin layer of silica gel (CAST, 2003). This plate is then placed into a specific solvent so that the sample end is submerged. As the solvent is absorbed through the non-submerged portion of the gel, various compounds in the extract and standards will be drawn through the gel based on their absorption and solubility properties. Thin layer chromatography is one of the most widely used techniques for analyzing contamination by DON and other trichothecenes (Krska and Welzig, 2006). This method is also commonly used to screen for AF, FUM, and ZEA.

Liquid chromatography is coupled with mass spectrometry as another method of mycotoxin contamination analysis. Currently, LCMS is a promising technique for screening and identifying a large number of toxins (Pascale and Visconti, 2008). This method is useful for simultaneous analysis of multiple toxins in a sample, but it is expensive and does not always produce accurate, sensitive, or precise results. Another mycotoxin detection method used more commonly in technical laboratory settings is GC (CAST, 2003). This analysis is good for determining certain trichothecenes which cannot be readily isolated using HPLC. Compounds are determined via their affinity for a stationary column or a mobile inert gas. Detecting mycotoxins via GC provides excellent sensitivity and can be used to determine multiple mycotoxins, but it is expensive and specialized expertise is required (Pascale and Visconti, 2008).

Immunological assays, such as ELISAs, can also successfully detect mycotoxin contamination. These assays use monoclonal or polyclonal antibodies against major known mycotoxins to determine quantitative and qualitative measurements of contamination (Pascale and Visconti, 2008). Due to kit detection limits, this method lacks accuracy when mycotoxin concentrations are very low. However, it is a fast and inexpensive procedure, especially useful in small laboratories or for direct field analysis. There are many commercially available ELISA kits for the most common mycotoxins (Krska and Welzig, 2006).

Again, the accuracy of these analytical methods of mycotoxin analysis depends on the quality of the sample collected. Suitable techniques must also be chosen based on the availability of testing machinery, the mycotoxins being analyzed, and the detection limit desired. Proper sampling and testing is important for determining food and feed safety before consumption.

REDUCING MYCOTOXIN CONTAMINATION

Pre-harvest

Pre-harvest control of fungal infection is one of the most effective ways to reduce mycotoxin contamination of grains since fungi development is most likely to occur during the growth of grains. In the US, pre-harvest control occupies the majority of resources in the effort to control mycotoxin contamination (Bhatnagar et al., 2004). Mycotoxin reduction strategies include both traditional and novel approaches, such as maintaining general crop health, reducing insect damage, applying a suitable fertilizer, and properly irrigating plants to reduce drought stress. Research has shown that by irrigating crops, *A. flavus* contamination can be reduced by about 78% (Diener et al., 1987). For many grains, pre-harvest selection of hybrids, plant density, time of planting, and harvest time can have an impact on toxin contamination (Diener et al., 1987; Magan and Aldred, 2007). Application of competitive fungi, which are nontoxic, can also be useful (CAST, 2003; Bhatnagar et al., 2004). However, these control practices may not always be applicable due to costs of production, geographic location, or production system.

Grain growth is an important factor in determining whether mycotoxin producing fungi will develop. The literature suggests that early maturing varieties of corn can have 3 to 4 folds lower DON and ZEA contamination than late maturing plants (Magan and Aldred, 2007). Planting time can also greatly alter fungal growth, where late sowing can produce crops with up to 4 fold higher toxin levels. Grain harvest time follows a similar trend, where early harvested crops have reduced mycotoxin contamination. Together, early maturation and early harvesting of crops can reduce mycotoxin contamination by decreasing the period of time that fungi can grow.

Breeding insect and mycotoxin resistant corn has become increasingly successful. One type of insect resistant corn is Bt maize, which contains the bacterium *Bacillus thuringiensis* (Bt). This bacterium has a gene encoding a toxic protein that can reduce insect damage due to the formation of a resistant germplasm. This insect resistance can subsequently decrease fungal growth by reducing the number of insect made entry points into the kernel (Bhatnagar et al., 2004). The development of exclusively fungal resistant corn is difficult because resistance may involve several genes. However, one study has shown that naturally AF resistant corn does exist. This corn contains high levels of a 14 kDa trypsin inhibitor protein, while nonresistant varieties either do not contain this protein or

only express it in very low concentrations (Chen et al., 1999). The action of the trypsin inhibitor against *Aspergillus flavus* may be due to the inhibition of the fungal α-amylase enzyme, which would subsequently limit access to the corn's simple sugars needed for fungal growth. Another strategy for reducing mycotoxin contamination of crops is the use of atoxigenic (non-mycotoxin producing) strains of fungi (Bhatnagar et al., 2004). This control method has been most promising for AF, where highly aggressive nontoxic strains of *Aspergillus flavus* and *A. parasiticus* can out-compete the toxin producing forms (CAST, 2003). Fumonisin and DON free fungi have been isolated, but are inconsistent in their ability to dominate mycotoxin producing strains and may sometimes produce toxins themselves (Bhatnagar et al., 2004).

Post-harvest

After harvest, grains can become contaminated with mycotoxin producing fungi or can undergo further mycotoxin production. As crops are transferred into feed products they may be ground, mixed, and stored in bins for later use, and the environmental conditions at each of these steps can influence the fungal contamination of grains. Generally, fungus growth can be controlled by lowering moisture, keeping grains fresh, and equipment clean (Jones et al., 2007). Of these techniques, moisture control may play the most important role. After harvest, grains must first be quickly dried to moisture levels of 14% or less before they are properly stored to remain at low moisture (CAST, 2003; Jones et al., 2007). Magan and Aldred (2007) found that corn left at 25% moisture for 7 days prior to drying and storage had a 77% increase in FUM and an even higher contamination of ZEA. During feed production, pelleting is another way to reduce fungi growth because moisture is removed in this process (Jones et al., 2007). However, fungal spores are not removed, and therefore improper pelleting or storage of feed can result in fungi growth and subsequent mycotoxin production.

Broken kernels and grain dust often contain the highest levels of mycotoxin contamination (Richard, 2007). Thus, physical methods of grain detoxification are beneficial by mechanically screening these highly contaminated particles to separate them from a lot of grain. Although AF is heat stable, roasting processes can be used for partial destruction of this toxin (CAST, 2003). Irradiation by UV light has also been shown to decrease AF concentration in peanut oils and milk, but some data also show that these toxins may transform into more harmful mutagens during this process (CAST, 2003). Mycotoxins can be efficiently extracted from grains through the use of certain solvents including 90% aqueous acetone, 80% isopropanol, henane-ethanol, hexane-methanol, and several others (CAST, 2003). Despite the efficiency of these solvents to reduce mycotoxin contamination in grains, this extraction process can be expensive and impractical in most situations.

Chemical detoxification is another way to reduce mycotoxin contamination. Toxin degradation using ammonia is a feasible method of detoxifying AF contaminated products (CAST, 2003). Ammoniation works by irreversibly converting AF B1 to less toxic products such as AF D1. When gaseous ammonia or ammonium hydroxide are added under certain conditions, ammoniation can decrease AF levels by 99% (CAST, 2003). Ozonation, a reaction with ozone (O_3) gas, is another chemical method of detoxification (CAST, 2003). Ozone gas can degrade AF in corn, cottonseed meal, and aqueous solutions. It can also alter other mycotoxins such as DON, and is useful for decontamination of bulk material at a low cost. Ozone degrades to oxygen, and it does not greatly affect nutrient composition of the grain. However, ozone gas has a short half-life of about 20 minutes, so it must be produced at the location it is to be used. Finally, mold inhibitors such as organic acids, salts of organic acids, and copper sulfate can also control fungi growth (Jones et al., 2007). Each of these chemicals can significantly reduce mycotoxin contamination, but they increase costs and are only effective if they are completely distributed throughout the entire feed.

Feed additives: mycotoxin binders

Frequently, mycotoxins are not removed from feed before they are ingested by animals. To prevent the harmful effects of consuming mycotoxins, feed additives can be incorporated into diets. Depending on their function, materials used as additives can be classified into two major categories: mycotoxin adsorbing agents or mycotoxin transforming agents (EFSA, 2009). Adsorbing materials are those that bind mycotoxins in the gastrointestinal tract to form a stable complex that will not dissociate as it passes through the animal. On the other hand, some materials can transform mycotoxins as a way to reduce exposure. Microorganisms, such as bacteria and fungi, can contain enzymes that degrade mycotoxins into non-toxic compounds (EFSA, 2009). In these ways, mycotoxin exposure to the animal can be reduced.

Adsorbing agents primarily include natural clay products and yeasts (EFSA, 2009). Silicate products, known as aluminosilicates, are the largest group of mycotoxin adsorbing materials. Several types of aluminosilicate clays exist, including bentonites, montmorillonites, zeolites, and hydrated sodium calcium aluminosilicates (HSCAS) (Jones et al., 2007; EFSA, 2009). These clays contain aluminates, silicates, and interchangeable ions including alkali metal and alkaline earth metal ions (Huwig et al., 2001). Their structure commonly contains a SiO_4 unit which is electrically neutral, and an AlO_4 unit which carries a

negative charge. This negative charge then allows for mycotoxin adsorption in the gastrointestinal tract of the animal. Aluminosilicates have particular affinity for the polar mycotoxin AF, although some clay products can have a binding capacity for other mycotoxins (Huwig et al., 2001; Sabater-Vilar et al., 2007; Thieu et al., 2008).

The clay bentonite originates from the weathering of volcanic ash and contains several interchangeable ions including sodium, potassium, calcium, and magnesium (Ramos et al., 1996). Bentonites contain a layered crystalline structure which allows adsorption of other molecules. This adsorptive capability has made bentonite a widely used compound in industrial, engineering, and agricultural industries. For example, the use of bentonite in mycotoxin contaminated poultry diets can improve growth rate, feed efficiency, egg size, and egg shell quality while it can decrease mortality (Ramos et al., 1996). The addition of bentonite to animal diets is especially beneficial for reducing AF contamination of feed, as it is able to absorb up to 100% of the AF within the animal's body fluids such as milk, blood, stomach digesta, and rumen fluid.

Montmorillonite is another type of layered silicate clay which can adsorb organic materials either on its surface or within its interlaminar spaces by the exchange of cations present in these spaces (Ramos et al., 1996). This clay is commonly the main component of bentonite, although it often contains a stronger adsorptive capacity alone than when incorporated into bentonite (Ramos et al., 1996; EFSA, 2009). Montmorillonites have a strong affinity for AF B1, but have also been shown to attract ZEA (Ramos et al., 1996; Lemke et al., 1998).

Zeolites are silicates with a three-dimensional structure that contains crystalline hydrated aluminosilicates of alkali and alkaline earth cations (Ramos et al., 1996; EFSA, 2009). They are able to reversibly gain and lose water, as well as exchange cations without structural change (Ramos et al., 1996; Papaioannou et al., 2002). Zeolites have the strong ability to adsorb AF, as well as provide some protection against ZEA (Smith, 1980; Ramos et al., 1996).

The material HSCAS, a phyllosilicate, also has a high affinity for AF B1 (Huwig et al., 2001). The function of HSCAS is based on the fact that it that contains positive charge deficiencies that create a potential for adsorbing positively charges compounds, such as AF (Ramos et al., 1996). Subsequently, it acts like a sponge to absorb this toxin in the gastro-intestinal tract of animals (Huwig et al., 2001). The complex which is formed with AF is very stable at temperatures of 25 to 37°C, in a wide pH range of 2 to 10. Although HSCAS is commercially sold to feed manufactures for its anticaking properties, when added to animal feeds, it can also adsorb mycotoxins (EFSA, 2009). When HSCAS is added to diets contaminated with AF, growth reduction caused by this toxin can be greatly reduced. Although very effective at preventing aflatoxicosis, HSCAS is limited in its ability to adsorb other mycotoxins (Ramos et al., 1996).

Yeast and yeast cell wall components are another type of adsorption additive which reduce the harmful effects of mycotoxins (Fink-Gremmels, 2006). These materials rapidly bind to the toxins they come in contact with, and have a particular affinity for DON, OCH, and ZEA (Huwig et al., 2001). Products containing only yeast cell walls have a stronger ability for mycotoxin adsorption then whole yeast because their cell walls, which contain polysaccharides, lipids, and proteins, are more accessible (Huwig et al., 2001). These cell wall compounds have adsorption centers which bind the toxin via hydrogen bonding, or ionic and hydrophobic interactions. The cell wall component β-D-glucans appear to play the strongest role in this adsorption capability (Yiannikouris et al., 2004). This polysaccharide, which consists of 50 to 60% of the cell wall dry weight, can adopt helical conformations that can provide various adsorption sites for mycotoxins. Typically, it is possible to bind 2.7 mg of ZEA per gram of yeast cell wall product (Huwig et al., 2001).

One strain of yeast, *Trichosporon mycotoxinivorans*, can detoxify OCH by cleavage of the phenylalanine moiety to form the derivate OCHα (Schatzmayr et al., 2006). This metabolite is virtually nontoxic compared to the parent compound. Recently, this *T. mycotoxinivorans* yeast has been shown to have very high capability to degrade ZEA (Vekiru et al., 2001). The metabolite of breakdown, ZOM-1, is formed when *T. mycotoxinivorans* opens of the macrocyclic ring of ZEA at the ketone group at the 6th carbon. *In vitro*, ZOM-1 does not show estrogenic activity in a yeast bioassay even at a concentration 1,000 fold higher than that of ZEA (Vekiru et al., 2001). This metabolite also did not interact with the human estrogen receptor in an *in vitro* assay. Due to the fact that *T. mycotoxinivorans* can be fermented, concentrated, freeze-dried, and stabilized without losing its deactivating capacity, its utilization as a feed additive for OCH and ZEA detoxification seems practical.

Bacterial enzymes can also act as mycotoxin detoxifiers, although these act as mycotoxin biotransforming agents (Huwig et al., 2001; EFSA, 2009). Prior to mycotoxin absorption in the animal's intestinal tract, the enzymes secreted from bacterial microorganisms can work to transform mycotoxins into nontoxic metabolites which can be absorbed by the animal with no toxic effects (Schatzmayr et al., 2006). Whereas clay mineral additives have particular affinity for AF, bacteria and their enzymes can have an affinity for DON, OCH, and ZEA due to their ability to alter the toxin's ring structure. Deoxynivalenol can be enzymatically tranformed to the nontoxic metabolite DOM-1 by an epoxidase of *Eubacterium* BBSH 797, a

gram positive, anaerobic bacterium (Schatzmayr et al., 2006; EFSA, 2009). Other enzymes which can transform mycotoxins include proteases, carboxypeptidases, and lactonohydrolyases (EFSA, 2009). Several fungal species can also contain enzymes which degrade mycotoxins. One fungi, *Gliocladium roseum*, can open structural lactone rings to detoxify ZEA by 80 to 90% (Schatzmayr et al., 2006; EFSA, 2009). However, there is currently little use of these fungi in practical feed application, making their use limited and questionable.

MYCOTOXINS AND THE SWINE INDUSTRY

Common mycotoxins affecting swine

Of the major and minor classes of mycotoxins, AF, DON, and ZEA are among the most concerning for swine, since swine are the most sensitive species and these mycotoxins are extremely prevalent in the United States (Goyarts et al., 2005; Richard, 2007; Sabater-Vilar et al., 2007; Meissonnier et al., 2008). A survey from the North Carolina Cooperative Extension Service found that 34% of corn tested contained more than 20 μg/kg AF and over 60% of feed contained DON (Jones et al., 2007). In past years, the financial losses in the Southeastern United States due to the mycotoxin AF alone totaled $97 million for grain producers and another $100 million in production losses for swine producers (Hussein and Brasel, 2001). Production losses can be due to reduced growth, sickness due to immune suppression and organ damage, or death of the animal (FDA, 1994; Richard, 2007; Sabater-Vilar et al., 2007; Chen et al., 2008). Although less prominent, FUM and fusaric acid are two other mycotoxins which can be problematic to the swine industry.

Effects of mycotoxin consumption by swine

The consumption of AF and DON by pigs has been shown to decrease body weight and ADG at both low and high concentrations of 120 to 3,000 μg/kg AF (Harvey et al., 1989; Schell et al., 1993; van Heugten et al., 1994; Marin et al., 2002; Thieu et al., 2008; Chaytor et al., 2010; Chaytor et al., 2011) and 600 to 9,570 μg/kg DON (Harvey et al., 1989; Smith et al., 1997; Swamy et al., 2002; Doll et al., 2003; Goyarts et al., 2005; Cheng et al., 2006; Tiemann et al., 2006; Chaytor et al., 2010; Chaytor et al., 2011). Chaytor et al. (2011) indicated that a diet with as low as 60 μg/kg AF in combination with 300 μg/kg DON caused a 12% reduction of ADG, while a diet containing 180 μg/kg AF and 900 μg/kg DON resulted in a 21% decreased in ADG of pigs in a 33 day study.

Feed intake has been reported to be reduced when pigs consumed contaminated feed with both high and low AF and DON concentrations (Rotter at al., 1994; van Heugten et al., 1994; Smith et al., 1997; Swamy et al., 2002; Goyarts et al., 2005; Cheng et al., 2006; Thieu et al., 2008; Chaytor et al., 2011). As indicated by van Heugten et al. (1994) even a concentration as low as 140 μg/kg AF decreased feed intake by 3.5%. Although ADG and feed intake are frequently altered, feed efficiency is not commonly effected by the mycotoxins AF and DON (Harvey et al., 1991; van Heugten et al., 1994; Smith et al., 1997; Swamy et al., 2002; Doll et al., 2003). However, Chaytor et al. (2010) showed 175 μg/kg AF in combination with 900 μg/kg DON reduced feed efficiency of pigs.

The immune system of the pig is greatly compromised by AF and DON contamination of feed. Harvey et al. (1989) showed that ingestion of a high AF concentration (3,000 μg/kg) resulted in an increase in white blood cell numbers above normal range when pigs were fed this diet for 28 days. Increased monocyte and hematocrit levels for pigs fed 900 to 2,500 μg/kg DON have also been reported (Pinton et al., 2008; Chaytor et al., 2010). Other immunological parameters such as the immunoglobulins IgA, IgG, and IgM can be altered by mycotoxins as well. Many studies have shown that high concentrations of 2,200 to 6,800 μg/kg DON incorporated into the diet caused an increase in serum IgA and IgM (Swamy et al., 2002; Goyarts et al., 2005; Tiemann et al., 2006; Pinton et al., 2008). Other research has shown variable results with low mycotoxins concentrations of 120 to 280 μg/kg AF or 280 to 900 μg/kg DON, where effects on immunoglobulin subsets may or may not occur (van Heugten et al., 1994; Accensi et al., 2006; Chaytor et al., 2010; Chaytor et al., 2011). The cytokine tumor necrosis factor alpha (TNFα) was altered when pigs consumed a diet containing both 180 μg/kg AF and 900 μg/kg DON (Chaytor et al., 2011). TNFα, a pro-inflammatory cytokine, has a regulatory role in inflammation responses (Wood, 2006). An increase in TNFα may indicate an inflammation response due the toxicity of AF and DON. As a result of these immune system challenges by mycotoxins, secondary problems can also arise if the animal is compromised and there is increased incidence of other disease infections (Fink-Gremmels, 2008).

Organ damage is another common problem in pigs consuming AF and DON. High AF (3,000 μg/kg) in the diet caused liver lesions and hepatocellular cytoplasmic vacuolation with early portal fibrosis and bile duct hyperplasia (Harvey et al., 1991). It has previously been documented that 1,000 μg/kg DON causes damage including necrosis, blood vessel thickening and hemorrhage (Cheng et al., 2006; Chen et al., 2008). Whether low concentrations of these two mycotoxins will cause organ damage in pigs is less clear, but some research suggests that as low as 175 μg/kg AF and 900 μg/kg DON combined causes hepatic fibrosis, bile ductule hyperplasia,

megakaryosis, and vacuolation (Chaytor et al., 2010; Chaytor et al., 2011).

The effects of ZEA on the swine industry are also substantial. Zearalenone exhibits estrogenic effects, and thus is especially detrimental to reproduction (Richard, 2007). In a study by Doll et al. (2003), weanling pigs fed 420 μg/kg ZEA for 37 days had increased cervix and vulva swelling. The relative uterus weight of pigs may also by significantly increased by consuming diets containing 420 to 4,330 μg/kg ZEA (Etienne and Jemmali, 1982; Doll et al., 2003). Uterine tissue damage, such as hyperplasia, hypertrophy, and metaplasia of the myometrium, is documented when feeding ZEA (Tiemann and Danicke, 2007).

High concentrations of 2,200 to 22,090 μg/kg ZEA fed to breeding gilts has harmful effects on their reproductive performance by altering embryo development and reducing the number of live born piglets (Tiemann and Danicke, 2007). These effects are shown by Etienne and Jemmali (1982) where high concentrations of 4,330 μg/kg ZEA fed to gilts before puberty can result in anestrus, causing the pigs to not ovulate after puberty. In this case, corpora lutea were maintained like they would be in pregnant gilts and the uterus showed a pseudopregnancy condition. Conclusively, this pseudopregnancy resulted in 45% of gilts not coming into estrus at 50 days prior to puberty (Etienne and Jemmali, 1982). Low concentrations of 50 to 200 μg/kg ZEA in young gilts is also shown to be detrimental to the ovaries, where the development and maturation of the follicles is altered through the activation of an apoptotic process in the granulosa layer (Tiemann and Danicke, 2007; Vekiru et al., 2010). The serum concentration of the FSH, a gonadotropic hormone, is also shown to decrease when female pigs are fed as low as 50 μg/kg ZEA (Doll et al., 2003; Gutzwiller et al., 2009). Decreased FSH could lead to reduced follicular development and subsequently reduced ovary weight.

The effects of ZEA can also be observed in boars, where consumption of contaminated grains can depress serum testosterone, spermatogenesis, and a 30% reduction in the weight of the testes (D'Mello et al., 1999; Minnervina and Dell'Aquila, 2008). A reduction in sperm viability and quality is also a common symptom. All of these effects can result in feminisation and suppressing of libido.

Fumonisin contamination of feeds can result in a diverse range of damages to swine tissues, including lesions to the esophagus, gastrointestinal tract, liver, lungs, and brain (Casteel et al., 1993; D'Mello et al., 1999). Porcine pulmonary edema (PPE) is one such disease that is thought to be caused by FUM. Features of PPE include elevated serum cholesterol, increased hepatic enzymes concentrations, and pulmonary hypertension caused by hypoxic vasoconstriction (D'Mello et al., 1999). Pigs fed FUM showed greater pulmonary artery pressure, and decreased heart rate, cardiac output, and mixed venous O_2 tension. The consumption of high FUM (100,000 μg/kg) is shown to be damaging to the liver as well, where weanling pigs developed hepatic nodule hyperplasia (Casteel et al., 1993). At lower levels of FUM, reduced feed intake and ADG is not well documented, where there will be an effect in some cases and not in others (D'Mello et al., 1999). At low concentrations, an increase in carcass fat content is documented in some cases. As a side effect of FUM ingestion, Oswald et al. (2003) showed that ingestion of 500 μg/kg FUM B1 by weanling pigs increased the intestinal colonization of *E. coli* in the small and large intestines. Due to the fact that FUM causes changes in sphingolipid metabolism, ingestion of this mycotoxin could modify bacterial receptors on the surfaces of epithelial cells (Oswald et al., 2003). These changes may contribute to increased bacterial colonization of the intestinal tract.

Although not one of the major mycotoxins, fusaric acid is becoming increasingly concerning for the swine industry. This mycotoxin, classified as a phytotoxin, is produced by *Fusarium moniliforme* which is a common infector of corn (Smith and MacDonald, 1991). It is of particular concern due to the fact that it may have synergistic relationships with other *Fusarium* mycotoxins to increase overall toxicity (Bacon et al., 1996). Fusaric acid is shown to be a potent inhibitor of dopamine-beta-hydroxylase, the enzyme involved in the brain during the synthesis of norepinephrine (Smith and MacDonald, 1991). When pigs consume high concentrations of this mycotoxin, symptoms include decreased growth, vomiting, lethargy, and alterations to the hypothalamus including elevation of tryptophan and serotonin (Smith and MacDonald, 1991; Swamy et al., 2002).

Use of feed additives to reduce mycotoxin effects

Although compounds that binder or destroy mycotoxins are not approved by the FDA for use in animal diets, the addition of feed additives in experimental settings indicate that they are a promising method to reduce the negative effects of mycotoxins ingested by pigs. Clay products, including bentonites, HSCA, and zeolites, are particularly affective at reducing the effects of AF but are shown to reduce effects of some other mycotoxins. Quang et al. (2008) found that the addition of 0.4% bentonite clay to piglet diets containing 200 μg/kg AF for the 41 day trial period increased BW, ADG, and G:F compared to a diet containing only AF. Sodium bentonite and calcium bentonite at up to 0.5% were also found to improve ADG and ADFI of pigs consuming a highly contaminated diet with 800 μg/kg AF (Lindemann et al., 1993; Schell et al., 1993). Calcium bentonite is also shown to reduce the release of the liver enzymes aspartate aminotransferase,

gamma glutamyltransferase, and alkaline phosphatase. Under the same high AF concentration, the addition of HSCA at 0.5% had no effect on ADG, ADFI, or any of the liver enzymes.

Research has shown that the use of clays to reduce the effect of DON do not improve BW, ADG, or ADFI when added to pig diets contaminated with 900 μg/kg DON (Chaytor et al., 2010). However, clay products may be beneficial on biochemical and histological parameters affected by this mycotoxins (Ramos et al., 1996). Chaytor et al. (2010) showed that clay additives reduced the combined effects of AF and DON on moncytes, IgG, and IgM. Certain clay products, such as zeolite, can also be beneficial at reducing ZEA in swine diets although benefits on growth may occur in some situations and not in others (Papaioannou et al., 2002). In a study by Papaioannou et al. (2002), the reproductive performance of sows and the performance of their litters were evaluated after feeding ZEA and zeolite. It was found that the addition of this clay significantly improved reproductive performance, and that the clay itself did not have any effect on the sows. Montmorillonite clay added to diets containing 1,300 μg/kg ZEA is also shown to reduce multi-organ toxicities caused by ZEA (Jiang et al., 2010). The addition of this clay at 5 g/kg diet had the greatest efficiency in comparison to higher and lower inclusion rates. In this study, montmorillonite clay was an effective adsorbent without any toxic effect itself.

Yeast products are another feed additive with potential for reducing the effects of mycotoxins. At an inclusion rate of 0.2% of the diet, yeast cell wall products prevented some neurochemical changes, such as depressed norepinephrine, that occurred when feeding 5,500 μg/kg DON and 400 μg/kg ZEA (Swamy et al., 2002). Yeast products also prevented an increase in monocyte, IgA, and IgM concentrations due to AF and DON (Swamy et al., 2002; Chaytor et al., 2010). It has been documented that hepatic bile ductule hyperplasia, megakaryosis, and vacuolation can all be reduced through the use of yeast additives to AD and DON contaminated swine diets (Chaytor et al., 2010).

In a study by Cheng et al. (2006), a mycotoxin degrading enzyme containing epoxidase, esterase, and peptidase activities provided a partial or complete elimination of toxic effect. Pigs were fed diets containing 1,000 μg/kg DON and 250 μg/kg ZEA, which resulted in altered growth and feed intake, and caused multi-organ toxicity. The enzyme spared mycotoxin effects for criteria including growth performance, alveolar macrophage activities, antibody and cytokine increases, and tissue damage (Cheng et al., 2006). Chaytor et al. (2010) reported that enzyme additives reduced the effects of 175 μg/kg AF in combination with 900 μg/kg DON for parameters such as IgG, IgM, and hepatic damage such as megakaryosis.

Although not shown as of yet to be effective in swine, other materials have been indicated to be beneficial feed additives in reducing mycotoxin contaminations. One of these materials, whey protein concentrate, was shown to reduce the affects of AF consumed by rats (Saleh et al., 2007). The addition of the whey protein to AF contaminated diet improved growth performance, feed efficiency, and altered biochemical parameters. Fiber has been shown to have some efficacy in decreasing the toxicity of ZEA (Lemke et al., 1998). When alfalfa was present at levels of at least 25% in the diet, it helped to improve body weight, feed intake, and feed efficiency in contrast to diets containing only ZEA (Smith, 1980; Lemke et al., 1998). Increasing dietary protein may also be helpful at reducing challenges by ZEA in rats.

Another material, cholestyramine, is a quaternary ammonium anion exchange resin with ability to reduce the effects of ZEA on the prepuberal mouse uterine weight (Lemke et al., 1998). With future research, each of these materials as well as many potential others, may be beneficial at alleviating mycotoxin effects on swine.

CONCLUSION

Mycotoxicoses in animals is a global issue, and there is increasing concern for effects of mycotoxins on animal health and well being. Although acute ingestion of high levels of mycotoxins can be very harmful to the animal, long term consumption of low concentrations of mycotoxins can also be damaging. Aflatoxin, deoxynivalenol, zearalenone, fumonisin, and ochratoxin are the five major mycotoxins that are commonly found on grains throughout the United States and around the world. The swine industry may be especially concerned about the mycotoxins AF, DON, and ZEA since swine are the most sensitive commodity species to these contaminants. When consumed by pigs and other animals, these mycotoxins can cause reduced growth and feed intake, organ damage, altered neurotransmitter function, and immune challenges. There are several ways of reducing mycotoxin concentrations both pre and post-harvest, including the addition of feed additives such as, but not limited to, natural clays, yeasts, and enzymes. Since mycotoxins can be so detrimental to the swine industry, further determination of sustainable way to combat the global mycotoxin problem is important for maintaining animal health, as well as reducing economic impacts on farmers and producers.

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