ABSTRACT

CHAYTOR, ALEXANDRA CLARE. Growth and Immune Responses of Pigs under Chronic Exposure to Low Levels of Mycotoxins in Naturally Contaminated Feed, and the Role of Various Feed Additives in Ameliorating Mycotoxin Effects. (Under the direction of Sung Woo Kim).

The objectives of this research was to: (1) investigate growth and immune responses of pigs fed diets containing corn naturally contaminated with low concentrations aflatoxin (AF) and deoxynivalenol (DON), and (2) examine 3 feed additives with a potential ability to detoxify the mycotoxins AF and DON.

For the first objective, diets contaminated with low AF and DON were fed to 60 gilts (13.9 ± 0.2 kg BW) for 33 days. Pigs were randomly assigned to 4 treatments: A (control without AF and DON); B (a diet with 60 μg/kg AF and 300 μg/kg DON); C (a diet with 120 μg/kg AF and 600 μg/kg DON); and D (a diet with 180 μg/kg AF and 900 μg/kg DON). Concentrations of mycotoxins were below FDA regulatory levels for growing swine. Feed intake and BW were recorded weekly and pigs were bled and euthanized on day 33 for blood analysis and evaluation of tissue damage. Pigs fed C and D tended to have reduced ADG (0.43 and 0.41 kg/d, $P = 0.058$) and ADFI (0.92 and 0.88 kg/d, $P = 0.061$) than A (0.52 and 1.04). White blood cell count of pigs fed D was greater (23.4 x10^3 cells/μL, $P < 0.05$) than A (18.4), B (18.5) and C (16.8). Serum tumor necrosis factor alpha of pigs fed D was greater (335 pg/mL, $P < 0.05$) than A (299). Pigs fed D had greater ($P < 0.05$) liver fibrosis than A. This experiment showed diets containing 120 to 180 μg/kg AF and 600 to 900 μg/kg DON reduced growth, altered immunity, and damaged the liver of pigs.

For the second objective, 225 gilts (8.8 ± 0.4 kg BW) were allotted to 5 treatments: PC (positive control without mycotoxins); NC (negative control with 175 µg/kg AF and 900 µg/kg DON); A (NC + a clay based additive); B (NC + a clay and yeast cell wall based additive); and C (NC + a clay and enzyme based additive). Feed intake and BW were recorded weekly and blood samples collected every other week. On day 42, pigs were euthanized to obtain organ tissues.

Pigs fed NC had decreased BW (24.6 kg, $P < 0.05$) and ADG (0.37 kg/d) than PC (26.6 and 0.42), while feed additives did not differ from NC. Pigs fed NC had greater IgG (15.1 mg/mL, $P < 0.05$) than PC (10.5) and A (8.6). Pigs in NC had greater IgM (4.4 mg/mL, $P < 0.05$) than PC (3.0), A (2.7) and B (3.2). Pigs in NC had greater liver bile ductule hyperplasia, megakaryosis and vacuolation than PC ($P < 0.05$). All additives differed from NC for megakaryosis, and B had decreased bile ductule hyperplasia and vacuolation. Kidney vacuolation tended to increase ($P = 0.066$) in NC compared with PC, B, and C. Results indicate diets with low AF and DON reduced growth and increased tissue damage and immune challenges. The use of 3 feed additives improved organ and immune health, but did not improve growth performance.

Collectively, these experiments show AF and DON can be harmful to pigs at concentrations below FDA regulations. At contamination as low as 120 µg/kg AF and 600 µg/kg DON, growth performance was reduced, tissue damage occurred, and immune health was challenged. The addition of specific feed additives to swine diets may be an effective method to combat the effects of low AF and DON contamination.

Growth and Immune Responses of Pigs under Chronic Exposure to Low Levels of Mycotoxins in Naturally Contaminated Feed, and the Role of Various Feed Additives in Ameliorating Mycotoxin Effects

by
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BIOGRAPHY

Alexandra Clare Chaytor was born in Mombasa, Kenya on February 11, 1986 and was raised in Stonington, Maine. Growing up around horses and goats led to participation in 4-H, where Alexandra showed dairy goats in United States Dairy Goat Association competitions. Alexandra attended high school at Stoneleigh Burnham School in Greenfield, Massachusetts until graduating in 2004, when she then enrolled at Elon University in Elon, North Carolina. During her time at Elon University, Alexandra served as captain of the Intercollegiate Dressage Association Equestrian Team for three years, and won Nationals in 2005. In 2008 she received her Bachelor of Science in Biology as a member of the Tri Beta Biological Honors Society. With a desire to further her education in the biological sciences, Alexandra began her Master of Science degree in Animal Science with a minor in Nutrition at North Carolina State University under the direction of Dr. Sung Woo Kim. Alexandra received a first place award from the North Carolina State University Graduate Student Research Symposium and a second place award from the American Society of Animal Science Midwest conference based on research related to mycotoxins. After completing her Master's Degree, Alexandra plans to continue her education at North Carolina State University in order to obtain her Doctor of Philosophy in Animal Science.

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LITERATURE REVIEW

Introduction

The United States swine industry continually faces challenges. Production costs, profits, and losses can drastically change from year to year. One of the factors that can cause problems for pork producers are mycotoxins. These toxic metabolites of certain fungi are estimated to contaminate 25% of the world's crops (Schatzmayr et al., 2006; Veldman, 2004). When mycotoxins enter the diet, they can greatly affect animal health and can play a large role in farm production. Mortality is one severe result of mycotoxin ingestion which instantly affects producer's profits, but the economic downside of these fungal contaminants extends far beyond death. Overall health and production can be altered due to decreased growth and feed intake, an increase in organ damage, and immune suppression (CAST, 2003).

Feed represents a large portion of total swine farming cost. Although expensive, grains are essential for providing an energy source in the diets of pigs, where corn alone can account for 91% of the grains used in the animal feed industry. However, current high grain prices are greatly affecting swine production (Stillman et al., 2009). Recent costs have increased to record highs, where from 2006 to 2008 the value of corn per bushel nearly doubled and the prices for barley and oats more than doubled (Baker et al., 2010). The demand for grains is one important factor in this recent price inflation. The ethanol industry, a large user of corn, has grown dramatically over a short period of time. Historically, high feed costs were due to poor grain yields, but recent high corn prices are linked to the rapid growth in the use of corn for ethanol (Plain, 2008). Currently, roughly 27% of total corn

production goes solely to this biofuel industry (Anderson et al., 2008; Baker et al., 2010; Plain, 2008). As a result of this high demand, corn production grew by 4.9 million hectares in 2007, and price jumped to over $5 per bushel in early 2008. By the end of 2009, corn price dropped slightly from the previous year, but still remained high at $3.45 to $3.95 per bushel. Despite the rising corn costs, profitable output for ethanol producers has encouraged increased ethanol production. Consequently, corn prices continue to rise, leaving farmers to face the challenge of affordably feeding their animals (Anderson et al., 2008; Wu and Munkvold, 2008). For swine producers, high feed expenditures have increased the cost of producing market hogs by more than 30% (Plain, 2008). The United States Department of Agriculture (USDA) report for the 2010 livestock, dairy and poultry outlook suggests that a combination of decreased swine production and increased grain costs will cause pork producers to only break even during 2010 (Johnson, 2010). Projections are not guaranteed however, and if corn costs continue to increase, then farmers may find themselves losing money.

As grain prices increase, the use of inexpensive grains may increase in order to reduce feed costs. However, these grains are often of lower quality, and can have a high mycotoxin contamination. These grains may be from a previous year's crop which was not stored properly or from a particular growing location where weather conditions favor fungal growth. When grains are contaminated with mycotoxins they can greatly alter animal health which can further result in economic losses for both grain producers and animal farmers in animal production. The United States Food and Drug Administration (FDA) has estimated

that the annual cost of crop losses due to all mycotoxins can total $932 million, with research and monitoring adding another $500 million to $1.5 billion (CAST, 2003; Marasas et al., 2008). Production losses for hog 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 of pigs.

Mycotoxins

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 fungus, but they can negatively impact the health of animals and humans who consume them (Hussein and Brasel, 2001). 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. Mycotoxins greatly affect the health and economic stability of many farm industries, including swine production.

Mycotoxins are ubiquitous throughout the United States and around the world. Growth of the fungus can occur under a variety of environmental, temperature, and moisture conditions both pre- and post-harvest (Binder et al., 2007). 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 (**ZON**) (Huwig at al., 2001; Marasas et al., 2008; Richard, 2007).

Major Classes of Mycotoxins

Aflatoxin. Aflatoxin 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* (Dersjant-Li et al., 2003; CAST, 2003). Normally, these fugal strains reside in soil as saprobic microorganisms (Gourama and Bullerman, 1995). The fungi which produce AF can be found on many major crops all over the world such as corn, peanuts, cotton, seeds, and tree nuts (CAST, 2003; Richard, 2007). Growth of *A. parasiticus*, thrives under warmer climates in subtropical and tropical regions, and commonly contaminates peanut crops. Another subspecies, *A. flavus* is most common in temperate latitudes of 25° to 35° north and south of the equator, making it an infector of corn in many of the high cereal producing states of the Southern and Midwestern United States (CAST, 2003; Gourama and Bullerman, 1995).

Aspergillus 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 (Agag, 2004; CAST, 2003; Diener et al., 1987; Richard, 2007). This disease caused immense losses of poultry in England after they consumed AF contaminated grains. Thereafter, the toxin produced by the fungus *A. flavus* was named aflatoxin, and the four

different toxin components were identified. The four forms of AF include 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 also be produced in animal tissues and fluids (such as milk) after they are hydroxylated from AF B1 and AF B2 (Dersjant-Li et al., 2003; CAST, 2003; Richard, 2007). Of these 6 forms, AF B1 (Figure 1) is considered to be the most toxic after both acute and chronic ingestion.

Growth of AF producing fungi is highly dependent on environmental conditions and 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). Any wounding of the plant tissue by insects or birds will interfere with the kernel seed coat and increase entry of the fungal organism (Agag, 2004; CAST, 2003; 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 development of AF. Fungal growth is enhanced when grains are stored in warm (20 to 30°C) and damp (>14% moisture) conditions (Dersjant-Li et al., 2003; CAST, 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 in the Corn Belt region of the Midwest (Richard, 2007). 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).

Aflatoxin ingestion by animals can result in many problems, one of which is decreased growth rates. Liver damage and immune suppression can also commonly occur. Decreased immunity is problematic because it can result in a heightened susceptibility to other diseases such as coccidiosis, salmonellosis, and candidiasis (CAST, 2003). Aflatoxin is also considered carcinogenic, and can result in death when consumed in high concentration.

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. Once in the liver, AF can bind to nucleic acids to block RNA polymerase and ribosomes, as well as covalently bind to proteins to alter their structural and enzymatic functions (Agag, 2004). 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 if the animals have consumed feed highly contaminated with AF (Agag, 2004; Richard, 2007). The ratio of AF M1 excreted in milk is estimated at about 1:75, and it 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

highly concentrated casein. Human consumption of the M1 metabolite can result in similar growth reduction and tissue damage as the other forms of AF.

Although any age and species can be affected by AF, younger animals are generally affected to a greater degree and certain species more than others. Pigs are the most susceptible commodity species to AF, but the toxin can also affect other species such as fish, cattle, poultry, horses, rodents, companion animals, and humans (Agag, 2004; Hussein and Brasel, 2001; Meissonnier et al., 2008; Richard, 2007; 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 in ruminants when the mycotoxin is consumed at high concentrations or for extended periods of time.

To protect humans and animals from the harmful effects of mycotoxin ingestion, the FDA is responsible for mycotoxin regulation. One statute which the FDA is accountable for is the Federal Food, Drug, and Cosmetic Act (FDCA), which has the mission of protecting public health from poisonous substances found in human food or animal feeds (CAST, 2003). Under Section 402(a)(1) of the FDCA, a food source is considered contaminated "if it bears or contains any poisonous or deleterious substance that may render it injurious to health; but in case the substance is not an added substance such food shall not be considered adulterated." Some mycotoxins, such as AF, are considered added poisons because their presence in human and animal feeds can be partially avoided by good pre- and post-harvest practices (CAST, 2003). 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 weigh on the unavoidability of the mycotoxin. 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 groups. Concentrations of AF are allowed in feed 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 can contain up to 200 µg/kg, and beef cattle diets can 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). Despite these regulations, both high and low levels of AF can commonly contaminate feed sources and alter animal health.

Deoxynivalenol. The trichothecenes are a group of mycotoxins produced by several strains of fungi in the *Fusarium* family including *F. sporotrichioides*, *F. graminearum*, *F. poae*, and *F. culmorum* (Hussein and Brasel, 2001). Deoxynivalenol is the most common trichothecenes, but others include T-2 toxin, diacetoxyscirpenol, and nivalenol (Hussein and Brasel, 2001; Richard, 2007). These 4 toxins are grouped together based on their similar structures containing sesquiterpene rings characterized by a 12,13-epoxy-trichothec-9-ene nucleus (Figure 1). Most of these toxins are infrequently found in the United States, however

DON is of significant importance. Produced primarily by *F. graminearum* or *F. culmorum*, DON contaminates corn, wheat, oats, and barley worldwide (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 (Meissonnier et al., 2008; Thieu et al., 2008; Wu, 2007). Deoxynivalenol may commonly co-exist with other mycotoxins, such as zearalenone, which is also produced by strains of *Fusarium* fungi.

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 seen 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).

DON and other trichothecenes are effective inhibitors of DNA, RNA, and protein synthesis (Hussein and Brasel, 2001; CAST, 2003). When DON binds to polysomes and ribosomes within an animal cell, 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 apoptosis (programmed cell death) as well as increased immune and inflammatory responses (Pestka, 2007). Deoxynivalenol causes this immune suppression when it affects cells in tissues such as bone marrow and lymph nodes (CAST, 2003). The result is that this toxin is found to increase an animal's susceptibility toward bacterial and viral contaminants (Fink-Gremmels, 2006). Deoxynivalenol is also shown to alter brain functioning by increasing the neurotransmitters tryptophan, serotonin, and 5-hydroxyindoleacetic acid concentrations which can lead to decreased feed intake and decreased weight gain (Cheng et al., 2006; Swamy et al., 2002). The most commonly known reaction to ingesting grains highly contaminated with DON is vomiting, giving it the nickname vomitoxin (Richard, 2007). Kidney damage is also a frequent symptom of toxicity by DON (Chen et al., 2008; Richard, 2007; Sabater-Vilar et al., 2007). Despite its many damaging affects, 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 of DON that has had its ring structure altered (Pestka, 2007; Schatzmayr et al., 2006).

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 beef and feedlot cattle, and at 5 mg/kg for grains used in swine diets (FAO, 2003; FDA, 1994). 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 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 on other grains such as sorghum and rice (CAST, 2003; Dersjant-Li et al., 2003).

Research has not yet shown the exact conditions needed for disease occurrence since FUM was not discovered until the late 1980s (FAO, 2003). It is known that the fungi producing this mycotoxin appear to grow well when crops are subjected to drought stress followed later by warm, wet weather (Richard, 2007). Detection of contamination by *Fusarium* is difficult, as some grains may show physical damage and others may not. If observed on corn, FUM 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 often rise to 10 mg/kg (Dersjant-Li et al., 2003), although higher levels can occasionally be found.

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; Richard, 2007; Taranu et al., 2005). 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 and biochemical processes involved in liver functioning (Richard, 2007). The FUM toxin is also shown to affect other mechanisms 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 their 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 (CAST, 2003; FDA, 1994). The FDA has FUM contamination of corn for human consumption set to between 2 and 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 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 has a wide range of commodities that it can infect, 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 hard 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 by 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 Nephrophy is one such kidney disease in humans that is caused by OCH. 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 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. The final of the top 5 mycotoxins is ZON. 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 ZON (Figure 1) contains a phenolic resorcyclic acid lactone (Hussein and Brasel, 2001; Richard, 2007). This structure plays a role in ZON'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 ZON, 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, ZON 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 ZON can disrupt reproductive processes in pre-pubertal, cycling, and pregnant animals (Chen et al., 2008; Cheng et al., 2006). 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 ZON 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 (CAST, 2003; Hussein and Brasel, 2001; Richard, 2007). Recently, ZON has been linked to stimulating growth of breast cancer cells in humans which have estrogen response receptors (Fink-Gremmels, 2006; Hussein and Brasel, 2001). Despite these effects on reproduction, a metabolite of ZON, α-zearalanol, is used as a commercial feed additive for fattening of cattle.

Although the mycotoxin ZON is harmful to reproductive systems, animal mortality of those consuming ZON is not an issue. In the United States there are currently no regulations for the occurrence and contamination of ZON in feeds as risk assessments have indicated that regulatory standards are not needed (CAST, 2003). In the European Union however, there are ZON regulatory levels of up to 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 has indicated that regulations are not currently warranted (CAST, 2003). Some of these minor mycotoxins include T-2 toxin, cyclopiazonic 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 infect 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. Cyclopiazonic 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. Cyclopiazonic 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. Another mycotoxin produced by species of *Penicillium* and *Aspergillus* is patulin (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 source 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 in laboratory settings. 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 is one of the oldest known mycotoxicoses. The fungi which produce this mycotoxin thrives in several grasses, most commonly tall

fescue (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 these animals most at risk. Fescue toxicosis in cattle 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 alkaloids 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 small 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 1000 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, ZON, 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 ZON.

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).

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 growth of grains. In the U.S., 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 (Bhatnagar et al., 2004; CAST, 2003). 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 ZON 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 also follows a similar trend, where early harvested crops have reduced mycotoxin contamination. Early maturation can allow for crops to be harvested at the beginning of the season, which decreases 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 bacteria 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 limit access to the corn's simple sugars needed for fungus 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 outcompete the toxin producing form (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 ZON. 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.

Physical methods of grain detoxification are also beneficial, as contaminated kernels can be mechanically separated 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 shows that these toxins may transform into more harmful mutagens during this process (CAST, 2003). Mycotoxins can also be efficiently extracted from grains through the use of certain solvents, including 90% aqueous acetone, 80% isopropanol, henane-ethanol, hexane-methanol, as well as several others (CAST 2003). The effectiveness of this process is demonstrated by the ethanol industry, where the byproduct dried distillers' grain and solubles (DDGS) can have a 3 fold higher concentration of mycotoxins than the original corn (Wu and Munkvold, 2008). Despite the effectiveness 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). Ozonization, 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

chemical detoxifiers can significantly reduce mycotoxin contamination, but they are only effective if they are completely distributed throughout the entire feed.

Feed additives. 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 minimized.

Adsorbing agents primarily include natural clay products and yeasts (EFSA, 2009). Silicate products, known as aluminosilicates, are the largest group of mycotoxin adsorbing materials. These clays are materials containing 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 animal's gut. Aluminosilicates have particular affinity for the polar mycotoxin AF, although some clays can also have a binding capacity for other mycotoxins (Huwig et al., 2001; Sabater-Vilar et al., 2007; Thieu et al., 2008). For clay materials, the efficiency of adsorption

depends on mycotoxin concentration in the feed, as well as animal weight gain and feed intake. Several types of aluminosilicate clays exist, including bentonites, montmorillonites, zeolites, and hydrated sodium calcium aluminosilicates (**HSCAS**) (EFSA, 2009; Jones et al., 2007).

One of these clays, 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 (EFSA, 2009; Ramos et al., 1996). Montmorillonites have a strong affinity for AF B1, but have also been shown to attract ZON (Lemke et al., 1998; Ramos et al., 1996).

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

The material HSCAS also has a high affinity for AF B1, acting like a sponge to absorb this toxin in the gastro-intestinal tract of animals (Huwig et al., 2001). Hydrated sodium calcium aluminosilicate is a phyllosilicate that contains positive charge deficiencies that create a potential for adsorbing positively charges compounds, such as AF (Ramos et al., 1996). 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 (Huwig et al., 2001). Although HSCAS is commercially sold 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 HSCAS is very effective at preventing aflatoxicosis, it 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 have an affinity for DON, OCH and ZON, and rapidly bind to the toxins to which they come in contact (Huwig et al., 2001). One strain, *Trichosporon mycotoxinivorans,* is a yeast that can detoxify OCH by cleavage of the phenylalanine moiety to form the derivate OCH alpha

(Schatzmayr et al., 2006). This metabolite is virtually nontoxic compared to the parent compound. 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 ZON per gram of yeast cell wall product (Huwig et al., 2001).

Bacteria can also act as mycotoxin detoxifiers, although these act as mycotoxin biotransforming agents (EFSA, 2009; Huwig et al., 2001). 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 trichothecenes (DON), OCH, and ZON 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 (EFSA, 2009; Schatzmayr et al., 2006). 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 ZON by 80 to 90% (EFSA, 2009; Schatzmayr et al., 2006). 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 and DON are among the most concerning for swine, since swine are the most sensitive species and these two mycotoxins are extremely prevalent in the United States on corn, barley, and wheat grains (Goyarts et al., 2005; Meissonnier et al., 2008; Richard, 2007; Sabater-Vilar et al., 2007). 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). Also, since swine are the most susceptible to both of these mycotoxins, the negative effects of AF and DON can be very damaging for swine production. In past years, the financial losses in the Southeastern Unites States due to the mycotoxin AF totaled $97 million for grain producers and another $100 million in production losses for hog farmers (Hussein and Brasel, 2001). Production losses can be due to reduced growth, sickness due to immune suppression, or death of the animal.

Effects of AF and DON Consumption

Ingestion of mycotoxins by swine can have severe impacts on health (Binder et al., 2007; CAST, 2003). Above and below FDA action level of 200 µg/kg AF and allowance levels of 5 mg/kg DON, these toxins can cause multiple problems such as weight loss, decreased growth, organ damage, immune suppression, and death (Chen et al., 2008; FDA, 1994; Richard, 2007; Sabater-Vilar et al., 2007). These effects are detrimental to the swine industry.

The consumption of AF and DON by pigs is shown to decrease body weight at both low and high concentrations of 140 to 3,000 µg/kg AF (Harvey et al., 1989; Marin et al., 2002; Thieu et al., 2008; van Heugten et al., 1994) and 1000 to 4600 µg/kg DON (Cheng et al., 2006; Harvey et al., 1989; Smith et al., 1997; Swamy et al., 2002). Dersjant-Li et al. (2003) showed that AF at 300 µg/kg and DON at 600 µg/kg can each cause 5% reductions the in growth of pigs. ADG of swine can also be reduced after consuming feed containing a range of 200 to 800 µg/kg AF (Schell et al., 1993; Thieu et al., 2008) or 210 to 9,570 µg/kg DON (Cheng et al., 2006; Doll et al., 2003; Goyarts et al., 2005; Tiemann et al., 2006). Feed intake is also reduced when pigs consume contaminated feed with high concentrations (≥ 200 µg/kg AF and ≥ 500 µg/kg DON) of both mycotoxins (Cheng et al., 2006; Goyarts et al., 2005; Rotter at al., 1994; Smith et al., 1997; Swamy et al., 2002; Thieu et al., 2008; van Heugten et al., 1994). Feed intake can decrease by 3.5% when pigs are fed diets with AF as low as 140 µg/kg (van Heugten et al., 1994). Feed efficiency is not commonly altered by the

mycotoxins AF and DON, but can occasionally be reduced as well (Doll et al., 2003; Harvey et al., 1991; Smith et al., 1997; Swamy et al., 2002; van Heugten et al., 1994).

A pig's immune system is greatly altered by AF and DON contamination of feed. Harvey et al. (1989) showed that high AF ingestion can result in an increase in white blood cell numbers above normal range when pigs are fed 3,000 µg/kg AF for 28 days. Increased monocyte and hematocrit levels for pigs fed 2,200 to 2,500 µg/kg DON have also been reported (Pinton et al., 2008). 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 can cause an increase in serum IgA and IgM (Goyarts et al., 2005; Pinton et al., 2008; Swamy et al., 2002; Tiemann et al., 2006). Other research has shown that low mycotoxins concentrations of 140 to 280 µg/kg AF or 280 to 840 µg/kg DON cause no effects on immunoglobulin subsets in pigs (Accensi et al., 2006; van Heugten et al., 1994). When the immune system is challenged by mycotoxins, problems can arise as there may be an increased incidence of secondary disease infections (Fink-Gremmels, 2008).

Organ damage is another common problem in pigs consuming AF and DON. The mycotoxin AF is known to target the liver, and DON the kidneys (Richard, 2007). High AF (3,000 µg/kg) in the diet is shown to cause liver lesions and hepatocellular cytoplasmic vacuolation with early portal fibrosis and bile duct hyperplasia (Harvey et al., 1991). It is previously documented that 1,000 µg/kg DON causes damage including necrosis, blood vessel thickening and hemorrhage (Chen et al., 2008; Cheng et al., 2006). It is not well

documented whether low concentrations of these two mycotoxins will cause organ damage, but it is possible that low AF and DON are still toxic to internal organs if consumed chronically.

Use of Feed Additives to Reduce Mycotoxin Effects

The use of feed additives is a promising method to reduce the negative effects of mycotoxins that are ingested by pigs. Quang et al. (2008) found that the addition of bentonite clay to piglet diets containing 200 µg/kg AF could reduce mycotoxin effects. When supplemented at 0.4 % of the diet for the 41 day trial period, bentonite increased BW, ADG, and G:F compared to a diet containing only AF. Sodium bentonite at 0.5 % was also found to improve ADG and ADFI of pigs consuming a highly contaminated diet with 800 µg/kg AF (Lindemann et al., 1993). The clay HSCAS is also shown to be capable of ameliorating the toxic effects of AF on growth performance, as well as biochemical and histological parameters (Ramos et al., 1996).

Supplementation of a yeast cell wall product to the diets of young swine has shown to provide mycotoxin ameliorating effects. 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 ZON (Swamy et al., 2002). This yeast cell wall product also prevented an increase in IgA and IgM concentrations. The addition of enzyme additives have also shown to be beneficial in reducing mycotoxin effects. 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 ZON, 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 increase, cytokine production, and tissue damage (Cheng et al., 2006).

Summary

Mycotoxicosis in animals is a global issue, and there is increasing concern for effects of mycotoxins on animal health and well being. The swine industry is certainly one production system which is affected by mycotoxins because swine are often the most sensitive commodity species to these mycotoxins. Although acute ingestion of high levels of contamination can be very harmful to the animal, long term consumption of low concentrations of mycotoxins can also be damaging. The mycotoxins AF and DON are of most concern as they are commonly found on grains throughout the United States and the world. When these mycotoxins are consumed, pigs and other animals can have reduced growth, organ damage, and immune challenges. Determining a sustainable way to combat the global mycotoxin problem is important economically for farmers and producers.

Literature Cited

Accensi, F., P. Pinton, P. Callu, N. Abella-Bourges, J. -F. Guelfi, F. Grosjean, and I. P. Oswald. 2006. Ingestion of low doses of deoxynivalenol does not affect hematological, biochemical, or immune responses of piglets. J. Anim. Sci. 84:1935-1942.

Agag, B. I. 2004. Mycotoxins in foods and feeds: aflatoxins. Ass. Univ. Bull. Environ. Res. 7:173-205.

Anderson, D., J. D. Anderson, and J. Sawyer. 2008. Impact of the ethanol boom on livestock and dairy industries: what are they going to eat? J. Agric. App. Econ. 40:573-579.

Baker, A., E. Allen, and H. Lutman. 2010. Corn use for ethanol increased this month. *Feed Outlook*, Economic Research Service, USDA. FDS-10b, February 16, 2010.

Bhatnagar, D., G. A. Payne, T. E. Cleveland, and J. F. Robens. 2004. Mycotoxins: current issues in U.S.A. Page 275-280 in Meeting the Mycotoxin Menace, D. Barug, H. van Egmond, R. Lopez-Garcia, T. van Osenbruggen, and A. Visconti, ed. Wageningen Academic Publishers, The Neatherlands.

Binder, E. M., L. M. Tan, L. J. Chin, J. Handl, and J. Richard. 2007. Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. Anim. Feed Sci. Technol. 137:265-282.

CAST. 2003. Mycotoxins: risks in plant, animal, and human systems. Council for Agri. Sci. Technol. Task Force Report No. 139. Ames, IA.

Chen, Z. Y., R. L. Brown, J. S. Russin, A. R. Lax, and T. E. Cleveland. 1999. A corn trypsin inhibitor with antifungal activity inhibits *Aspergillus flavus* α–amylase. Phytopathol. 89:902-907.

Chen, F., Y. Ma, C. Xue, J. Ma, Q. Xie, G. Wang, Y. Bi, and Y. Cao. 2008. The combination of deoxynivalenol and zearalenone at permitted feed concentrations causes serious physiological effects in young pigs. J. Vet. Sci. 9:39-44.

Cheng, Y. H., C. F. Weng, B. J. Chen, and M. H. Chang. 2006. Toxicity of different Fusarium mycotoxins on growth performance, immune responses and efficacy of a mycotoxin degrading enzyme in pigs. Anim. Res. 55:579–590.

Cotty, P. J., and R. Jaime-Garcia. 2007. Influences of climate on aflatoxin producing fungi and aflatoxin contamination. Int. J. Food Microbiol. 199:109-115.

Dersjant-Li, Y., M. W. A. Verstegen, and W. J. J. Gerrits. 2003. The impact of low concentrations of aflatoxin, deoxynivalenol or fumonisin in diets on growing pigs and poultry. Nutr. Res. Rev. 16:223–239.

Diener, U. L., R. J. Cole, T. H. Sanders, G. A. Payne, L. S. Lee, and M. A. Klich. 1987. Epidemology of aflatoxin formation by *Aspergillus flavus*. Ann. Rev. Phytopathol. 25:249-270.

Doll, S., S. Da Nicke, K. H. Ueberschar, H. Valenta, U. Schnurrbusch, M. Ganter, F. Klobasa, and G. Flachowsky. 2003. Effects of graded levels of Fusarium toxin contaminated maize in diets for female weaned piglets. Arch. Anim. Nutr. 57:311 – 334.

EFSA. 2009. Review of mycotoxin-detoxifying agents used as feed additives: mode of action, efficacy and feed/food safety. Sci. Report, Parma, Italy.

FAO. 2003. Worldwide regulation for mycotoxins in food and feed in 2003. Food and Agriculture Organization of the United Nations, Rome, Italy.

FDA. 1994. FDA regulatory guidance for toxins and contaminants. Food and Drug Administration, Silver Spring, MD.

Fink-Gremmels, J. 2006. Moulds and mycotoxins as undesirable substances in animal feeds. Page 37-50 in The Mycotoxin Factbook, D. Barug, D. Bhatnagar, H. P. van Egmond, J. W. van der Kamp, W. A. van Osenbruggen, and A. Visconti, ed. Wageningen Academic Publishers, The Netherlands.

Gourama, H., and L. B. Bullerman. 1995. Aspergillus flavus and Aspergillus parasiticus: aflatoxigenic fungi of concern in foods and feeds: a review. J. Food Prot. 58:1395-1404.

Goyarts, T., S. Danicke, H. J. Rothkotter, J. Spilke, U. Tiemann, and M. Schollenberger. 2005. On the effects of a chronic deoxynivalenol intoxication on performance, haematological and serum parameters of pigs when diets are offered either for ad libitum consumption or fed restrictively. J. Vet. Med. 52:305–314.

Harvey, R. B., L. F. Kubena, W. E. Huff, D. E. Corrier, D. E. Clark, and T. D. Phillips. 1989. Effects of aflatoxin, deoxynivalenol, and their combinations in the diets of growing pigs. Am. J. Vet. Res. 40:602-607.

Harvey, R. B., L. F. Kubena, M. H. Elissalde, D. E. Corrier, W. E. Huff, G. E. Rottinghaus, and B. A. Clement. 1991. Cocontamination of swine diets by aflatoxin and diacetoxyscirpenol. J. Vet. Diagn. Invest. 3:155-160.

Hussein, H. S., and J. M. Brasel. 2001. Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxocol. 167:101-134.

Huwig, A., S. Freimund, O. Kappeli, and H. Dutler. 2001. Mycotoxin detoxification of animal feed by different absorbents. Toxicol. Lett. 122:179-188.

Johnson, R. J. 2010. Red meat exports to strengthen in 2010. *Livestock, Dairy and Poultry Outlook*, Economic Research Service, USDA. LDP-M-187, January 20, 2010.

Jones, F. T., M. B. Genter, W. M. Hagler, J. A. Hansen, B. A. Mowrey, M. H. Poore, and L. W. Whitlow. 2007. Understanding and coping with effects of mycotoxins in livestock feed and forage. North Carolina Cooperative Extension Service, Raleigh, NC.

Krogh, P. 1977. Ochratoxins. Page 489–498 in Mycotoxins in Human and Animal Health, J. V. Rodricks, C. W. Hesseltine, and M. A. Mehlman, ed. Pathotox Publishers, Inc., Park Forest South, IL. .

Krska, R., and E. Welzig. 2006. Mycotoxin analysis: an overview of classical, rapid and emerging techniques. Page 225-247 in The Mycotoxin Factbook, D. Barug, D. Bhatnagar, H. P. van Egmond, J. W. van der Kamp, W. A. van Osenbruggen, and A. Visconti, ed. Wageningen Academic Publishers, The Netherlands.

Lemke, S. L., P. G. Grant, and T. D. Phillips. 1998. Adsorption of zearalenone by organophilic montmorillonite clay. J. Agric. Food Chem. 46:3789-3796.

Lindemann, M. D., D. J. Blodgett, E. T. Kornegay, and G. G. Schurig. 1993. Potential ameliorators of aflatoxicosis in weanling/growing swine. J. Anim. Sci. 71:171-178.

Magan, N., and D. Aldred. 2007. Post-harvest control strategies: minimizing mycotoxins in the food chain. Int. J. Food Microbiol. 119:131-139.

Marases, W. F., W. C. A. Gelderblom, G. S. Shephard, and H. F. Vismer. 2008. Mycotoxins: a global problem. Page 29-39 in Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade, J. F. Leslie, R. Bandyopadhyay, and A. Visconti, ed. CAB International, Oxfordshire, UK.

Marin, D. E., I. Taranu, R. P. Bunaciu, F. Pascale, D. S. Tudor, N. Avram, M. Sarca, I. Cureu, R. D. Criste, V. Suta, and I. P. Oswald. 2002. Changes in performance, blood parameters, humoral and cellular immune responses in weanling piglets exposed to low doses of aflatoxin. J. Anim. Sci. 80:1250-1257.

Meissonnier, G. M., P. Pinton, J. Laffitte, A. Cossalter, Y. Y. Gong, C. P. Wild, G. Bertin, P. Galtier, and I. P. Oswald. 2008. Immunotoxicity of aflatoxin B1: Impairment of the cell-mediated response to vaccine antigen and modulation of cytokine expression. Toxicol. Appl. Pharmacol. 231:142–149.

Murray, R. K., D. A. Bender, K. M. Botham, P. J. Kennelly, V. W. Rodwell, and P. A. Weil. 2009. Harper's illustrated biochemistry. 28th ed. Mc-Graw Hill Co., New York, NY.

Papaioannou, D.S., Kyriakis, C.S., Papasteriadis, A., Roumbies, N., 2002. A field study on the effect of in-feed inclusion of a natural zeolite (clinoptilolite) on health status and performance of sows/gilts and their litters. Res. Vet. Sci. 72:51-59.

Pascale, M., and A. Visconti. 2008. Overview of detection methods for mycotoxins. Page 171-183 in Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade, J. F. Leslie, R. Bandyopadhyay, and A. Visconti, ed. CAB International, Oxfordshire, UK.

Payne, G. A. 1998. Process of contamination by aflatoxin-producing fungi and their impact on crops. Page 279-306 in Mycotoxins in Agriculture and Food Safety, K. K. Sinha, and D. Bhatnagar, ed. Marcel Dekker, New York, NY.

Pestka, J. J. 2007. Deoxynivalenol: toxicity, mechanisms and animal health risks. Anim. Feed Sci. Technol. 137:283-298.

Pinton, P., F. Accensi, E. Beauchamp, A. Cossalter, P. Callu, F. Grosjean, and I. P. Oswald. 2008. Ingestion of deoxynivalenol (DON) contaminated feed alters the pig vaccinal immune responses. Toxicol. Lett. 177:215–222.

Plain, R. 2008. High feed prices and record slaughter brings red ink. Page 6-12 in National Hog Farmer 2008 State of the Industry Report. Penton Media, Minneapolis, MN.

Ramos, A-J., J. Fink-Gremmels, and E. Hernandez. 1996. Prevention of toxic effects of mycotoxins be means of nonnutritive adsorbent compounds. J. Food Prot. 59:631-641.

Richard, J. L. 2007. Some major mycotoxins and their mycotoxicoses: an overview. Int. J. Food Microbiol. 119:3-10.

Rotter, B. A., B. K. Thompson, M. Lessard, H. L. Trenholm, and H. Tryphona. 1994. Influence of low-level exposure to *Fusarium* mycotoxins on selected immunological and hematological parameters in young swine. Fund. Appl. Toxicol. 23:117-124.

Sabater-Vilar, M., H. Malekinejad, M. H. J. Selman, M. A. M. van der Doelen, and J. Fink-Gremmels. 2007. In vitro assessment of absorbents aiming to prevent deoxynivalenol and zearalenone mycotoxins. Mycopathol. 163:81-90.

Schatzmayr G., F. Zehner, M. Taubel, D. Schatzmayr, A. Klimitsch, A.P. Loibner, and E.M. Binder. 2006. Microbiologicals for deactivating mycotoxins. Mol. Nutr. Food Res. 50:543-551.

Schell, T. C., M. D. Lindemann, E. T. Kornegay, D. J. Blodgett, and J. A. Doerr. 1993. Effectiveness of different types of clay for reducing the detrimental effects of aflatoxin-contaminated diets on performance and serum profiles of weanling pigs. J. Anim. Sci. 71:1226-1231.

Smith, T. K. 1980. Influence of dietary fiber, protein and zeolite on zearalenone toxicosis in rats and swine. J. Anim. Sci. 50:278-285.

Smith, T. K., E. G. McMillan, and J. B. Castillo.1997. Effect of feeding blends of Fusarium mycotoxin-contaminated grains containing deoxynivalenol and fusaric acid on growth and feed consumption of immature swine. J. Anim. Sci. 75:2184-2191.

Stillman, R., M. Haley, and K. Mathews. 2009. Grain prices impact entire livestock production cycle. Amber Waves 7:24-27.

Swamy, H. V. L. N., T. K. Smith, E. J. MacDonald, H. J. Boermans, and E. J. Squires. 2002. Effects of feeding a blend of grains naturally contaminated with Fusarium mycotoxins on swine performance, brain regional neurochemistry, and serum chemistry and the efficacy of a polymeric glucomannan mycotoxin adsorbent. J. Anim. Sci. 80:3257-3267.

Taranu, I., D. E. Marin, S. Bouhet, F. Pascale, J. Bailly, J. D. Miller, P. Pinton, and I. P. Oswald. 2005. Mycotoxin fumonisin B1 alters the cytokine profile and decreases the vaccinal antibody titer in pigs. Toxicol. Sci. 84:301-307.

Thieu, N. Q., B. Ogle, and H. Pettersson. 2008. Efficacy of bentonite clay in ameliorating aflatoxicosis in piglets fed aflatoxin contaminated diets. Trop. Anim. Health Prod. 40:649–656.

Tiemann, U., K. P. Brüssow, L. Jonas, R. Pöhland, F. Schneider, and S. Dänicke. 2006. Effects of diets with cereal grains contaminated by graded levels of two Fusarium toxins on selected immunological and histological measurements in the spleen of gilts. J. Anim. Sci. 84:236-245.

van Heugten, E., J. W. Spears, M. T. Coffey, E. B. Kegley, and M. A. Qureshi. 1994. The effect of methionine and aflatoxin on immune function in weanling pigs. J. Anim. Sci. 72:658-664.

Veldman, B. 2004. Mycotoxins in the animal production chain. Page 275-280 in Meeting the Mycotoxin Menace, D. Barug, H. van Egmond, R. Lopez-Garcia, T. van Osenbruggen, and A.Visconti, ed. Wageningen Academic Publishers, The Netherlands.

Whitaker, T. B, A. B. Slate, and A. S. Johansson. 2005. Sampling Feeds for Mycotoxin Analysis. Page 1-23 in The Mycotoxin Blue Book, D. Diaz, ed. Nottingham University Press, Bath, England.

Wu, F. 2007. Measuring the economic impacts of Fusarium toxins in animal feeds. Anim. Feed Sci. Technol. 137:363-374.

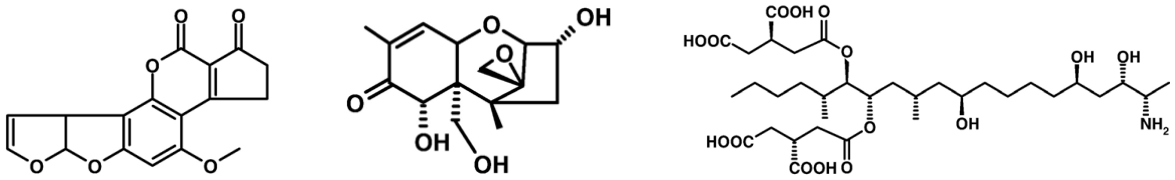
Wu, F., and G. P. Munkvold, 2008. Mycotoxins in ethanol co-products: modeling economic impacts on livestock industry and management strategies. J. Agric. Food Chem. 56:3900-3911.

Yiannikouris, A., J. Francois, L. Poughon, C.-G. Dussap, G. Bertin, G. Jeminet, and J.-P. Jouany. 2004. Adsorption of zearalenone by β-D-glucans in the *Saccharomyces cerevisiae* cell wall. J. Food Prot. 67:1195-1200.

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



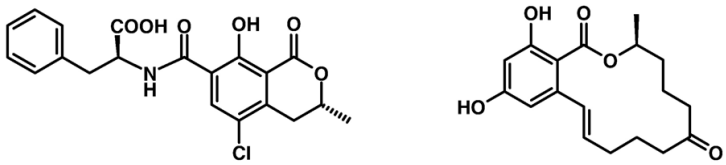


Figure 1. Structures of the major mycotoxins (adapted from Richard, 2007)

CHAPTER 2

EFFECTS OF CHRONIC EXPOSURE OF DIETS WITH LOW LEVELS OF AFLATOXIN AND DEOXYNIVALENOL ON GROWTH AND IMMUNE STATUS OF PIGS

Effects of chronic exposure of diets with low levels of aflatoxin and deoxynivalenol on growth and immune status of pigs

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ABSTRACT

This study investigated the growth and immune responses of pigs fed diets containing low concentrations of aflatoxin (**AF**) and deoxynivalenol (**DON**) from naturally contaminated corn. Sixty gilts (13.9 ± 0.2 kg BW) were randomly assigned to 4 treatments (5 replicates per treatment and 3 pigs per pen): **A** (a control diet without detectable AF and DON); **B** (a diet with 60 μg/kg AF and 300 μg/kg DON); **C** (a diet with 120 μg/kg AF and 600 μg/kg DON); and **D** (a diet with 180 μg/kg AF and 900 μg/kg DON). Pigs were allowed ad libitum access to feed and water for 33 d. Feed intake and BW were measured weekly and pigs were bled (8 mL) on d 33 to measure the numbers of blood cells, to conduct liver function tests, and to measure immunological parameters including IgG, IgM, interferon gamma, IL4, IL6, and tumor necrosis factor alpha (**TNFα**). One pig representing the average BW of each pen was euthanized to obtain the liver, kidneys, and spleen for weight, tissue color measurement and histological evaluation of tissue damage. Pigs in C and D tended to have reduced ADG (P = 0.058, 0.43, and 0.41 vs. 0.52 kg/d) and ADFI (P = 0.061, 0.92, and 0.88 vs. 1.04 kg/d) when compared to A. White blood cell count of pigs in D (23.4 x10^3 cells/μL) was greater (P < 0.05) than those in A, B and C (18.4, 18.5, and 16.8 x10^3 cells/μL). Serum TNFα

concentration of pigs in D (335 pg/mL) was greater ($P < 0.05$) than A and C (299 and 290 pg/mL). Pigs in B and D had greater ($P < 0.05$) fibrosis in liver tissues than those in A. Collectively this study shows that diets containing AF greater than 60 μg/kg and DON greater than 300 μg/kg may reduce growth and decrease feed intake, whereas diets containing 120 μg/kg AF and 600 μg/kg DON may result in altered immune health, systemic inflammation, and partial liver damage causing further reduction in growth of pigs.

Key words: aflatoxin, deoxynivalenol, growth, immune, pigs

Introduction

Corn is a highly demanded commodity and the most widely available feed energy source in the U.S. Swine producers are often limited to using corn that is contaminated with mycotoxins (Anderson et al., 2008; Wu and Munkvold, 2008). Mycotoxins are toxic metabolites of fungi commonly found on cereal grains (Binder et al., 2007). Of 300 toxins, aflatoxin (**AF**) and deoxynivalenol (**DON**) are of particular concern as they are prevalent in the U.S. (Richard, 2007; Sabater-Vilar et al., 2007). A survey from the NC Cooperative Extension found that 34% of corn tested contained more than 20 µg/kg AF and over 60% of feed contained DON (Jones et al., 2007).

Ingestion of mycotoxins by swine can have severe impacts on health, causing weight loss, decreased growth and production, liver and kidney damage, immune suppression, and death (Binder et al., 2007; CAST, 2003; Chen et al., 2008; FDA, 1994; Richard, 2007; Sabater-Vilar et al., 2007). For both mycotoxins, swine tend to be the most sensitive commodity species (Goyarts et al., 2005; Meissonnier et al., 2008). For large pork producing states, such as North Carolina, these mycotoxins are of great concern.

Few studies have examined the combined effects of low concentrations of AF and DON in naturally infected corn. Contamination by AF and DON often indicates the presence of other toxins as well because similar fungi may grow together. Natural infection models are of extreme importance especially at modest levels of contamination. It is hypothesized that pigs eating diets with low AF and DON may not be critically affected, but may have reduced weight gain, feed intake, and weakened immunity. The objectives of this study were

to investigate growth and immune responses of pigs consuming low concentrations of AF and DON together from naturally contaminated corn, and also to determine the minimum concentrations of these mycotoxins that negative effects cannot be observed.

Materials and Methods

Animals

Sixty gilts (American Landrace, 48.8 ± 1.0 d old) were obtained from Murphy Brown, LLC. (Warsaw, NC). They were housed in solid concrete floor indoor pens (1.42 m x 3.86 m) at the North Carolina Swine Evaluation Station (Clayton, NC) during the spring of 2009. Pigs were acclimatized for 6 d, and were then blocked into 5 groups based on their BW, before being randomly assigned to 4 treatments within each group. Initial average BW was 13.9 ± 0.2 kg. Each treatment had 5 replicates with 3 pigs per pen. Body weights were measured weekly on d 0, 7, 14, 21, 28, and 33. The protocol for the use of animals in this study was approved by the North Carolina State University Animal Care and Use Committee.

Experimental Diets

Corns naturally contaminated with AF and DON were used in this study to achieve targeted concentrations of each mycotoxin. Corn with an AF level of 270 µg/kg was obtained from Snow Hill, NC and corn with DON at 5,000 µg/kg was obtained from Manchester, NC. These two corn sources were used to manufacture experimental diets (Table 1) for this study. Samples of corn and diets were taken by subsampling from at least 10 different locations to obtain accurate mycotoxin concentrations (Munkvold et al., 2005;

Vincelli et al., 1995; Whitaker et al. 2005). Aflatoxin in corn was measured at Cargill, Inc (Raleigh, NC) using the Aflatest testing system (Vicam, Milford, MA), a monoclonal antibody-based ELISA. Levels of DON and fumonisins in each diet were measured via HPLC at Romer Laboratories (Union, MO).

Pigs were fed experimental diets based on their assigned treatment groups representing: A (a control diet without detectable AF and DON); B (a diet with 60 µg/kg AF and 300 µg/kg DON); C (a diet containing 120 µg/kg AF and 600 µg/kg DON); and D (a diet with 180 µg/kg AF and 900 µg/kg DON). All diets contained levels of toxins below the FDA action levels of 200 µg/kg AF and advisory levels of 5,000 µg/kg DON for finishing swine (FDA, 1994; FAO, 2003). Fumonisin concentrations found in diets (Table 1) were greatly below guidance levels of 20,000 µg/kg, and were thus considered not significant. During the 33 d of diet administration, all pigs had free access to feed and water. Nutrient concentrations met or exceeded requirements suggested from NRC (1998).

Blood Sampling

Blood samples were collected aseptically from the jugular vein on d 0, and again on d 33. Initial bleeding was performed on 20 randomly chosen pigs, and final bleeding included all pigs. Blood was collected in Monovette tubes (Sarstedt, Newton, NC) containing EDTA for homological and antibody analysis. Tubes without anticoagulant were used to collect serum for biochemical and cytokine measurements. Plasma samples were collected after centrifuging (3,000 x *g* for 15 min at 4°C) and stored at -80°C, and serum samples were collected and stored at -20°C until analyzed.

Hematological Measurements

Hematocrit, hemoglobin, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean corpuscular volume, mean platelet volume (**MPV**), platelet number, red blood cell count, red blood cell distribution width, white blood cell (**WBC**) count, neutrophils, lymphocytes, monocytes, eosinophils, basophils and large unstained cells were measured in plasma by the North Carolina State University Diagnostic Laboratories (College of Veterinary Medicine, Raleigh, NC).

Immunoglobulin Subsets

Total concentrations of the immunoglobulin subsets IgG and IgM were measured via ELISA, as described by the manufacturer (Bethyl, Montgomery, TX). Goat anti-pig IgG or goat anti-pig IgM were used as capture antibodies to coat wells. Plasma samples were diluted to 1:80,000 and 1:7,500 for IgG and IgM, respectively. Horseradish peroxidase goat anti-pig IgG or IgM was used as the detection antibody in combination with the TMB peroxidase enzyme substrate. A stop solution of 2 M sulfuric acid (H_2SO_4) was used to stop the enzyme-substrate reaction after 15 min. Absorbance was read at 450 nm using an ELISA plate reader (Synergy HT, Bio-tek instruments) and the KC4 data analysis software. Samples were quantified against the standard curve constructed with known amounts of pig immunoglobulin subset. Detection limits were 7.8 to 500 ng/mL for IgG, and 15.6 to 1,000 ng/mL for IgM.

Cytokine Measurements

Serum interferon gamma (**IFNγ**), IL4, IL6, and tumor necrosis factor alpha (**TNFα**) were measured by ELISA methods. Serum IFNγ concentrations were measured using a commercially available ELISA kit (Pierce Biotechnology, Rockford, IL) following protocol methods. The assay range for IFNγ was 8 to 500 pg/mL, with a sensitivity of < 2 pg/mL. Concentrations of cytokines were analyzed relative to their standard curve of known standard concentrations. Serum IL4 was analyzed using a commercially available ELISA kit (Alpco Immunoassays, Salem, NH). Following the manufactures protocol, standards and samples were added to IL4 antibody coated 96-well plate in combination with biotinylated anti-IL4. Streptavidin horseradish peroxidase and stabilized chromogen were used for detection, and absorbance was measured at 450 nm. The minimal detectable level of IL4 is 2 pg/mL.

Concentrations of IL6 were measured in serum following the manufacturer's procedure (R&D Systems, Minneapolis, MN). Equal volumes of stabilized hydrogen peroxide and stabilized chromogen were mixed, and used for color detection along with a stop solution of diluted hydrochloric acid. Absorbance was measured at 450 and 540 nm. The minimum detectable limit for this ELISA is 10 pg/mL of IL6.

Determination of TNFα concentrations in serum was completed following the manufacturers procedure (Pierce Biotechnology, Rockford, IL). First, 100 µL of 1:2 diluted standard or 100 µL of sample were added to microplate wells coated with capture antibody in conjunction with biotinylated antibody reagent. Detection occurred by the use of horseradish peroxidase (HRP), TMB substrate and a stop solution of 2 M H_2SO_4. Absorbance was read

at 450 nm and 550 nm by an ELISA plate reader and the KC4 data analysis software. Detection limit for TNFα is 5 pg/mL.

Biochemical Serum Assays

Concentrations of alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, calcium, chloride, cholesterol, creatinine, creatine phosphokinase, globulin, glucose, phosphorus, potassium, sodium and urea nitrogen were measured (Antech Diagnostics, Cary, NC) for determination of liver function.

Histological Measurements

Liver, kidney and spleen tissues were collected from 20 pigs (one pig per pen, median BW pig) on d 33 for weight, color and damage evaluation. Tissue color was measured from three locations on each tissue via a Minolta Colorimeter, which measured values of lightness (L*), redness (a*) and yellowness (b*). Samples from the liver, kidneys and intestine were fixed in 10% buffered formalin, and sent to the North Carolina State University Histopathology Laboratory (College of Veterinary Medicine, Raleigh, NC) for hematoxylin and eosin (H & E) staining and observation of tissue damage. Liver damage can include hydropic degeneration, vacuolation, necrosis, inflammation, megakaryosis, fibrosis, and bile ductule hyperplasia. Possible kidney damage includes vacuolation, necrosis, inflammation, regeneration, protein casts, and fibrosis. Microscopic examinations of damage were measured on the degree of change observed, with values: 1. normal to minimal damage (0 to 5%); 2. mild (5 to 15%); 3. moderate (15 to 40%); 4. severe (higher than 40%).

Statistical Analysis

Data were analyzed using the GLM procedures of SAS (SAS Inst., Inc., Cary, NC) following a randomized complete block design. A pen was considered as the experimental unit. Separation of means was done using the PDIFF option of SAS. Probability values less than 0.05 were considered statistically significant and between 0.05 and 0.10 as trends.

Results

Growth Performance

Initial BW of pigs did not differ ($P = 0.971$) among treatments. During the first 14 d, BW of pigs did not differ among treatments (Table 2). At the end of d 21, pigs in C and D tended to have a decreased BW ($P = 0.051$) compared with pigs in A by 7.7 and 9.9%, respectively, whereas BW did not differ among pigs in B, C, and D. At the end of d 28, pigs in C and D tended to have reduced BW ($P = 0.054$) in contrast to A by 8.7 and 10.9%, respectively, and BW did not differ among pigs in B, C, and D. At the end of study (d 33), pigs in C and D tended to have decreased BW ($P = 0.084$) from pigs in A by 8.6 and 11.5%, respectively, whereas pigs in B, C, and D did not differ.

Average daily gain of pigs in C and D was reduced ($P < 0.05$) compared with A by 18.4 and 23.7%, during the first 7 d, whereas ADG of pigs in B, C, and D did not differ. Average daily gain of pigs did not differ among treatments during d 7 to 14. Average daily gain of pigs in C and D was decreased ($P < 0.05$) compared with A by 19.3 and 33.3% during d 14 to 21, while ADG of pigs in B, C, and D did not differ. Treatments did not differ for

ADG during d 21 to 33. However, during the entire 33 d period, ADG of pigs in C and D tended to be reduced ($P = 0.058$) compared with those in A by 17% and 21%, respectively. Throughout this time, the ADG of pigs in B, C, and D did not differ.

Average daily feed intake of pigs did not differ among treatments during the first 14 d. However, ADFI of pigs in C and D was decreased ($P < 0.05$) from the ADFI of pigs in A by 15.9 and 20.6% during d 14 to 21, and by 11.9 and 18.6% during d 21 to 28. Average daily feed intake of pigs in B, C, and D did not differ during d 21 to 28. No treatment differences were found in ADFI during d 28 to 33. For the entire period however (d 0 to 33), pigs in C and D tended to consume less feed ($P = 0.061$) than pigs in A by 11.5 and 15.4%, respectively, while ADFI of pigs in B, C, and D did not differ.

The gain to feed ratio of pigs in D tended to be reduced ($P = 0.084$) compared with pigs in A by 14.0% throughout the first 7 d of this study. During this time, G:F in B and C pigs did not differ from A. Gain to feed did not differ among treatments during other weeks or during the entire 33 d period.

Hematological Measurements

Pigs fed diet D had a 27.2% increase in WBC numbers ($P < 0.05$) compared with pigs in A, and a 26.5% and 39.3% increase compared with pigs fed B and C diets (Table 3). Pigs fed B and C did not differ from A. Mean platelet volume for pigs in D tended to be greater ($P = 0.064$) than pigs in A and C but not B, whereas treatments B and C did not differ from A. All other blood hematological measurements showed no differences among treatments.

Immunological Evaluation

Immunological parameters IgG, IgM, and IFNγ showed no differences between treatments (Table 4). Concentrations of IL4 and IL6 were not measurable in any treatments. Serum concentrations of TNFα in pigs fed D increased ($P < 0.05$) by 12.1% in contrast to A and 15.5% in contrast to C, whereas it showed no difference to B. Treatments A, B, and C did not differ in TNFα concentrations.

Biochemistry, Histology, Color, and Weight of Internal Organs

Blood biochemistry for liver function test (Table 5) showed that pigs fed D had increased globulin levels by 27.8% ($P < 0.05$) compared with A, whereas B, C, and D did not differ. Treatment D also showed a 58.7% increase ($P < 0.01$) in alkaline phosphatase in comparison to pigs fed A, and a 67.5% and 72.7% increase over B and C pigs, respectively. Treatments A, B, and C did not differ in alkaline phosphatase. Cholesterol measurements showed pigs fed D differed from all treatments, and had a 73.7% increase ($P < 0.01$) compared with A. Sodium concentrations showed a trend ($P = 0.053$) for D to have increased levels compared with pigs in B, but D was not different from A or C diets. All other measurements of serum liver function were not different between treatments.

Weights of the kidney, liver, and spleen were not affected by dietary treatments (Table 6), nor were organ weights as percent of total BW. There were no treatment differences for color scores of the liver or kidneys. Spleen color measurements did not differ among treatments for redness or yellowness, but pigs in D showed a decrease ($P < 0.05$) in

spleen lightness (L*) compared with A, while pigs in B and C were not different from either A or D treatments.

Histological evaluation of the liver of pigs fed diet D showed increased ($P < 0.05$) fibrosis by 50.0% in contrast to A, whereas D did not differ from pigs in B and C (Table 7). Pigs fed C did not differ from A, but those fed B had increased fibrosis compared with A and C. All other measurements of liver damage showed no difference between treatments, and kidney histopathology showed no damage and no difference between treatments.

Discussion

In this study, AF and DON were the chosen mycotoxins because they are commonly found in North American grains such as corn, wheat, and barley (CAST, 2003). When ingested by livestock, these mycotoxins can cause decreased growth, organ damage or immune suppression which may result in increased disease incidence. Together, these effects result in significant economic losses for swine producers.

Low dietary concentrations of AF and DON were chosen in the current study in order to represent amounts which could frequently occur in feed. Though much research has examined the effects of feeding pigs diets containing high AF or DON, few have investigated feeding low concentrations of these mycotoxins as we have done. Our research also aimed to determine the effects of mycotoxins in naturally contaminated corn rather than supplementing purified forms of mycotoxins in the diet. Supplementation of purified sources of mycotoxins to diets can be an accurate way of understanding effects of one specific

mycotoxin, but grains are often found to be contaminated by more than one mycotoxin at a time. This study used two corn sources naturally contaminated with AF and DON in order to simulate what pigs may frequently consume in production conditions.

The consumption of AF by pigs is shown to decrease BW at both low and high concentrations of 140 to 3,000 µg/kg AF (Harvey et al., 1989; Marin et al., 2002; Thieu et al., 2008; van Heugten et al., 1994). Results for DON ingestion show similar outcomes of decreased weight gain in swine (Cheng et al., 2006; Harvey et al., 1989; Smith et al., 1997; Swamy et al., 2002). Dersjant-Li et al. (2003) showed that AF at 300 µg/kg and DON at 600 µg/kg can cause 5% reductions in growth of pigs. In the current study, pigs in diets C and D also showed a trend for reduced BW starting at d 21 of diet administration, and by the final week these pigs had decreased BW by 8.6 and 11.5%. Our results show that there may be a chronic effect of AF and DON on BW because differences among treatments were not observed until after 3 wk (d 21) of diet consumption by pigs.

Pigs in treatments C and D had reduced ADG than A pigs by 11.5 and 15.4%, respectively. Similar results were found when pigs were fed diets with a range of 210 to 9,570 µg/kg DON (Cheng et al., 2006; Doll et al., 2003; Goyarts et al., 2005; Tiemann et al., 2006), and with a range of 200 to 800 µg/kg AF contamination (Schell et al., 1993; Thieu et al., 2008). However, this adverse effect of AF on ADG has not been well documented when AF levels are less than 200 µg/kg. Other research shows varying mycotoxin effects on ADG depending on duration of diet administration. Rotter et al. (1994) found that pigs fed diets with 750 to 3,000 µg/kg DON had decreased ADG during the first week of the study but this

reduction in ADG disappeared after the first week, indicating that pigs may adapt to their DON contaminated diets. Our study shows different results, where feeding 120 to 180 µg/kg AF in combination with 600 to 900 µg/kg DON resulted in reduced ADG during d 0 to 7 and d 14 to 21, and a tendency for pigs fed C and D to have reduced ADG by 17 and 21% during the entire 33 d trial period.

Feeding diets contaminated by multiple mycotoxins may cause a reduction in ADG of pigs, with each mycotoxin influencing growth to a different extent. Dersjant-Li et al. (2003) provided prediction equations for the weight gain reduction contributed from AF ($Y = 15.6 \times X + 12.7$) and DON ($Y = 8.45 \times Z + 1.71$), where Y is a percent reduction of weight gain, X is the amount of AF (mg/kg), and Z is the amount of DON (mg/kg). Using these equations, and considering analyzed values of AF (0.182 mg/kg) and DON (0.768 mg/kg) in diet D of this study, it can be predicted that the percent weight gain reductions would be 15.5% from AF and 8.2% from DON, or 23.7% from both AF and DON together. Thus AF and DON contributed 65% (15.5/23.7) and 35% (8.2/23.7), respectively, of the weight gain reduction. This estimate of total gain reduction (23.7%) from Dersjant-Li et al. (2003) is similar to the 21% reduction of weight gain obtained in our study.

Decreased ADG may be partly due to the reduced ADFI. Contamination of feed by high concentrations ($\geq$ 200 µg/kg AF and $\geq$ 500 µg/kg DON) of both mycotoxins has been shown to cause reduced feed consumption by pigs (Cheng et al., 2006; Goyarts et al., 2005; Rotter at al., 1994; Smith et al., 1997; Swamy et al., 2002; Thieu et al., 2008; van Heugten et al., 1994). Feed intake has also been shown to decrease when pigs are fed diets with low AF

concentrations. van Heugten et al. (1994) found a 3.5% decrease in feed intake when pigs were fed diets with relatively low (140 µg/kg) AF. However, DON alone did not decrease feed intake at a concentration as low as 280 µg/kg (Accensi et al., 2006).

Despite the growth performance differences observed between treatments, feed efficiency was not a contributing factor to changes in BW and feed intake since pigs showed no difference in their gain to feed ratio. This result is typically found to be the case for AF and DON contamination (Doll et al., 2003; Harvey et al., 1991; Smith et al., 1997; Swamy et al., 2002; van Heugten et al., 1994).

Swine WBC numbers can vary greatly. Previous research has shown that healthy pigs age 7 to 16 weeks can have a range of values from 20.1 to 27.3 x 10^3/µL (Chmielowiec-korzeniowska et al., 2008; Feldman et al., 2000; Klem et al., 2009). In these studies, individual WBC values were described to be as low as 16.4 x 10^3/µL and as high as 46.0 x 10^3/µL. Hematological evaluation shows that pigs fed diet D in our study had an increased WBC count by 27.7% compared with pigs fed A. Although 180 µg/kg AF and 900 µg/kg DON increased pig WBC numbers compared with pigs fed no mycotoxins, all pigs in this study had WBC counts falling within the previously described range. Harvey et al. (1989) showed that high AF ingestion can result in an increase in WBC numbers above normal range when pigs were fed 3,000 µg/kg AF for 28 days. DON alone at 3,000 µg/kg did not result in an increase in WBC compared to the control pigs. However, WBC count increased above normal range for pigs fed a diet containing 3,000 µg/kg of both AF and DON. These results by Harvey et al. (1989) indicate that AF may have a stronger effect on WBC count

then DON. Our D diet with 180 µg/kg AF and 900 µg/kg DON falls well below the mycotoxin concentrations used by Harvey et al. (1989), but showed a trend for similar results. We speculate that pigs fed D may have developed WBC counts similar to Harvey et al. (1984) if consumption of low concentrations of mycotoxins had continued over a longer period of time.

In contrast to increased WBC counts found for high mycotoxin ingestion, Marin et al. (2002) observed a decrease in WBC numbers when pigs were fed diets containing as low as 140 µg/kg AF. Diet C in our study had a similar AF concentration (120 µg/kg AF), and also observed a numerical decrease in WBC count although it was not statistically different from A. Low AF and DON may decrease WBCs by causing other problems to the immune system, which may result in decreased WBC production (CAST, 2003). These results show that WBC numbers can be affected in different ways depending on the concentration of mycotoxin, and may in turn cause subsequent effects on the body as a whole.

The only other hematological parameter found to be different between treatments in the current study was MPV. Platelets secrete a large number of substances that are important mediators of inflammation, coagulation, and thrombosis (Chu et al., 2009). The MPV is a measure of platelet size, with larger platelets being more metabolically active with a greater prothrombic potential and increased platelet aggregation. The effects of AF and DON on MPV have not been discussed in previous research, however Accensi et al. (2006) reported that there was no difference in platelet number when feeding 280 µg/kg DON. In our study, pigs fed diet D had increased platelet size over pigs fed A and C diets, whereas platelet

numbers did not differ between treatments. This result indicates that although the number of platelets was similar for all pigs, D treatment pigs may have had more active platelets as they mediate an inflammatory response to the mycotoxins.

A healthy immune system is important for growing pigs, especially in an industry setting where pigs are often challenged by many different pathogens. Deoxynivalenol in particular has powerful effects on the immune system. Many studies have shown that 2,200 to 6,800 µg/kg DON incorporated into the diet cause an increase in serum IgA and IgM, without causing any change in serum IgG concentration (Goyarts et al., 2005; Pinton et al., 2008; Swamy et al., 2002; Tiemann et al., 2006). Contradictory results were shown in other studies when medium to high dietary concentrations of DON from 800 to 3,900 µg/kg, resulted in no change in serum IgA, IgM, or IgG concentrations (Doll et al., 2003). Aflatoxin is not seen to alter immunoglobulin subsets, as shown by Meissonnier et al. (2008), where 385 to 1,807 µg/kg AF did not result in immunoglobulin increase or decrease. In agreement with the results of our study, other research showed no effects on immunoglobulin subsets when pigs consume low concentrations of 140 to 280 µg/kg AF or 280 to 840 µg/kg DON (Accensi et al., 2006; van Heugten et al., 1994).

Serum concentrations of cytokines TNFα, IFNγ, IL4, and IL6 were used to further understand immune system status. Pro-inflammatory cytokines such as TNFα and IL6 are important in tissue macrophage control and inflammatory response, whereas IL4 inhibits macrophage activation (Wood, 2006). The cytokine IFNγ is produced by Th1 lymphocytes and can be involved in cell-mediated immune responses in some cases, and have regulatory

and anti-inflammatory effects in others (Wood and Sawitzki, 2006). The present study showed that serum concentrations of IFNγ did not differ between treatments, and IL4 and IL6 were not measurable for any pigs due to levels being below the reference standard's detection limit. Little research has been conducted to determine the effects of the mycotoxins AF and DON on cytokine production, although Accensi et al. (2006) found that 280 to 840 µg/kg DON did not affect serum concentrations of IL4 or IFNγ in pigs. Concentrations of these two cytokines were not altered in our study, but analysis of TNFα showed that pigs fed D (180 µg/kg AF and 900 µg/kg DON) had increased serum concentrations compared with pigs in A. The increase (12.1%) in serum TNFα may indicate an acute-phase reaction in response to inflammation caused by the toxic effects of AF and DON.

Liver function can be evaluated via blood biochemistry. Research has documented that mycotoxins can alter liver function, though not all results are in agreement. Concentrations of 140 to 280 µg/kg AF were shown to increase globulin levels in one study (Marin et al., 2002), but to decrease gobulin, albumin, and total protein in another (Thieu et al., 2008). Results have shown that 800 to 3,000 µg/kg AF can decrease cholesterol, calcium, magnesium, potassium and phosphorous in some cases (Harvey et al., 1989), but to have no effect on cholesterol, albumin, total protein, calcium, magnesium, potassium, phosphorous or sodium in others (Harvey et al., 1991; Schell et al., 1993). Studies on DON have shown similar results. Rotter et al. (1994) found increased total protein, globulin, calcium and phosphorous at levels from 750 to 3,000 µg/kg DON. Other research has shown

lower total protein, albumin and globulin at 1,000 µg/kg DON (Chen et al., 2008; Cheng et al., 2006), or no change in liver function parameters at 2,200 to 2,500 µg/kg DON (Pinton et al., 2008). Pigs fed diet D in our study had increased globulin, alkaline phosphatase and cholesterol compared with pigs fed A. These elevated measurements may indicate that there is abnormal excretion of liver metabolites due to mild liver damage. With such a wide range of reported data, it is difficult to determine the effects AF and DON have on liver function. However, our results collectively show that a moderate level combination of the mycotoxins AF and DON in swine diets may work together to potentiate an increase in certain biochemical parameters of liver function, whereas one individual mycotoxin may not cause the same effect.

In this study, AF and DON in diets did not alter liver, kidney, or spleen weights. Few studies have analyzed the effects of these two mycotoxins on organ weights. It is documented in one study, that pigs fed 750 to 3,000 µg/kg DON did not have altered organ weights (Rotter et al., 1994). In another study, pigs fed 4,600 µg/kg DON in combination with other mycotoxins such as zeralanone, fusaric acid, and 15-acetyldeoxynivalenol, had a decrease in liver and kidney weights while spleen weight was not affected (Swamy et al., 2002). In contrast, 3,000 µg/kg AF levels have shown to increase liver and spleen weights as a percentage of BW (Harvey et al., 1991). In light of these contrasting results and the data shown in our study, it appears that effects on organ weights by mycotoxins can vary. Alterations may be related to the particular type of mycotoxin, as well as the dose and exposure time of pigs to that mycotoxin.

Tissue color measurements were collected as one way to analyze internal organs for damage. No color differences were seen between treatments for the liver and kidneys, or for redness and yellowness measurements of the spleen. However difference was observed in spleen lightness, where pigs fed D had a lower numerical reading for lightness than A. The lower lightness value indicates a darker color (Konica Minolta, 2007). Though pigs fed 180 µg/kg AF and 900 µg/kg DON had normal redness and yellowness readings compared to A, their spleens were darker in color. Though significant, this color change was minor and was not visually observable. Few others have researched organ tissue color change related to AF and DON, especially at low concentrations. One study found liver color of pigs was altered when fed 2,500 µg/kg AF (Harvey et al., 1991), but due to a lack of comprehensive data it is unknown if pig organ coloring will be affected at both low and high AF or DON levels in feed.

It has previously been documented that 1,000 µg/kg DON causes multi-organ damage including necrosis, blood vessel thickening, and hemorrhage (Chen et al., 2008; Cheng et al., 2006). High AF (3,000 µg/kg) in the diet has also been shown to cause liver lesions and hepatocellular cytoplasmic vacuolation with early portal fibrosis and bile duct hyperplasia (Harvey et al., 1991). The effect of low AF and DON on tissue damage is not well documented, but one study found that at 280 to 840 µg/kg DON, no damage occurred (Accensi et al., 2006). In our study however, pigs fed diet D had increased liver fibrosis after consuming a similar level of DON (900 µg/kg). Conclusions can thus be made that consuming moderate levels of both AF and DON together can potentiate the effects of these

mycotoxins on liver damage. Interestingly, pigs fed B also had increased fibrosis over A. Although this change between treatments is statistically significant, pigs in B and D are both considered to have normal to mild damage (values 1-2). All other measurements of liver and kidney damage in this study were considered normal for all treatments.

Collectively, growth performance of pigs at 10 to 20 kg body weight can be reduced by consuming diets containing a combination of mycotoxins at concentrations as low as 120 µg/kg AF and 600 µg/kg DON. Further analysis showed that the effects on growth were observed after 3 wk of diet administration, indicating AF and DON together can cause chronic rather than acute response on pig growth. Pig immune status can also be challenged by increased inflammatory responses at 180 µg/kg AF and 900 µg/kg DON. These results show a negative impact of low to moderate levels of AF and DON on growth and immune status of pigs. Finally, it is concluded that 60 µg/kg AF and 300 µg/kg DON may be a lower threshold for mycotoxin effects on pig growth (BW and ADG) or immune status (WBC and TNFα) because no statistical differences were seen in comparison to pigs fed the diet A with no mycotoxin contamination.

Literature Cited

Accensi, F., P. Pinton, P. Callu, N. Abella-Bourges, J. -F. Guelfi, F. Grosjean, and I. P. Oswald. 2006. Ingestion of low doses of deoxynivalenol does not affect hematological, biochemical, or immune responses of piglets. J. Anim. Sci. 84:1935-1942.

Anderson, D., J. D. Anderson, and J. Sawyer. 2008. Impact of the ethanol boom on livestock and dairy industries: what are they going to eat? J. Agric. App. Econ. 40:573-579.

Binder, E. M., L. M. Tan, L. J. Chin, J. Handl, and J. Richard. 2007. Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. Anim. Feed Sci. Technol. 137:265-282.

Chen, F., Y. Ma, C. Xue, J. Ma, Q. Xie, G. Wang, Y. Bi, and Y. Cao. 2008. The combination of deoxynivalenol and zearalenone at permitted feed concentrations causes serious physiological effects in young pigs. J. Vet. Sci. 9:39-44.

Cheng, Y. H., C. F. Weng, B. J. Chen, and M. H. Chang. 2006. Toxicity of different Fusarium mycotoxins on growth performance, immune responses and efficacy of a mycotoxin degrading enzyme in pigs. Anim. Res. 55:579–590.

Chmielowiec-korzeniowska, A., M. Babicz, and M. Pyrz. 2008. Levels of hematological parameters of pigs over the fattening period. Annales, Universitatis Mariae Curie-Skłodowska 26:19-24.

Chu, S. G., R. C. Becker, P. B. Berger, D. L. Bhatt, J. W. Eikelboom, B. Konkle, E. R. Mohler, M. P. Reilly, and J. S. Berger. Mean platelet volume as a predictor of cardiovascular risk: a systematic review and meta-analysis. J Thromb Haemost. 2010:148–56.

Dersjant-Li, Y., M. W. A. Verstegen, and W. J. J. Gerrits. 2003. The impact of low concentrations of aflatoxin, deoxynivalenol or fumonisin in diets on growing pigs and poultry. Nutr. Res. Rev. 16:223–239.

Doll, S., S. Da Nicke, K. H. Ueberschar, H. Valenta, U. Schnurrbusch, M. Ganter, F. Klobasa, and G. Flachowsky. 2003. Effects of graded levels of Fusarium toxin contaminated maize in diets for female weaned piglets. Arch. Anim. Nutr. 57:311 – 334.

Dubreuil, P., and H. Lapierre. 1997. Biochemistry reference values for Quebec lactating dairy cows, nursing sows, growing pigs and calves. Can. J. Vet. Res. 61:235-239.

FAO. 2003. Worldwide regulation for mycotoxins in food and feed in 2003. Food and Agriculture Organization of the United Nations, Rome, Italy.

FDA. 1994. FDA regulatory guidance for toxins and contaminants. Food and Drug Administration, Silver Spring, MD.

Feldman, B. F., J. G. Zinkl, and N. C. Jain. 2000. Schalm's Veterinary Hematology. 5th ed. Lippincott Williams & Wilkins, Philadelphia, PA.

Goyarts, T., S. Danicke, H. J. Rothkotter, J. Spilke, U. Tiemann, and M. Schollenberger. 2005. On the effects of a chronic deoxynivalenol intoxication on performance, haematological and serum parameters of pigs when diets are offered either for ad libitum consumption or fed restrictively. J. Vet. Med. 52:305–314.

Harvey, R. B., L. F. Kubena, W. E. Huff, D. E. Corrier, D. E. Clark, and T. D. Phillips. 1989. Effects of aflatoxin, deoxynivalenol, and their combinations in the diets of growing pigs. Am. J. Vet. Res. 40:602-607.

Harvey, R. B., L. F. Kubena, M. H. Elissalde, D. E. Corrier, W. E. Huff, G. E. Rottinghaus, and B. A. Clement. 1991. Cocontamination of swine diets by aflatoxin and diacetoxyscirpenol. J. Vet. Diagn. Invest. 3:155-160.

Jones, F. T., M. B. Genter, W. M. Hagler, J. A. Hansen, B. A. Mowrey, M. H. Poore, and L. W. Whitlow. 2007. Understanding and coping with effects of mycotoxins in livestock feed and forage. North Carolina Cooperative Extension Service, Raleigh, NC.

Klem, T. B., E. Bleken, H. Morberg, S. I. Thoresen, and T. Framstad. 2009. Hematologic and biochemical reference intervals for Norwegian crossbreed grower pigs. Vet Clin. Path.1-6.

Konica Minolta. 2007. Precise color communication: color control from perception to instrumentation. Konica Minolta Sensing, INC, Japan.

Marin, D. E., I. Taranu, R. P. Bunaciu, F. Pascale, D. S. Tudor, N. Avram, M. Sarca, I. Cureu, R. D. Criste, V. Suta, and I. P. Oswald. 2002. Changes in performance, blood parameters, humoral and cellular immune responses in weanling piglets exposed to low doses of aflatoxin. J. Anim. Sci. 80:1250-1257.

Marin, D. E., I. Taranu, R. P. Bunaciu, F. Pascale, D. S. Tudor, N. Avram, M. Sarca, I. Cureu, R. D. Criste, V. Suta, and I. P. Oswald. 2002. Changes in performance, blood parameters, humoral and cellular immune responses in weanling piglets exposed to low doses of aflatoxin. J. Anim. Sci. 80:1250-1257.

Meissonnier, G. M., P. Pinton, J. Laffitte, A. Cossalter, Y. Y. Gong, C. P. Wild, G. Bertin, P. Galtier, and I. P. Oswald. 2008. Immunotoxicity of aflatoxin B1: Impairment of the cell-mediated response to vaccine antigen and modulation of cytokine expression. Toxicol. Appl. Pharmacol. 231:142–149.

Munkvold, G., C. Hurburgh, and J. Meyer. 2005. Aflatoxins in corn. Iowa State University Extension, Ames, IA. NRC. 1998. Nutrient requirements of swine. 10th rev. ed. Natl. Acad. Press, Washington, DC.

Pinton, P., F. Accensi, E. Beauchamp, A. Cossalter, P. Callu, F. Grosjean, and I. P. Oswald. 2008. Ingestion of deoxynivalenol (DON) contaminated feed alters the pig vaccinal immune responses. Toxicol. Letters 177:215–222.

Richard, J. L. 2007. Some major mycotoxins and their mycotoxicoses: an overview. Int. J. Food Microbiol. 119:3-10.

CAST. 2003. Mycotoxins: risks in plant, animal, and human systems. Council for Agri. Sci. Technol. Task Force Report No. 139. Ames, IA.

Rotter, B. A., B. K. Thompson, M. Lessard, H. L. Trenholm, and H. Tryphona. 1994. Influence of low-level exposure to *Fusarium* mycotoxins on selected immunological and hematological parameters in young swine. Fund. Appl. Toxicol. 23:117-124.

Sabater-Vilar, M., H. Malekinejad, M. H. J. Selman, M. A. M. van der Doelen, and J. Fink-Gremmels. 2007. In vitro assessment of absorbents aiming to prevent deoxynivalenol and zearalenone mycotoxins. Mycopathol. 163:81-90.

Schell, T. C., M. D. Lindemann, E. T. Kornegay, D. J. Blodgett, and J. A. Doerr. 1993. Effectiveness of different types of clay for reducing the detrimental effects of aflatoxin-contaminated diets on performance and serum profiles of weanling pigs. J. Anim. Sci. 71:1226-1231.

Smith, T. K., E. G. McMillan, and J. B. Castillo.1997. Effect of feeding blends of Fusarium mycotoxin-contaminated grains containing deoxynivalenol and fusaric acid on growth and feed consumption of immature swine. J. Anim. Sci. 75:2184-2191.

Swamy, H. V. L. N., T. K. Smith, E. J. MacDonald, H. J. Boermans, and E. J. Squires. 2002. Effects of feeding a blend of grains naturally contaminated with Fusarium mycotoxins on swine performance, brain regional neurochemistry, and serum chemistry and the efficacy of a polymeric glucomannan mycotoxin adsorbent. J. Anim. Sci. 80:3257-3267.

Thieu, N. Q., B. Ogle, and H. Pettersson. 2008. Efficacy of bentonite clay in ameliorating aflatoxicosis in piglets fed aflatoxin contaminated diets. Trop. Anim. Health Prod. 40:649–656.

Tiemann, U., K. P. Brüssow, L. Jonas, R. Pöhland, F. Schneider, and S. Dänicke. 2006. Effects of diets with cereal grains contaminated by graded levels of two Fusarium toxins on selected immunological and histological measurements in the spleen of gilts. J. Anim. Sci. 84:236-245.

van Heugten, E., J. W. Spears, M. T. Coffey, E. B. Kegley, and M. A. Qureshi. 1994. The effect of methionine and aflatoxin on immune function in weanling pigs. J. Anim. Sci. 72:658-664.

Vincelli, P., G. Parker, and S. McNeill. 1995. Aflatoxins in corn. University of Kentucky Cooperative Extension Service, Lexington, KY.

Whitaker, T. B, A. B. Slate, and A. S. Johansson. 2005. Sampling Feeds for Mycotoxin Analysis in The Mycotoxin Blue Book, ed. D. Diaz, Nottingham University Press, Bath, England, pp.1-23.

Wood, P. 2006. Understanding Immunology. 2nd ed. Pearson Education Limited, Harlow, England.

Wood, K. J., and B. Sawitzki. 2006. Interferon γ: a crucial role in the function of induced regulatory T cells *in vivo*. Trends Immun. 27:183-187.

Wu, F., and G. P. Munkvold. 2008. Mycotoxins in ethanol co-products: modeling economic impacts on livestock industry and management strategies. J. Agric. Food Chem. 56:3900-3911.

Table 1. Composition of experimental diets (as-fed basis)

	Treatment[1]			
Diet	A	B	C	D
Ingredient, %				
Ground yellow corn	84.67	56.45	28.23	0.00
Ground yellow corn with AF[2]	0.00	22.22	44.44	66.67
Ground yellow corn with DON[3]	0.00	6.00	12.00	18.00
Soybean meal, dehulled	11.90	11.90	11.90	11.90
Salt	0.30	0.30	0.30	0.30
Vitamin premix[4]	0.03	0.03	0.03	0.03
Trace mineral premix[5]	0.15	0.15	0.15	0.15
Dicalcium phosphate	1.00	1.00	1.00	1.00
Ground limestone	0.70	0.70	0.70	0.70
L-Threonine	0.14	0.14	0.14	0.14
L-Lysine HCl	0.46	0.46	0.46	0.46
DL-Methionine	0.08	0.08	0.08	0.08
L-Tryptophan	0.04	0.04	0.04	0.04
L-Valine	0.03	0.03	0.03	0.03
Poultry fat	0.50	0.50	0.50	0.50
Calculated composition				
DM, %	89.5	89.5	89.5	89.5
ME, Mcal/kg	3.37	3.37	3.37	3.37
CP, %	13.3	13.3	13.3	13.3
Lys, %	0.94	0.94	0.94	0.94
Cys + Met, %	0.55	0.55	0.55	0.55
Trp, %	0.17	0.17	0.17	0.17
Thr, %	0.60	0.60	0.60	0.60
Calcium, %	0.59	0.59	0.59	0.59
Available phosphorus, %	0.23	0.23	0.23	0.23
Total phosphorus, %	0.50	0.50	0.50	0.50
Aflatoxin, µg/kg	0	60	120	180
Deoxynivalenol, µg/kg	0	300	600	900
Fumonisin, µg/kg	0	0	0	0
Analyzed composition				
DM, %	89.6	88.0	87.5	87.3
CP, %	13.2	13.0	14.0	14.3
Aflatoxin, µg/kg[6]	0	64	124	182
Deoxynivalenol, µg/kg[6]	0	320	548	768
Fumonisin, µg/kg[6]	0	42	84	128

Table 1. Continued.

[1] A: a diet with no supplemental aflatoxin and deoxynivalenol; B: a diet with supplemental aflatoxin (60 µg/kg) and deoxynivalenol (300 µg/kg); C: a diet with supplemental aflatoxin (120 µg/kg) and deoxynivalenol (600 µg/kg); D: a diet with supplemental aflatoxin (180 µg/kg) and deoxynivalenol (900 µg/kg)

[2] Corn contained 270 µg/kg aflatoxin

[3] Corn contained 5,000 µg/kg deoxynivalenol

[4] The vitamin premix provided the following per kilogram of complete diet: 6613.8 IU of vitamin A as vitamin A acetate; 992.0 IU of vitamin D_3; 19.8 IU of vitamin E; 2.64 mg of vitamin K as menadione sodium bisulfate; 0.03 mg of vitamin B_{12}; 4.63 mg of riboflavin; 18.52 mg of D-pantothenic acid as calcium panthonate; 24.96 mg of niacin; 0.07 mg of biotin

[5] The trace mineral premix provided the following per kilogram of complete diet: 4.0 mg of Mn as manganous oxide; 165 mg of Fe as ferrous sulfate; 165 mg of Zn as zinc sulfate; 16.5 mg of Cu as copper sulfate; 0.30 mg of I as ethylenediamine dihydroiodide; and 0.30 mg of Se as sodium selenite

[6] Dietary mycotoxin concentration analyzed by Romer Laboratories (Union, MO).

Table 2. Growth performance of pigs fed diets containing various concentrations of aflatoxin and deoxynivalenol

	Treatment[1]					
Item	A	B	C	D	SEM	*P* value
Body weight, kg						
d 0	13.93	13.97	14.00	13.85	0.22	0.971
d 7	16.58	16.38	16.19	15.89	0.30	0.447
d 14	19.67	18.95	18.60	18.61	0.44	0.317
d 21	23.62[a]	22.45[ab]	21.79[b]	21.27[b]	0.55	0.051
d 28	27.64[a]	26.06[ab]	25.23[b]	24.63[b]	0.71	0.054
d 33	30.92[a]	29.02[ab]	28.27[b]	27.36[b]	0.90	0.084
Average daily gain, kg/d						
d 0 to 7	0.38[c]	0.35[cd]	0.31[d]	0.29[d]	0.02	0.040
d 7 to 14	0.44	0.37	0.34	0.39	0.03	0.271
d 14 to 21	0.57[c]	0.50[cd]	0.46[de]	0.38[e]	0.04	0.020
d 21to 28	0.58	0.51	0.49	0.48	0.03	0.161
d 28 to 33	0.66	0.59	0.61	0.55	0.07	0.700
d 0 to 33	0.52[a]	0.46[ab]	0.43[b]	0.41[b]	0.03	0.058
Average daily feed intake, kg/d						
d 0 to 7	0.74	0.69	0.66	0.66	0.03	0.201
d 7 to 14	0.92	0.82	0.79	0.81	0.04	0.194
d 14 to 21	1.07[c]	1.01[cd]	0.90[de]	0.85[e]	0.05	0.025
d 21 to 28	1.18[c]	1.10[cd]	1.04[d]	0.96[d]	0.05	0.031
d 28 to 33	1.42	1.34	1.32	1.23	0.08	0.409
d 0 to 33	1.04[a]	0.97[ab]	0.92[b]	0.88[b]	0.04	0.061
Gain:feed						
d 0 to 7	0.50[a]	0.49[a]	0.47[ab]	0.43[b]	0.02	0.084
d 7 to 14	0.49	0.45	0.43	0.48	0.02	0.356
d 14 to 21	0.53	0.50	0.51	0.47	0.02	0.313
d 21to 28	0.50	0.47	0.47	0.50	0.01	0.354
d 28 to 33	0.45	0.44	0.46	0.44	0.03	0.952
d 0 to 33	0.50	0.47	0.47	0.47	0.01	0.138

[1] A: a diet with no supplemental aflatoxin and deoxynivalenol; B: a diet with supplemental aflatoxin (60 µg/kg) and deoxynivalenol (300 µg/kg); C: a diet with supplemental aflatoxin (120 µg/kg) and deoxynivalenol (600 µg/kg); D: a diet with supplemental aflatoxin (180 µg/kg) and deoxynivalenol (900 µg/kg)

[a-b] Means within a row with different superscripts tend to differ ($0.05 \leq P < 0.10$)

[c-e] Means within a row with different superscripts differ ($P < 0.05$)

Table 3. Hematology of pigs fed diets containing various concentrations of aflatoxin and deoxynivalenol

	Treatment[1]					
Item	A	B	C	D	SEM	*P value*
Hematocrit, %	37.70	40.60	41.06	41.34	3.85	0.904
Hemoglobin, g/dL	11.80	12.98	12.86	13.00	1.17	0.865
MCH[2], pg	16.50	16.06	16.00	16.46	0.40	0.701
MCHC[2], g/dL	31.30	32.08	31.38	31.42	0.25	0.166
MCV[2], fL	52.80	44.08	51.00	52.44	3.49	0.300
MPV[2], fL	8.12[a]	8.66[ab]	7.90[a]	9.28[b]	0.35	0.064
Platelet, 10^3/µL	515.4	474.4	467.4	313.0	74.4	0.283
RDW[2], %	19.10	19.32	19.00	19.24	0.58	0.980
RBC[2], 10^6/µL	7.11	8.09	8.04	7.89	0.70	0.740
WBC[2], 10^3/µL	18.4[c]	18.5[c]	16.8[c]	23.4[d]	1.3	0.023
Basophil, 10^3/µL	0.07	0.07	0.06	0.24	0.07	0.246
Eosinophil, 10^3/µL	0.16	0.17	0.22	0.20	0.03	0.489
Lymphocyte, 10^3/µL	12.70	11.13	9.65	13.84	1.63	0.333
Monocyte, 10^3/µL	0.98	0.99	0.63	1.06	0.16	0.269
Neutrophil, 10^3/µL	4.27	6.06	6.10	5.87	0.83	0.386
LUC[2], 10^3/µL	0.17	0.12	0.17	0.26	0.04	0.192

[1] A: a diet with no supplemental aflatoxin and deoxynivalenol; B: a diet with supplemental aflatoxin (60 µg/kg) and deoxynivalenol (300 µg/kg); C: a diet with supplemental aflatoxin (120 µg/kg) and deoxynivalenol (600 µg/kg); D: a diet with supplemental aflatoxin (180 µg/kg) and deoxynivalenol (900 µg/kg)

[2] MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; MPV: mean platelet volume; RDW: red blood cell distribution width; RBC: red blood cells; WBC: white blood cells; LUC: large unstained cells

[a-b] Means within a row with different superscripts tend to differ ($0.05 \leq P < 0.10$)

[c-d] Means within a row with different superscripts differ ($P < 0.05$)

Table 4. Immunological parameters of pigs fed diets containing various concentrations of aflatoxin and deoxynivalenol

	Treatment[1]					
Item	A	B	C	D	SEM	*P value*
IgG[2], mg/mL	9.85	12.04	10.89	14.91	2.28	0.462
IgM[2], mg/mL	1.32	1.20	1.10	1.51	0.31	0.806
IFNγ[2], pg/mL	6.13	4.65	6.03	6.10	1.64	0.900
TNFα[2], pg/mL	298.9[a]	308.0[ab]	290.1[a]	335.1[b]	9.1	0.023

[1] A: a diet with no supplemental aflatoxin and deoxynivalenol; B: a diet with supplemental aflatoxin (60 µg/kg) and deoxynivalenol (300 µg/kg); C: a diet with supplemental aflatoxin (120 µg/kg) and deoxynivalenol (600 µg/kg); D: a diet with supplemental aflatoxin (180 µg/kg) and deoxynivalenol (900 µg/kg)

[2] IgG: immunoglobulin G; IgM: immunoglobulin M; IFNγ: interferon gamma; TNFα: tumor necrosis factor α

[a-b] Means within a row with different superscripts differ ($P < 0.05$)

Table 5. Biochemical blood assays of pigs fed diets containing various concentrations of aflatoxin and deoxynivalenol

	Treatment[1]					
Item	A	B	C	D	SEM	*P value*
Albumin, g/dL	2.66	2.76	2.80	2.98	0.18	0.663
Albumin:Globulin	1.38	1.22	1.20	1.24	0.07	0.354
Alkaline Phosphatase, U/L	214.0[a]	202.8[a]	196.6[a]	339.6[b]	25.8	0.006
ALT[2], U/L	24.60	23.80	20.80	28.40	2.55	0.263
AST[2], U/L	60.60	29.60	32.00	58.60	12.69	0.214
Bilirubin, mg/dL	0.10	0.10	0.10	0.10	0.00	1.000
BUN:Creatinine[2]	6.20	5.00	6.00	6.00	0.62	0.537
Calcium, mg/dL	9.08	9.54	8.58	10.16	0.66	0.408
Chloride, mEq/L	101.6	101.2	99.6	98.6	1.0	0.189
Cholesterol, mg/dL	67.8[a]	85.4[b]	78.4[ab]	117.8[c]	6.1	0.001
CPK[2], U/L	2,464.8	688.2	819.0	809.6	660.5	0.230
Creatinine, mg/dL	0.94	1.00	0.88	0.98	0.06	0.550
Globulin, g/dL	1.94[a]	2.32[b]	2.36[b]	2.48[b]	0.11	0.029
Glucose, mg/dL	135.0	128.0	109.4	116.8	10.1	0.328
Na:K[2]	23.4	21.2	22.0	22.4	1.8	0.853
Phosphorous, mg/dL	9.86	9.22	9.88	9.74	0.30	0.400
Potassium, mEq/L	6.30	6.70	7.04	6.62	0.75	0.920
Sodium, mEq/L	144.2[d]	141.6[e]	142.6[d]	143.6[d]	0.62	0.053
Total protein, g/dL	4.60	5.08	5.16	5.46	0.26	0.180
Urea nitrogen, mg/dL	5.80	5.00	5.20	5.80	0.62	0.729

[1] A: a diet with no supplemental aflatoxin and deoxynivalenol; B: a diet with supplemental aflatoxin (60 µg/kg) and deoxynivalenol (300 µg/kg); C: a diet with supplemental aflatoxin (120 µg/kg) and deoxynivalenol (600 µg/kg); D: a diet with supplemental aflatoxin (180 µg/kg) and deoxynivalenol (900 µg/kg)

[2]ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN:Creatinine: blood urea nitrogen to creatinine ratio; CPK: creatine phosphokinase; Na:K: sodium to potassium ratio

[a-c] Means within a row with different superscripts differ ($P < 0.05$)

[d-e] Means within a row with different superscripts tend to differ ($0.05 \leq P < 0.10$)

Table 6. Tissue analysis of pigs fed diets containing various concentrations of aflatoxin and deoxynivalenol

	Treatment[1]					
Item	A	B	C	D	SEM	*P value*
Organs weight, g						
Liver	827.5	752.3	798.3	796.5	45.9	0.677
Kidney	136.5	137.4	145.5	125.6	9.6	0.556
Spleen	65.7	61.8	56.0	61.9	5.1	0.626
Organs, % of body weight						
Liver	2.72	2.58	2.85	2.83	0.10	0.247
Kidney	0.44	0.47	0.52	0.45	0.03	0.237
Spleen	0.21	0.21	0.21	0.22	0.02	0.918
Color liver						
Lightness (L*)	36.84	36.64	37.04	35.73	0.86	0.715
Redness (a*)	13.70	14.56	13.30	14.67	0.45	0.145
Yellowness (b*)	4.76	5.31	4.54	4.07	0.54	0.469
Color kidney						
Lightness (L*)	47.50	44.62	46.92	45.07	1.45	0.456
Redness (a*)	12.06	15.73	12.15	14.60	1.35	0.196
Yellowness (b*)	8.68	8.32	8.80	8.14	1.01	0.963
Color spleen						
Lightness (L*)	37.38[a]	39.14[ab]	36.88[ab]	35.38[b]	0.76	0.030
Redness (a*)	16.75	17.51	17.13	16.58	0.43	0.458
Yellowness (b*)	0.96	1.87	1.91	1.34	0.41	0.348

[1] A: a diet with no supplemental aflatoxin and deoxynivalenol; B: a diet with supplemental aflatoxin (60 µg/kg) and deoxynivalenol (300 µg/kg); C: a diet with supplemental aflatoxin (120 µg/kg) and deoxynivalenol (600 µg/kg); D: a diet with supplemental aflatoxin (180 µg/kg) and deoxynivalenol (900 µg/kg)

[a-b] Means within a row with different superscripts differ ($P < 0.05$)

Table 7. Histopathology of pigs fed diets containing various concentrations of aflatoxin and deoxynivalenol

	Treatment[1]					
Item	A	B	C	D	SEM	*P value*
Liver[2]						
Bile Ductule Hyperplasia	1.2	1.8	1.6	1.6	0.2	0.303
Fibrosis	1.2[a]	2.0[b]	1.4[ac]	1.8[bc]	0.2	0.014
Hydropic Degeneration	1.8	2.0	2.0	2.2	0.2	0.537
Inflammation	1.0	1.2	1.0	1.0	0.1	0.426
Megakaryosis	1.0	1.2	1.0	1.4	0.1	0.195
Necrosis	1.0	1.0	1.0	1.0	0.0	1.000
Vacuolation	1.2	1.4	1.2	1.4	0.2	0.791
Kidney[2]						
Fibrosis	1.0	1.0	1.0	1.0	0.0	1.000
Inflammation	1.2	1.0	1.0	1.0	0.1	0.426
Necrosis	1.4	1.4	1.3	1.6	0.2	0.777
Protein Casts	1.0	1.0	1.0	1.0	0.0	1.000
Regeneration	1.2	1.0	1.6	1.2	0.2	0.230
Vacuolation	1.4	1.7	1.6	1.4	0.2	0.602

[1] A: a diet with no supplemental aflatoxin and deoxynivalenol; B: a diet with supplemental aflatoxin (60 µg/kg) and deoxynivalenol (300 µg/kg); C: a diet with supplemental aflatoxin (120 µg/kg) and deoxynivalenol (600 µg/kg); D: a diet with supplemental aflatoxin (180 µg/kg) and deoxynivalenol (900 µg/kg)

[2] Liver and kidney tissue microscopic examinations measured on degrees of the change: 1. normal to minimal (0-5%); 2. mild (5-15%); 3. moderate (15-40%); 4. severe (>40%)

[a-c] Means within a row with different superscripts are different ($P < 0.05$)

CHAPTER 3

EFFICACY OF FEED ADDITIVES TO REDUCE THE EFFECTS OF CHRONIC EXPOSURE TO AFLATOXIN AND DEOXYNIVALENOL ON GROWTH AND IMMUNE STATUS OF PIGS

Efficacy of feed additives to reduce the effects of chronic exposure to aflatoxin and deoxynivalenol on growth and immune status of pigs

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ABSTRACT

Three feed additives with potential ability to detoxify mycotoxins were tested to determine the effects on growth and immune responses of pigs fed diets containing aflatoxin (**AF**) and deoxynivalenol (**DON**) for 42 d. Gilts (n = 225, 8.8 ± 0.4 kg BW) were allotted to 5 treatments (15 replicates, 3 pigs per pen): **PC** (positive control without AF and DON); **NC** (negative control with 175 µg/kg AF and 900 µg/kg DON); **A** (NC + a clay based additive); **B** (NC + a clay and yeast based additive); and **C** (NC + a clay and enzyme based additive). Feed intake and BW were recorded weekly, and blood was sampled on d 0, 14, 28, and 42. On d 42, pigs were euthanized to obtain liver, kidney, and spleen. Pigs in NC had decreased BW ($P < 0.05$, 24.6 kg) and ADG (0.372 kg/d) compared with PC (26.6 kg and 0.421 kg/d) but was not different from other treatments. Pigs in NC tended to have reduced ADFI ($P = 0.090$, 0.749 kg/d) and G:F ($P = 0.052$, 0.495) in contrast to PC (0.813 kg/d and 0.520) but was not different from other treatments. Antibody titer testing of H3N2 showed no difference between NC and all other treatments. On d 42, pigs in NC tended to have a greater basophil count (160 cells/uL) than PC (90, $P = 0.097$), but were not different from other treatments. Pigs in NC had a greater ($P < 0.05$) monocyte count (1,430 cells/uL) than

PC (970), A (1,050), and B (950). On d 42, pigs in NC had a greater serum IgG (15.1 mg/mL, $P < 0.05$) than PC (10.5) and A (8.6), and tended to have greater IgG than pigs fed C (11.1, $P = 0.078$). Pigs in NC had a greater serum IgM (4.39 mg/mL, $P < 0.05$) than PC (3.02), A (2.71), and B (3.19) and tended to have a greater serum IgM than C (3.23, $P = 0.062$). Serum tumor necrosis factor alpha concentration was not affected by dietary treatments. Pigs in NC had a greater liver weight as % of BW (3.29%, $P < 0.05$) than PC (2.71%) and B (2.80%). Pigs in NC had greater liver bile ductule hyperplasia, megakaryosis, and vacuolation than PC ($P < 0.05$), and all treatments differed from NC for megakaryosis. Pigs fed diet B also had decreased bile ductule hyperplasia and vacuolation. Collectively, feeding diets with 175 µg/kg AF and 900 µg/kg DON to pigs for a 42 d period reduced growth performance and increased liver damage and immune challenges. Use of these feed additives ameliorated tissue damage and immune challenges but did not consistently improve growth performance.

Key words: alfatoxin, deoxylnivalenol, feed additives, pigs

Introduction

Mycotoxins are toxic metabolites of fungi commonly found on grains which cause severe impacts on animal health when ingested (CAST, 2003). Of 300 toxins, aflatoxin (**AF**) and deoxynivalenol (**DON**) are of particular concerns as they are prevalent in the U.S. (Richard, 2007). For both toxins, swine are the most sensitive commodity species (Goyarts et al., 2005; Meissonnier et al., 2008). In states with intensive pork production, mycotoxins are of great concerns since they can cause decreased growth, organ damage, and immune challenges at levels both above and below FDA regulations (Chaytor et al., 2010; FDA, 1994; Richard, 2007; Sabater-Vilar et al., 2007). It is estimated that 25% of the world's crops are contaminated to some extent by mycotoxins (Schatzmayr et al., 2006; Veldman, 2004). A survey from the NC Cooperative Extension Service found that 34% of corn tested in North Carolina contained more than 20 µg/kg AF and over 60% of feed contained DON (Jones et al., 2007).

One effective method to reduce problems with mycotoxin contamination is the use of feed additives. Yeast components and specific types of clay have been shown to inactivate mycotoxins by binding, whereas specific enzymes are shown to inactivate mycotoxins by hydrolysis in the animal's gut (Huwig et al., 2001; Jones et al., 2007). Efficacy of these feed additives depends on the types of mycotoxins and concentrations. It is hypothesized that pigs eating diets naturally contaminated with low concentrations of AF and DON will have reduced weight gain, feed intake, and an altered immune system, and that the use of certain feed additives will reduce these negative effects. The objective of this study was to

investigate efficacy of 3 uniquely different types of feed additives with a potential ability to detoxify mycotoxins on growth and immune responses of pigs fed diets naturally contaminated with low concentration of both AF and DON.

Materials and Methods

Animals, Design, and Diets

Two hundred twenty five gilts (8.8 ± 0.4 kg BW, crossbred pigs, Smithfield Premium Genetics, Rose Hill, NC) were used in this study during the fall of 2009. They were housed in solid concrete floor indoor pens (1.42 m x 3.86 m) at the North Carolina Swine Evaluation Station (Clayton, NC). Pigs were randomly assigned to 5 treatments. Each treatment had 15 replicates with 3 pigs per pen.

Corn naturally contaminated with AF (270 µg/kg AF) and barley naturally contaminated with DON (30,000 µg/kg) were used in this study to achieve targeted concentrations of each mycotoxin. These grains were used to make a contaminated negative control diet with 175 µg/kg AF and 900 µg/kg DON (Table 1). These concentrations were based on Chaytor et al. (2010) who demonstrated that these AF and DON concentrations resulted in at least a 15 % reduction in growth performance or change in hematology, biochemical, and immune measurements. Three feed additives including clay, yeast, and enzyme materials were then added to the negative control diet. Mycotoxin analysis of corn, barley, and diets were completed by obtaining 10 samples from different locations to obtain representative samples (Munkvold et al., 2005; Vincelli et al., 1995; Whitaker et al. 2005).

Aflatoxin, DON, and other mycotoxin contamination and in grains and diets was measured by North Dakota State Veterinary Diagnostic Laboratory (Fargo, ND) using HPLC. Non-contaminated corn and barley were also used in order to formulate a positive control without added mycotoxins.

Pigs were fed experimental diets based on their assigned treatment groups representing: **PC** (positive control without AF and DON); **NC** (negative control with 175 µg/kg AF and 900 µg/kg DON); **A** (NC + a clay based additive, 2 mg/kg); **B** (NC + a clay and yeast based additive, 1.5 mg/kg); and **C** (NC + a clay and enzyme based additive, 1.1 mg/kg). Diet A contained a montmorillonite clay product, whereas the diet B additive was composed of clay and yeast. The additive in diet C contained both clay minerals and an enzyme epoxidase of the bacteria *Eubacterium*. During the 42 d of diet administration, all pigs had free access to feed and water. Concentrations of essential nutrients met or exceeded requirements suggested by NRC (1998). Body weights and feed intake were measured weekly on d 0, 7, 14, 21, 28, 35, and 42. The protocol for the use of animals in this study was approved by North Carolina State University Animal Care and Use Committee.

Vaccination and Antibody Titer

On d 1 and d 14, all pigs were vaccinated with 2 mL of porcine End-FLUence 2 swine influenza vaccine (Intervet, Inc., Millsboro, DE) intramuscularly for antibody titer testing. Antibodies against H3N2 were measured on d 0 and d 42 by hemagglutination inhibition (**HI**) analysis at the Iowa State University Veterinary Diagnostic Libratory (Ames, IA).

Blood Sampling

Blood samples were collected aseptically from the jugular vein for all pigs on d 0, 14, 28, and 42 for antibody titer testing. A pig with the largest initial BW from each pen was also bled on d 28 and 42 for hematological, biochemical, and immunological analysis. Blood was collected in Monovette tubes (Sarstedt, Newton, NC) containing EDTA for hematological analysis. Tubes without anticoagulant were used to collect serum for measuring antibody titer, liver biochemistry, immunoglobulin, and cytokine concentrations. Serum samples were allowed to clot overnight at 4°C before centrifuging for 15 min at 3,000 *g* (4°C), and were finally stored at -80°C until analyzed.

Hematological Measurements

Whole blood with EDTA was sent to Antech Diagnostics (Cary, NC) for complete blood count on d 28 and 42. Measurements included hematocrit, hemoglobin, mean corpuscular hemoglobin (**MCH**), mean corpuscular hemoglobin concentration (**MCHC**), mean corpuscular volume (**MCV**), platelet number, red blood cell count, white blood cell (**WBC**) count, basophils, eosinophils, lymphocytes, monocytes, and neutrophils.

Biochemical Serum Assays

Concentrations of alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin, BUN to creatinine ratio (**BUN:creatinine**), calcium, chloride, cholesterol, creatinine, creatine phosphokinase (**CPK**), globulin, glucose, phosphorus, potassium, sodium, and urea nitrogen were measured (Antech Diagnostics, Cary, NC) for determination of liver function on d 35 and 42.

Immunoglobulin Subsets

Total concentrations of the immunoglobulin subsets IgG and IgM were measured via ELISA, as described by the manufacturer (Bethyl, Montgomery, TX). Goat anti-pig IgG or goat anti-pig IgM were used as capture antibodies to coat wells. Serum samples were diluted to 1:140,000 and 1:20,000 for IgG and IgM, respectively. Horseradish peroxidase goat anti-pig IgG or IgM was used as the detection antibody in combination with the TMB enzyme substrate. A stop solution of 0.18 M sulfuric acid (H_2SO_4) was used to stop the enzyme-substrate reaction. Absorbance was read at 450 nm using an ELISA plate reader (Synergy HT, Bio-tek instruments) and the KC4 data analysis software. Samples were quantified relative to the standard curve constructed with known amounts of pig immunoglobulin subset. Detection limits were 7.8 to 500 ng/mL for IgG, and 15.6 to 1,000 ng/mL for IgM.

Cytokine Measurements

Serum tumor necrosis factor alpha (**TNFα**) was measured by ELISA method following the manufactures procedure (R&D Systems, Minneapolis, MN). A total of 50 µL assay dilute RD1-63 was added to microplate wells coated with a monoclonal antibody specific to porcine TNFα, followed by 50 µL of standard, control, or sample. Detection occurred by the use of a color reagent substrate and a stop solution of diluted hydrochloric acid. Absorbance was read at 450 nm and 540 nm by an ELISA plate reader and the KC4 data analysis software. The minimal detection limit range for TNFα is 2.8 to 5.0 pg/mL.

Histological Measurements

On d 42, a pig with the largest initial BW from each pen was euthanized to collect liver, kidney, and spleen for weight, color, and tissue damage evaluation. Tissue color was measured from 3 locations on each tissue via a Minolta Colorimeter (Konica Minolta, Ramsey, NJ), which measured values of lightness (L*), redness (a*), and yellowness (b*). Samples from the liver and kidneys were fixed in 10% buffered formalin, and sent to the North Carolina State University Histopathology Laboratory (College of Veterinary Medicine, Raleigh, NC) for hematoxylin and eosin (H & E) staining and observation of tissue damage. Liver damage measurement included bile ductule hyperplasia, fibrosis, hydropic degeneration, inflammation, megakaryosis, necrosis, and vacuolation. Kidney damage measurement included fibrosis, inflammation, necrosis, protein casts, regeneration, and vacuolation. Microscopic examinations of damage were measured blind, based on the degree of change observed with values of 1: normal to minimal damage (0 to 5%); 2: mild (5 to 15%); 3: moderate (15 to 40%); 4: severe (higher than 40%).

Statistical Analysis

Data were analyzed using the GLM procedures of SAS (SAS Inst., Inc., Cary, NC) following a completely randomized design. Growth performance was analyzed with initial BW as a covariate. A pen was considered as the experimental unit. Separation of means was completed using the PDIFF option of SAS. Contrasts for all measurements were conducted for NC versus each other treatment. Probability values less than 0.05 were considered statistically significant and between 0.05 and 0.10 as trends.

Results

Growth Performance

Initial BW of pigs was similar between all treatments (Table 2). For the first 4 weeks of this study, there was no difference in BW between pigs fed NC and pigs fed any other treatment. On d 35, pigs in PC tended to have a greater BW ($P = 0.060$, 21.6 kg) compared with pigs in NC (20.0 kg). By d 42, NC pigs had a decreased BW ($P < 0.05$, 24.6 kg) in contrast to pigs fed PC (26.6 kg). During the entire period, the BW of pigs fed feed additives was not different from pigs fed NC.

Average daily gain of pigs in all treatments did not differ from NC during d 0 to 21 (Table 2). During d 21 to 28, pigs in NC had a tendency for decreased ADG by 23.3% compared with PC ($P = 0.053$) and had decreased ADG by 24.6% compared with A ($P < 0.05$). There was no difference between NC and other treatments for d 28 to 35. Average daily gain of pigs in NC was decreased by 9.8% compared with PC during d 35 to 42, whereas there were no other differences compared with NC. During the entire 42 d period, pigs fed NC had an 11.6% decrease in ADG ($P < 0.05$) compared with PC but no difference in contrast to treatments A, B, and C which contained feed additives.

Average daily feed intake was not different between NC and all other treatments for d 0 to 14 (Table 2). During d 14 to 28, pigs fed NC had decreased ADFI by 15.8% in contrast to pigs fed PC, while there was no difference between NC and the treatments A, B, and C. Average daily feed intake did not differ between NC and all other treatments during d 28 to 42. Throughout the entire 42 d experimental period, pigs in NC had a tendency for a

decrease in ADFI by 7.9% ($P = 0.090$) compared with PC, whereas there was no difference between NC and treatments A, B, and C.

During d 0 to 21, there was no difference between NC and all other treatments for G:F (Table 2). Pigs fed NC had decreased G:F by 20.7% ($P < 0.05$) compared with pigs fed A during d 21 to 28, but did not differ from all other treatments. During d 28 to 35, pigs in NC had a tendency for decreased G:F ($P = 0.079$) compared with PC, while these pigs were not different from pigs fed A, B, or C diets. There were no treatment differences for G:F during the final trial week, d 35 to 42. During the entire trial period (d 0 to 42), pigs in NC tended to have decreased G:F by 4.8% compared with PC ($P = 0.052$) and 4.3% compared with B ($P = 0.086$), while they did not differ from A or C.

Antibody Titer Evaluation

Antibody analysis of H3N2 on d 0 before vaccination indicated there was no difference between NC and all other treatments for concentration in both numerical and logarithmic form (Table 3). On d 42, after both vaccinations, the antibody production increased in all pigs but was not different between NC and all other treatments. Pigs fed NC tended to increase ($P = 0.060$, 5.82) from C (4.58), but only in logarithmic form on d 42.

Hematological Measurements

Analysis of d 28 blood samples showed that pigs fed NC had increased MCV ($P < 0.05$) and MCH compared with PC (Table 4). The NC pigs also had greater ($P < 0.05$) MCHC compared with A and B, and a tendency for greater MCHC in contrast to PC ($P = 0.063$) and C ($P = 0.052$). Platelet number was greater ($P < 0.05$) by 39.3% and monocyte

number tended to be greater ($P = 0.073$) by 29.1% for pigs fed NC than pigs fed C, but these numbers were not different from other treatments. Basophil count of pigs in NC had a tendency to be decreased by 50.0% and 48.4% compared with A and B, respectively, while it was not different from PC and C treatments.

Hematocrit percentage in the blood on d 42 (Table 5) was increased in pigs fed NC ($P < 0.05$, 37.5 %) compared with PC (34.7 %) and B (34.9 %), and tended to be increased in contrast to C ($P = 0.092$, 35.4 %). On d 42, pigs in NC continued to have greater MCV by 7.8% compared with PC, as well as a 3.8% and 5.2% increase over A and B, respectively. MCHC levels in pigs fed NC decreased ($P < 0.05$, 30.7 g/dL) on d 42 compared with A (31.8 g/dL) and C (31.9 g/dL), while it did not differ from PC and B. Platelet count of pigs in NC was no longer increased over C, but showed a tendency for a 24.6% increase in contrast to B. The WBC count of pigs in NC tended to be greater by 24.6% ($P = 0.070$) compared with B, but was not different from any other treatments. Pigs in NC had a 42.4, 36.2, and 50.5% increase in monocytes in contrast to PC, A, and B, but was not different from C. On d 42, basophil count of NC pigs showed a tendency for a 77.8% increase ($P = 0.097$) compared with PC, with no difference between NC and A, B, or C.

Immunological Evaluation

Measurement of immunological parameters IgG, IGM, and TNFα on d 28 showed that pigs in NC had a 37.3% decreased ($P < 0.05$) in IgG compared to B, and a 29.5% decrease ($P < 0.05$) in TNFα in contrast to A (Table 6). All other treatments were not

different from NC for these immune measurements. There was no difference between NC and all other treatments for IgM on d 28.

On d 42, the concentration of serum IgG in NC pigs was increased ($P < 0.05$) by 44.6% and 75.4% in contrast to PC and A, respectively, while NC tended to be increased ($P = 0.078$) by 36.6% compared with C and did not differ from B (Table 6). IgM measurement showed that pigs in NC had greater ($P < 0.05$) IgM compared with PC, A, and B (45.4, 62.0, and 37.6%), and a tendency for a 35.9% ($P = 0.062$) increase in IgM when contrasted to C. Analysis of the cytokine TNFα showed that NC had a tendency for decreased ($P = 0.052$) serum TNFα compared with B and did not differ from PC, A, and C treatments. Cytokines interferon gamma (**IFNγ**), IL4, and IL6 were not measured in this study based on data from Chaytor et al. (2010) showing IFNγ was not altered when pigs were fed 180 µg/kg AF and 900 µg/kg DON. Cytokines IL4 and IL6 were not measureable for any pigs in this earlier experiment, so they also were not measured in our present study.

Liver Biochemistry

Albumin measurements from pigs on d 28 showed that NC pigs had a 12.7% greater ($P < 0.05$) amount of serum albumin compared with PC, and tendency for a 10.1% increase ($P = 0.096$,) in albumin in contrast to B (Table 7). Other treatments were not different from NC for albumin. Serum urea nitrogen of pigs in NC increased by 17.4%, and BUN:Creatinine by 22.7%, compared with PC, whereas other treatments did not differ from NC. Pigs fed NC had a tendency for greater ($P = 0.098$,10.2 mg/dL) calcium in contrast to treatment B (9.8 mg/dL), but was not different from all others. Levels of CPK in NC pigs

tended to be reduced ($P = 0.055$, 436.7 U/L) compared with A (767.9 U/L) but was not different from other treatments.

Serum analysis on d 42 showed that albumin concentration in pigs fed NC was still greater ($P < 0.05$, 2.8 g/dL) compared with pigs fed PC (2.5 g/dL) but was not different from other diets (Table 8). Urea nitrogen levels were decreased in NC pigs by 11.0% compared with pigs fed C. Pigs in NC had a tendency for increased ($P = 0.075$, 9.6 mg/dL) phosphorous compared to PC (9.0 mg/dL), but was not different from other treatments. Serum calcium for pigs fed NC on d 42 increased ($P < 0.05$) by 4.3% compared with PC and again showed a tendency to be greater ($P = 0.071$) than B, whereas it did not differ from A or C treatments.

Weight, Color, and Histology of Internal Organs

Differences in the weights of the liver, kidneys, and spleen were not observed when NC was contrasted with all other treatments (Table 9). Liver weight as a percent of BW increased ($P < 0.05$) by 21.4% in pigs fed NC compared with pigs fed PC, and by 17.5% compared with B. Pigs in A and C were not different from NC for liver weight as a percent of BW. Kidney and spleen weights as a percent of BW were not different between NC and all other treatments. Minolta color measurements of lightness and redness for the liver, kidneys, and spleen of NC pigs were not different compared with all other treatments. The numerical yellowness measurement (b*) for the liver was decreased in pigs fed NC in contrast with C ($P < 0.05$), but not compared with other treatments. Kidney yellowness score of NC pigs was also decreased ($P < 0.05$) compared with B, but was not different from other

treatments. Yellowness values for the spleen showed no difference between NC and all other treatments.

Histological analysis (Table 10) of tissue damage showed that pigs fed NC had an increased ($P < 0.05$) amount of hepatic hydropic degeneration compared with pigs fed A, but was not different from other treatments. Pigs fed NC had increased liver vacuolation compared with PC ($P < 0.05$) and tended to have increased levels compared with B ($P = 0.053$). Megakaryosis occurrence was greater ($P < 0.05$) in the livers of pigs fed NC in contrast to all other treatments. The livers of NC fed pigs also showed increased ($P < 0.05$) bile ductule hyperplasia compared with pigs fed PC and B, and tended to have greater damage compared with A and C ($P = 0.083$). All other measurements of liver damage did not differ between NC and all other treatments. Vacuolation was the only form of kidney damage found, where pigs in NC had a tendency for greater ($P = 0.066$) vacuolation in compared with PC, B and C. No difference was observed between NC and A.

Discussion

In this study, AF and DON were the chosen mycotoxins because they are commonly found in North American grains such as corn, wheat, and barley (CAST, 2003). When ingested by livestock, these mycotoxins can cause reductions in growth and feed intake, organ damage, and immune challenge (CAST, 2003; Chen et al., 2008; Cheng et al., 2006; Richard, 2007). Together, these effects result in significant economic losses for swine producers. Low dietary concentrations of mycotoxins were chosen in the current study in

order to represent amounts which could frequently be consumed by pigs. Feed containing 175 µg/kg AF and 900 µg/kg DON was used based a previous study (Chaytor et al., 2010) showing that growth performance and immune health were challenged when pigs consumed feeds contaminated with these levels of mycotoxins.

Currently there are many commercially available feed ingredients with potential abilities to reduce the toxicity of mycotoxins (Huwig et al., 2001; Ramos et al., 1996; Schatzmayr et al., 2006). These feed additives can be categorized into 2 groups, mycotoxin adsorbing agents or mycotoxin biotransforming agents, based on their properties and functions (EFSA, 2009). The current research aimed to determine the ability of 3 uniquely different types of feed additives to ameliorate the negative effects of feeding diets containing 175 µg/kg AF and 900 µg/kg DON to pigs for 42 d. In our study, diet A consisted of a montmorillonite clay with properties that allows adsorption of the polar AF compound within its interlaminar spaces by exchange cations present in these spaces (Huwig et al., 2001; Ramos et al., 1996). Clay and yeast components comprised treatment B. Yeast material has a strong ability for mycotoxin absorption because it's cell walls contain polysaccharides, lipids, and proteins which have absorption centers that bind the mycotoxin through hydrogen and ionic bonding, or hydrophobic interactions (Huwig et al., 2001). The clay component of this B diet additive may absorb the polar AF and the yeast component has potential to absorb the non-polar DON.

Finally, diet C contained clay and enzyme materials. The major enzyme in this additive was a bacterial epoxidase which has the ability to transform mycotoxins into

nontoxic metabolites (Schatzmayr et al., 2006). Whereas clay mineral additives have particular affinity for AF, these enzymes have the capability to detoxify trichothecenes, ochratoxin, and zearalenone by altering the toxin's ring structure. For example, an epoxidase of *Eubacterium* BBSH 797 can reduce DON to a nontoxic metabolite de-epoxy-deoxynivalenol (Schatzmayr et al., 2006).

The consumption of AF and DON by pigs has been shown to decrease BW gain at both low and high concentrations (Chaytor et al., 2010; Cheng et al., 2006; Harvey et al., 1989; Marin et al., 2002; Swamy et al., 2002; Thieu et al., 2008; van Heugten et al., 1994). Dersjant-Li et al. (2003) showed that AF at 300 µg/kg and DON at 600 µg/kg can cause 5% reductions in growth of pigs. Chaytor et al. (2010) showed that pigs fed a diet containing 180 µg/kg AF and 900 µg/kg DON showed a trend for reduced BW gain on d 21 of diet administration, and by the final week these pigs had an 11.5% decreased BW compared with control pigs. In the current study, pigs fed NC had a decrease in BW by 7.7% when compared with pigs fed PC. Body weighs of pigs fed feed additives did not differ from the NC treatment. These results show that low AF and DON can decrease BW, and that the addition of feed additives may not significantly protect this BW reduction when low mycotoxins are fed.

Over the entire trial period, pigs in NC had an 11.6% decrease in ADG compared to pigs fed PC. This result is lower than Chaytor et al. (2010) where pigs fed mycotoxin concentrations of 180 µg/kg AF and 900 µg/kg DON had reduced ADG by 21% from pigs fed no mycotoxins. However, AF levels were lower (150 µg/kg) and DON higher (1,100

µg/kg) in the present study than the experiment by Chaytor et al. (2010). Other studies that have fed pigs a range of 200 to 800 µg/kg AF or 210 to 9,570 µg/kg DON has found mycotoxin effects on ADG comparable to our current study (Cheng et al., 2006; Doll et al., 2003; Goyarts et al., 2005; Schell et al., 1993; Thieu et al., 2008; Tiemann et al., 2006). In the current study, ADG of pigs fed feed additives were not significantly different from pigs fed NC, indicating that they may not play a role in ameliorating AF and DON effects on growth performance. However, in other studies feeding pigs a high mycotoxin concentration of 800 µg/kg AF, the clay feed additives HSCAS and bentonites have improved ADG (Lindemann et al., 1993; Schell et al., 1993).

Feed intake has been shown to decrease when pigs were fed diets with low AF concentrations. van Heugten et al. (1994) found a 3.5% decrease in feed intake for pigs fed diets with 140 µg/kg AF. Deoxynivalenol alone is not shown to decrease ADFI at concentrations as low as 280 µg/kg (Accensi et al., 2006). However, Chaytor et al. (2010) showed that low AF and DON together (180 µg/kg AF, 900 µg/kg DON) tended to reduce ($P = 0.061$) ADFI by 15.4% over a 33 d period. Our current study showed a similar change but to a lesser degree, where pigs fed NC tended to have decreased ADFI by only 7.9% compared with PC over the 42 d trial. The use of clay feed additives can be beneficial at reducing mycotoxin effects on ADFI (Schell et al., 1993). However, pigs fed treatments A, B, or C in our study did not show differences in ADFI compared with NC, again indicating that feed additives did not significantly reduce AF and DON effects on feed intake.

Gain to feed ratio of pigs fed NC tended to be decreased over the entire trial period compared to pigs fed PC. However, other research indicates that AF and DON do not effect G:F (Chaytor et al., 2010; Doll et al., 2003; Harvey et al., 1991; Smith et al., 1997; Swamy et al., 2002; van Heugten et al., 1994). During the entire 42 d, pigs fed B had a tendency for increased G:F compared with NC, while A and C did not differ from NC. This result indicates that an additive composed of clay and yeast may improve feed efficiency when added to low AF and DON contaminated swine diets.

Antibodies against swine H3N2 were analyzed as a measurement of immune function. Although several swine influenza antibodies were measureable (i.e., H1N1), these were influenced by maternal exposure or previous vaccinations before our study began. The H3N2 were likely not provided by either of these exposures, but rather the vaccinations given on d 1 and d 14 of the trial. No difference was found between treatments for H3N2 on d 0, and NC only tended to have greater logarithmic values than C on d 42. Due to the fact that NC and PC were not different for any measurements, it is difficult to determine immune response related to AF and DON based on antibody titer analysis. Other studies have also found the effectiveness of antibody titer measurements to be variable when determining immune status (Meissonnier et al., 2008; Taranu et al., 2005). Collectively, it appears that antibody titer analysis after vaccination may not be a reliable measure of immune function due to the impact of previous exposures.

Hematological analysis of blood samples showed several contrasts between pigs fed NC and those fed other treatments. Mean corpuscular volume represents the average volume

of red blood cells found in the blood, and can be important for classification of anemia and red blood cell disorders (Perkins, 2009). In the current study, NC pigs had elevated MCV compared with PC on d 28 and d 42. On the final week, treatments A and B had reduced MCV compared to NC. Although there are differences between treatments, all values for MCV fall within a normal range of 47 to 56 fL (Friendship et al., 1984; Klem et al., 2009). Previous research has also shown no effect on MCV when pigs are fed 280 to 840 µg/kg DON (Accensi et al., 2006).

Another hematological measurement showing differences between treatments is MCHC, which represents the average concentration of hemoglobin in a given volume of red blood cells (Perkins, 2009). On d 28, the MCHC of pigs fed NC was increased compared with all other treatments. However, on d 42, pigs fed NC had decreased concentrations compared with A and C treatments while it was not different from PC or B. Although inconsistencies are seen, MCHC values for all treatments for both weeks again fall within a normal range of 28.4 to 38.0 g/dL (Friendship et al., 1984; Klem et al., 2009). Similar MCHC values are shown for pigs consuming 3,000 µg/kg AF, or 280 to 840 µg/kg DON (Accensi et al., 2006; Harvey et al., 1989). Another blood parameter found to differ between treatments is MCH, a measure of the average hemoglobin content per red blood cell (Perkins, 2009). On d 28, pigs fed NC had greater MCH compared with PC. The MCH measurements were not abnormal, and difference was not seen again on d 42. Although not outside normal ranges for any pigs, both MCHC and MCH may be elevated by hyperlipidemia, a condition

of abnormal lipid metabolism which can indicate liver damage in some cases (Botham et al., 2009; Perkins, 2009).

On d 28, pigs fed NC had a tendency for a decreased basophils compared with A and B, respectively. However, on d 42 these differences were not seen again. On d 42, pigs fed NC also had increased monocytes compared with PC, A, and B, respectively. Hematocrit percentage was greater in NC compared with PC, B, and C treatments. Despite these changes, basophil, monocyte, and hematocrit levels for all treatments remained within normal ranges for pigs of this age (Friendship et al., 1984; Klem et al., 2009). Pinton et al. (2008) found simililar results for monocytes and hematocrit of pigs fed 2,200 to 2,500 µg/kg DON. Collectively, hematological analysis shows that NC often has differences in contrast to PC. Each feed additive treatment had differences from NC in multiple instances, but there does not appear to be a trend for one to cause more improvements than another. Due to the fact that all measurements were within normal ranges, it is also difficult to determine to what extent mycotoxin effects are reduced by the presence of clay, yeast, and enzyme additives.

Immunological analysis showed minimal changes between NC and other treatments on d 28, but by d 45 there were several differences. By this final week of our study, concentrations of IgG were found to increase in NC compared with PC, A, and C. Similar results were seen for IgM where NC had greater concentrations in contrast to all other treatments. These results are contradictory to other research, where pigs that consumed 140 to 280 µg/kg AF or 280 to 900 µg/kg DON had no change in IgG or IgM (Accensi et al., 2006; Chaytor et al., 2010; van Heugten et al., 1994). All concentrations of IgG and IgM

were greater in our study compared to this other research, although it is important to keep in mind that our pigs were given immunizations for antibody titer testing which may have increased immunoglobulin levels in response to this vaccination. Our other measurement of immune function, TNFα, was decreased in pigs fed NC compared with A on d 28 and B on d 45. The cytokine TNFα is a pro-inflammatory cytokine important in tissue macrophage control and inflammatory response (Wood, 2006). The liver is a major source of TNFα, so a decrease in this cytokine may indicate liver damage (AL-Anati et al., 2005). For both sampling periods, levels of TNFα were lower for all pigs in this study compared with TNFα concentrations found in Chaytor et al. (2010), which may agree with the fact that more liver damage occurred to pigs in our current study. Few studies have analyzed swine serum TNFα, so it is difficult to determine if concentrations found are typical. Immunoglobulin and cytokine data from our current study may indicate that pigs consuming 175 µg/kg AF and 900 µg/kg DON in treatment NC had an increased immune response or challenge compared with pigs fed no mycotoxins or those provided feed additives.

Analysis of serum for liver functioning showed several changes between pigs fed NC and pigs fed other treatments. Albumin levels on both d 28 and d 45 were greater in NC compared with PC. Although pigs fed NC had increased albumin, all pigs in this study had serum albumin levels within a normal range of 1.9 to 4.2 g/dL (Dubreuil and Lapierre, 1997; Friendship et al., 1984; Klem et al., 2009). Previous studies involving mycotoxins show inconsistencies for effects on albumin, although all studies reported albumin concentrations to fall within normal range. Chen et al. (2008) found that pigs fed 1,000 µg/kg DON and 250

µg/kg zearalenone had decreased serum albumin compared to control pigs, whereas Goyarts et al. (2005) found no change in albumin when pigs were fed 6,510 µg/kg DON. Albumin levels altered by mycotoxins has also been shown to be improved by the use of bentonite clays for pigs consuming 800 µg/kg AF (Schell et al., 1993). Pigs in our study may not have been extremely affected by the lower mycotoxin levels, so albumin production was not reduced. Serum calcium also tended to be greater in NC pigs compared with pigs fed B on d 28 and d 45, and became increased over pigs fed PC by d 45. In previous studies, only high AF (3,000 µg/kg) altered serum calcium (Harvey et al., 1989). Despite calcium increase in our study and in other research, concentrations still fall within the normal range of 8.6 to 12.8 mg/dL (Dubreuil and Lapierre, 1997; Friendship et al., 1984; Klem et al., 2009). However, these data show that the clay and yeast based feed additive (diet B) may help to maintain calcium levels in pigs fed mycotoxins over several weeks.

Urea nitrogen concentrations were also found to differ between treatments, although concentrations always remained in a normal range (Friendship et al., 1984; Klem et al., 2009; Odink et al., 1990). On d 28, pigs fed NC had increase urea nitrogen compared with pigs fed PC. However, by d 45 pigs fed NC were no longer different from PC but had lower urea nitrogen compared with C. This change may be due to the fact that urea nitrogen dropped in all pigs between these two time points, but NC pigs had a greater reduction. Other research has shown urea nitrogen was not altered at 280 to 2,500 µg/kg DON, so differences seen in our study may be due to AF. Although several liver function measurements were seen to change for pigs in our study, analyzed concentrations of these metabolites were never found

to be abnormal compared to ranges defined for healthy pigs in a similar age group. Data indicate that pigs fed NC were often different from PC, and that NC was different from B for serum albumin and calcium and treatment C for urea nitrogen. However, since NC pigs did not have abnormal measurements for liver function, it is again unclear if the additives in treatments B and C greatly decreased mycotoxin effects.

Tissue analysis of pigs showed no difference between NC and all other treatments for weights of the liver, kidneys, or spleen. Weights were similar to those found in Chaytor et al. (2010), where there was no difference between control pigs and pigs fed an AF and DON contaminated diet similar to NC. Organ weights obtained in our study were also comparable with data found in previous research for a wide range of 50 to 1,807 µg/kg AF and 750 to 6,510 µg/kg DON contamination (Dilkin et al., 2003; Goyarts et al., 2005; Meissonnier et al., 2008; Rotter et al., 1994). There was no difference between NC and other treatments for the kidneys or spleen weight as a percent of BW, but liver weight as a percent BW was increased in NC pigs compared with PC and B. Similar results are shown by Harvey et al. (1991) where 3,000 µg/kg AF increased liver and spleen weights as a percentage of BW. Organ color analysis showed that most color measurements did not differ between NC and other treatments, except for yellowness readings for the liver and kidneys. Although significant via the Minolta Colorimeter, this color change was not visually observable. A yellowness color change was not seen in the previous study by Chaytor et al. (2010), and few others have researched organ tissue color change related to AF and DON. One study did find that liver color was altered in pigs fed 2,500 µg/kg AF (Harvey et al., 1991), but due to a lack of

comprehensive data, it is unknown if pig organ coloring will always be affected by AF or DON in feed.

Although serum liver function analysis was not abnormal, liver and kidney damages were prevalent in the pigs fed the NC diet. These two organs were analyzed because AF is known to target the liver, and DON the kidneys (Richard et al., 2007). It is previously documented that 1,000 µg/kg DON causes organ damage including necrosis, blood vessel thickening, and hemorrhage (Chen et al., 2008; Cheng et al., 2006). High AF (3,000 µg/kg) in the diet has also shown to cause liver lesions and hepatocellular cytoplasmic vacuolation with early portal fibrosis and bile duct hyperplasia (Harvey et al., 1991).

In our current study, pigs fed NC also had increased megakaryosis compared with all other treatments. These NC pigs also had increased vacuolation in contrast to pigs in PC and B, and increased hydrophic degeneration compared with A. Pigs fed the mycotoxin contaminated NC diet also had increased bile ductle hyperplasia compared with PC and B, and a tendency for increased damage in contrast to pigs fed the A and C diets. Vacuolation was the only form of kidney damage observed, where pigs fed NC had a tendency for increased damage compared with PC, B, and C. In a previous study by Chaytor et al. (2010), liver fibrosis was the only tissue damage observed. Hepatic fibrosis was not seen to differ between NC and other treatments in our current study, but the amount of damage was similar to Chaytor et al. (2010). Together, the results of tissue damage analysis shows more damage to the liver than the kidneys, which may indicate that low AF is more damaging to a pig's organs than low concentrations of DON. These data also indicate that all feed additives

tested can be effective in reducing tissue damage due to mycotoxins. However, it appears that the feed additives in treatment B, composed of clay and yeast, may be the most effective at reducing organ damages because pigs consuming this diet differed more frequently from NC based on liver serum biochemistry, organ weight, color, and both liver and kidney damage.

Collectively, the results of our study show that low AF at 175 µg/kg and DON at 900 µg/kg can alter growth performance, immune health, and damage internal organs. These results are in agreement with the results from a previous study by Chaytor et al. (2010). When the negative effects of a blend of mycotoxins at low concentrations occurred, the 3 feed additives used in this study appeared to be beneficial in some circumstances. When AF and DON reduced growth performance, all additives did not significantly lessen the reduction in body weight gain and feed intake caused by the mycotoxins. Interestingly, however, when compared with the AF and DON contaminated NC diet, the clay and yeast additive in diet B tended to improved G:F over the entire trial period so that it was similar to the pigs fed PC.

Although growth performance was not significantly improved, the negative effects of low concentrations of AF and DON on other systems within these pigs, such as immune functions and organ health, appeared to be reduce by the additives. All additives helped to reduce immune and inflammatory challenges, as measured by the immune parameters IgG and IgM. Feed additives were also beneficial for liver functioning and tissue damage, in that they often reduced mycotoxin effects. Although all additives used in diets reduced tissue

damage compared to NC, the additives in diet B (clay and yeast) and C (clay and enzyme) reduced damages seen in both the liver and kidneys. Overall, it is concluded that AF and DON can be toxic even at low concentrations of contamination. It is also concluded that the selected feed additives can lessen negative effects of low concentrations of AF and DON on health and immune challenges.

Literature Cited

Accensi, F., P. Pinton, P. Callu, N. Abella-Bourges, J. -F. Guelfi, F. Grosjean, and I. P. Oswald. 2006. Ingestion of low doses of deoxynivalenol does not affect hematological, biochemical, or immune responses of piglets. J. Anim. Sci. 84:1935-1942.

Al-Anati, L., N. Katz, and E. Petzinger. 2005. Interference of arachidonic acid and its metabolites with TNF-α release by ochratoxin A from rat liver. Toxicol. 208:335-346.

Botham, K. M., and P. A. Mayes. 2009. Cholesterol synthesis, transport, and excretion. Page 224-233 in Harper's Illustrated Biochemistry 28th ed. R. K. Murray, D. A. Bender, K. M. Botham, P. J. Kennelly, V. W. Rodwell, and P. A. Weil., ed. McGraw-Hill Companies, Inc., New York, NY.

CAST. 2003. Mycotoxins: risks in plant, animal, and human systems. Council for Agri. Sci. Technol. Task Force Report No. 139. Ames, IA.

Chaytor, A. C., M. T. See, J. A. Hansen, A. L. P. de Souza, T. F. Middleton, and S. W. Kim. 2010. Effects of chronic exposure of diets with low levels of aflatoxin and deoxynivalenol on growth and immune status of pigs. J. Anim. Sci. (Submitted)

Chen, F., Y. Ma, C. Xue, J. Ma, Q. Xie, G. Wang, Y. Bi, and Y. Cao. 2008. The combination of deoxynivalenol and zearalenone at permitted feed concentrations causes serious physiological effects in young pigs. J. Vet. Sci. 9:39-44.

Cheng, Y. H., C. F. Weng, B. J. Chen, and M. H. Chang. 2006. Toxicity of different Fusarium mycotoxins on growth performance, immune responses and efficacy of a mycotoxin degrading enzyme in pigs. Anim. Res. 55:579–590.

Dersjant-Li, Y., M. W. A. Verstegen, and W. J. J. Gerrits. 2003. The impact of low concentrations of aflatoxin, deoxynivalenol or fumonisin in diets on growing pigs and poultry. Nutr. Res. Rev. 16:223–239.

Dilkin,P., P. Zorzete, C. A. Mallmann, J. D. F. Gomes, C. E. Utiyama, L. L. Oetting, and B. Correa. 2003. Toxicological effects of chronic low doses of aflatoxin B1 and fumonisin B1-containing *Fusarium moniliforme* culture material in weaned piglets. Food Chem. Toxicol. 41:1345-1353.

Doll, S., S. Da Nicke, K. H. Ueberschar, H. Valenta, U. Schnurrbusch, M. Ganter, F. Klobasa, and G. Flachowsky. 2003. Effects of graded levels of Fusarium toxin contaminated maize in diets for female weaned piglets. Arch. Anim. Nutr. 57:311 – 334.

Dubreuil, P., and H. Lapierre. 1997. Biochemistry reference values for Quebec lactating dairy cows, nursing sows, growing pigs and calves. Can. J. Vet. Res. 61:235-239.

EFSA. 2009. Review of mycotoxin-detoxifying agents used as feed additives: mode of action, efficacy and feed/food safety. Sci. Report, Parma, Italy.

FAO. 2003. Worldwide regulation for mycotoxins in food and feed in 2003. Food and Agriculture Organization of the United Nations, Rome, Italy.

FDA. 1994. FDA regulatory guidance for toxins and contaminants. Food and Drug Administration, Silver Spring, MD.

Friendship R.M., J.H. Lumsden, I. McMillan, and M.R. Wilson. 1984. Hematology and biochemistry reference values for Ontario swine. Can. J. Comp. Med. 48:390-393.

Goyarts, T., S. Danicke, H. J. Rothkotter, J. Spilke, U. Tiemann, and M. Schollenberger. 2005. On the effects of a chronic deoxynivalenol intoxication on performance, haematological and serum parameters of pigs when diets are offered either for ad libitum consumption or fed restrictively. J. Vet. Med. 52:305–314.

Harvey, R. B., L. F. Kubena, W. E. Huff, D. E. Corrier, D. E. Clark, and T. D. Phillips. 1989. Effects of aflatoxin, deoxynivalenol, and their combinations in the diets of growing pigs. Am. J. Vet. Res. 40:602-607.

Harvey, R. B., L. F. Kubena, M. H. Elissalde, D. E. Corrier, W. E. Huff, G. E. Rottinghaus, and B. A. Clement. 1991. Cocontamination of swine diets by aflatoxin and diacetoxyscirpenol. J. Vet. Diagn. Invest. 3:155-160.

Hussein, H. S., and J. M. Brasel. 2001. Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxocol. 167:101-134.

Huwig, A., S. Freimund, O. Kappeli, and H. Dutler. 2001. Mycotoxin detoxification of animal feed by different absorbents. Toxicol. Lett. 122:179-188.

Jones, F. T., M. B. Genter, W. M. Hagler, J. A. Hansen, B. A. Mowrey, M. H. Poore, and L. W. Whitlow. 2007. Understanding and coping with effects of mycotoxins in livestock feed and forage. North Carolina Cooperative Extension Service, Raleigh, NC.

Klem, T. B., E. Bleken, H. Morberg, S. I. Thoresen, and T. Framstad. 2009. Hematologic and biochemical reference intervals for Norwegian crossbreed grower pigs. Vet Clin. Path.1-6.

Lindemann, M. D., D. J. Blodgett, E. T. Kornegay, and G. G. Schurig. 1993. Potential ameliorators of aflatoxicosis in weanling/growing swine. J. Anim. Sci. 71:171-178.

Marin, D. E., I. Taranu, R. P. Bunaciu, F. Pascale, D. S. Tudor, N. Avram, M. Sarca, I. Cureu, R. D. Criste, V. Suta, and I. P. Oswald. 2002. Changes in performance, blood parameters, humoral and cellular immune responses in weanling piglets exposed to low doses of aflatoxin. J. Anim. Sci. 80:1250-1257.

Meissonnier, G. M., P. Pinton, J. Laffitte, A. Cossalter, Y. Y. Gong, C. P. Wild, G. Bertin, P. Galtier, and I. P. Oswald. 2008. Immunotoxicity of aflatoxin B1: Impairment of the cell-mediated response to vaccine antigen and modulation of cytokine expression. Toxicol. Appl. Pharmacol. 231:142–149.

Munkvold, G., C. Hurburgh, and J. Meyer. 2005. Aflatoxins in corn. Iowa State University Extension, Ames, IA.

NRC. 1998. Nutrient requirements of swine. 10th rev. ed. Natl. Acad. Press, Washington, DC.

Odink J., J. F. Smeets, I. J. Visser, H. Sandman and J. M. Snijders. 1990. Hematological and clinicochemical profiles of healthy swine and swine with inflammatory processes. J. Anim. Sci. 68:163-170.

Perkins, S. L. 2009. Examination of the blood and bone marrow. Page 1-20 in Wintrobe's Clinical Hematology 12th ed. J. P. Greer, J. Foerster, G. M. Rodgers, F. Paraskevas, B. Glader, D. A. Arber, and R. T. Means, Jr., ed. Lippincott Williams and Wilkins, Philadelphia, PA.

Pinton, P., F. Accensi, E. Beauchamp, A. Cossalter, P. Callu, F. Grosjean, and I. P. Oswald. 2008. Ingestion of deoxynivalenol (DON) contaminated feed alters the pig vaccinal immune responses. Toxicol. Lett. 177:215–222.

Ramos, A-J., J. Fink-Gremmels, and E. Hernandez. 1996. Prevention of toxic effects of mycotoxins by means of nonnutritive adsorbent compounds. J. Food Prot. 59:631-641.

Richard, J. L. 2007. Some major mycotoxins and their mycotoxicoses: an overview. Int. J. Food Microbiol. 119:3-10.

Rotter, B. A., B. K. Thompson, M. Lessard, H. L. Trenholm, and H. Tryphona. 1994. Influence of low-level exposure to *Fusarium* mycotoxins on selected immunological and hematological parameters in young swine. Fund. Appl. Toxicol. 23:117-124.

Sabater-Vilar, M., H. Malekinejad, M. H. J. Selman, M. A. M. van der Doelen, and J. Fink-Gremmels. 2007. In vitro assessment of absorbents aiming to prevent deoxynivalenol and zearalenone mycotoxins. Mycopathol. 163:81-90.

Schatzmayr, G., F. Zehner, M. Taubel, D. Schatzmayr, A. Klimitsch, A. P. Loibner, and E. M. Binder. 2006. Microbiologicals for deactivating mycotoxins. Mol. Nutr. Food Res. 50:543-551.

Schell, T. C., M. D. Lindemann, E. T. Kornegay, D. J. Blodgett, and J. A. Doerr. 1993. Effectiveness of different types of clay for reducing the detrimental effects of aflatoxin-contaminated diets on performance and serum profiles of weanling pigs. J. Anim. Sci. 71:1226-1231.

Smith, T. K., E. G. McMillan, and J. B. Castillo.1997. Effect of feeding blends of Fusarium mycotoxin-contaminated grains containing deoxynivalenol and fusaric acid on growth and feed consumption of immature swine. J. Anim. Sci. 75:2184-2191.

Swamy, H. V. L. N., T. K. Smith, E. J. MacDonald, H. J. Boermans, and E. J. Squires. 2002. Effects of feeding a blend of grains naturally contaminated with Fusarium mycotoxins on swine performance, brain regional neurochemistry, and serum chemistry and the efficacy of a polymeric glucomannan mycotoxin adsorbent. J. Anim. Sci. 80:3257-3267.

Taranu, I., D. E. Marin, S. Bouhet, F. Pascale, J-. D. Bailly, J. D. Miller, P. pinton, and I. P. Oswald. 2005. Mycotoxin fumonisin B1 alters the cytokine profile and decreases the vaccinal antibody titer of pigs. Toxicol. Sci. 84:301-307.

Thieu, N. Q., B. Ogle, and H. Pettersson. 2008. Efficacy of bentonite clay in ameliorating aflatoxicosis in piglets fed aflatoxin contaminated diets. Trop. Anim. Health Prod. 40:649–656.

Tiemann, U., K. P. Brüssow, L. Jonas, R. Pöhland, F. Schneider, and S. Dänicke. 2006. Effects of diets with cereal grains contaminated by graded levels of two Fusarium toxins on selected immunological and histological measurements in the spleen of gilts. J. Anim. Sci. 84:236-245.

van Heugten, E., J. W. Spears, M. T. Coffey, E. B. Kegley, and M. A. Qureshi. 1994. The effect of methionine and aflatoxin on immune function in weanling pigs. J. Anim. Sci. 72:658-664.

Veldman, B. 2004. Mycotoxins in the animal production chain. Page 275-280 in Meeting the Mycotoxin Menace, D. Barug, H. van Egmond, R. Lopez-Garcia, T. van Osenbruggen, and A.Visconti, ed. Wageningen Academic Publishers, The Netherlands.

Vincelli, P., G. Parker, and S. McNeill. 1995. Aflatoxins in corn. University of Kentucky Cooperative Extension Service, Lexington, KY.

Whitaker, T. B, A. B. Slate, and A. S. Johansson. 2005. Sampling Feeds for Mycotoxin Analysis. Page 1-23 in The Mycotoxin Blue Book, D. Diaz, ed. Nottingham University Press, Bath, England.

Wood, P. 2006. Understanding Immunology. 2nd ed. Pearson Education Limited, Harlow, England.

Table 1. Composition of experimental diets (as-fed basis)

	Treatment[1]	
	PC	NC
Ingredient, %		
Ground yellow corn	69.42	4.42
Ground barley	3.00	0.00
Ground yellow corn with aflatoxin[2]	0.00	65.00
Ground barley with deoxynivalenol[3]	0.00	3.00
Soybean meal, dehulled	25.00	25.00
Salt	0.30	0.30
Vitamin premix[4]	0.03	0.03
Trace mineral premix[5]	0.15	0.15
Dicalcium phosphate	0.90	0.90
Ground limestone	0.70	0.70
Poultry fat	0.50	0.50
Calculated composition		
DM, %	89.50	89.50
ME, Mcal/kg	3.36	3.36
CP, %	18.08	18.08
Lys, %	0.95	0.95
Cys + Met, %	0.61	0.61
Trp, %	0.21	0.21
Thr, %	0.68	0.68
Calcium, %	0.61	0.61
Available phosphorus, %	0.23	0.23
Total phosphorus, %	0.54	0.54
Aflatoxin, µg/kg	0.00	175
Deoxynivalenol, µg/kg	0.00	900
Fumonisin, µg/kg	0.00	0.00
Zearalenone, µg/kg	0.00	0.00
Analyzed composition		
DM, %	88.55	89.97
CP, %	16.71	19.34
Aflatoxin[6], µg/kg	< 20	150
Deoxynivalenol[6], µg/kg	< 500	1,100
Fumonisin[6], µg/kg	< 2000	3,000
Zearalenone[6], µg/kg	< 500	400

[1] PC: positive control without AF and DON; NC: negative control with AF and DON; A: NC + 2 mg/kg of a clay additive (Amlan International, Chicago, IL); B: NC + 1.5 mg/kg of a clay and yeast additive (Nutriad, Elgin, Il); C: NC + 1.1 mg/kg of a clay and enzyme additive (Biomin, San Antonio, TX)

[2] Corn contained 270 µg/kg aflatoxin

Table 1. Continued.

[3] Barley contained 30,000 µg/kg deoxynivalenol

[4] The vitamin premix provided the following per kilogram of complete diet: 6613.8 IU of vitamin A as vitamin A acetate; 992.0 IU of vitamin D_3; 19.8 IU of vitamin E; 2.64 mg of vitamin K as menadione sodium bisulfate; 0.03 mg of vitamin B_{12}; 4.63 mg of riboflavin; 18.52 mg of D-pantothenic acid as calcium panthonate; 24.96 mg of niacin; 0.07 mg of biotin

[5] The trace mineral premix provided the following per kilogram of complete diet: 4.0 mg of Mn as manganous oxide; 165 mg of Fe as ferrous sulfate; 165 mg of Zn as zinc sulfate; 16.5 mg of Cu as copper sulfate; 0.30 mg of I as ethylenediamine dihydroiodide; and 0.30 mg of Se as sodium selenite

[6] Dietary mycotoxin concentration based on average obtained from analysis by North Dakota State University Veterinary Diagnostic Laboratory (Fargo, ND).

Table 2. Growth performance of pigs fed diets containing mycotoxins and various feed additives

	Treatment[1]						NC vs.:[2]			
	PC	NC	A	B	C	SEM	PC	A	B	C
BW, kg										
d 0[3]	8.9	8.9	8.8	8.9	8.9	0.4				
d 7	9.7	9.7	9.7	9.9	9.8	0.2	0.953	0.974	0.476	0.739
d 14	11.5	11.5	11.7	11.6	11.8	0.3	0.989	0.723	0.820	0.526
d 21	13.9	13.8	13.6	14.0	14.0	0.4	0.768	0.748	0.758	0.686
d 28	17.6	16.6	17.3	16.9	17.2	0.5	0.148	0.285	0.595	0.360
d 35	21.6	20.0	20.8	20.7	20.4	0.6	0.060	0.335	0.424	0.654
d 42	26.6	24.6	25.8	25.4	25.1	0.7	0.033	0.217	0.401	0.601
ADG, g/d										
d 0 to 7	112	114	116	141	127	32	0.952	0.974	0.477	0.741
d 7 to 14	260	257	275	243	280	27	0.926	0.575	0.673	0.486
d 14 to 21	346	325	282	335	319	34	0.623	0.301	0.817	0.877
d 21 to 28	516	396	525	426	456	52	0.053	0.040	0.634	0.333
d 28 to 35	571	489	498	531	453	43	0.142	0.869	0.448	0.513
d 35 to 42	722	651	706	672	670	30	0.077	0.166	0.604	0.639
d 0 to 42	421	372	400	391	384	18	0.033	0.217	0.402	0.600
ADFI, g/d										
d 0 to 7	324	303	308	318	316	23	0.437	0.853	0.564	0.616
d 7 to 14	542	537	544	525	552	32	0.904	0.857	0.760	0.710
d 14 to 21	728	613	646	632	629	34	0.006	0.414	0.637	0.696
d 21 to 28	906	829	889	832	865	42	0.154	0.266	0.963	0.504
d 28 to 35	991	937	1,028	974	934	51	0.418	0.171	0.582	0.957
d 35 to 42	1,388	1,276	1,353	1,265	1,277	55	0.109	0.265	0.864	0.995
d 0 to 42	813	749	795	758	762	29	0.090	0.223	0.823	0.732

Table 2. Continued.

	Treatment[1]						NC vs.:[2]			
	PC	NC	A	B	C	SEM	PC	A	B	C
Gain/feed										
d 0 to 7	205	335	192	324	276	117	0.373	0.326	0.936	0.684
d 7 to 14	478	477	511	391	477	55	0.982	0.633	0.241	0.990
d 14 to 21	483	530	431	536	520	52	0.451	0.118	0.929	0.865
d 21 to 28	555	471	594	513	534	49	0.159	0.041	0.482	0.293
d 28 to 35	629	512	474	541	468	48	0.079	0.574	0.654	0.511
d 35 to 42	531	516	526	537	527	20	0.598	0.706	0.452	0.694
d 0 to 42	520	495	504	517	500	10	0.052	0.499	0.086	0.697

[1] PC: positive control without AF and DON; NC: negative control with 175 µg/kg AF and 900 µg/kg DON; A: NC + 2 mg/kg of a clay additive; B: NC + 1.5 mg/kg of a clay and yeast additive; C: NC + 1.1 mg/kg of a clay and enzyme additive

[2] *P* value of negative control (NC) contrasted to each treatment

[3] Initial body weight (kg) was used as covariate in a statistical analysis

Table 3. Antibody titer of H3N2 via hemagglutination inhibition (HI) for pigs fed diets containing mycotoxins and various feed additives measured on d 0 and d 42

	Treatment[1]						NC vs:[2]			
	PC	NC	A	B	C	SEM	PC	A	B	C
d 0										
H3N2 Titer	14.6	20.5	20.6	17.0	16.3	3.2	0.182	0.972	0.456	0.344
H3N2LG[3]	1.31	1.82	1.75	1.50	1.50	0.28	0.171	0.871	0.421	0.400
d 42										
H3N2 Titer	212.3	349.1	301.3	356.0	229.2	67.0	0.132	0.639	0.942	0.193
H3N2LG[3]	5.00	5.82	5.13	5.70	4.58	0.47	0.200	0.337	0.861	0.060

[1] PC: positive control without AF and DON; NC: negative control with 175 µg/kg AF and 900 µg/kg DON; A: NC + 2 mg/kg of a clay additive; B: NC + 1.5 mg/kg of a clay and yeast additive; C: NC + 1.1 mg/kg of a clay and enzyme additive

[2] *P* value of negative control (NC) contrasted to each treatment

[3] H3N2LG: data for H3N2 titer analysis in logarithmic form

Table 4. Hematology of pigs fed diets containing mycotoxins and various feed additives measured on d 28

	Treatment[1]						NC vs.:[2]			
	PC	NC	A	B	C	SEM	PC	A	B	C
Hematocrit, %	32.93	35.13	39.57	38.59	36.29	2.35	0.513	0.188	0.296	0.723
Hemoglobin, g/dL	10.26	12.04	12.68	12.33	11.63	0.81	0.202	0.581	0.796	0.720
MCH[3], pg	15.94	16.77	16.82	16.14	16.57	0.21	0.006	0.870	0.031	0.490
MCHC[3], g/dL	32.09	32.55	32.02	31.97	32.08	0.17	0.063	0.033	0.018	0.052
MCV[3], fL	49.71	51.49	52.51	50.51	51.70	0.60	0.039	0.236	0.243	0.805
Platelet, 10^3/µL	381.7	415.3	358.5	377.9	298.1	39.0	0.547	0.309	0.494	0.035
RBC[3], 10^6/µL	6.63	6.82	7.52	7.58	7.01	0.43	0.743	0.254	0.208	0.754
WBC[3], 10^3/µL	26.24	24.06	26.32	24.66	21.80	1.53	0.322	0.305	0.782	0.294
Basophil, 10^3/µL	0.23	0.16	0.32	0.31	0.19	0.06	0.385	0.053	0.058	0.707
Eosinophil, 10^3/µL	0.58	0.55	0.58	0.47	0.44	0.09	0.840	0.832	0.509	0.385
Lymphocyte, 10^3/µL	11.40	10.49	12.50	11.34	10.41	0.86	0.459	0.104	0.482	0.947
Monocyte, 10^3/µL	1.70	2.04	2.04	1.73	1.58	0.18	0.184	0.985	0.227	0.073
Neutrophil, 10^3/µL	11.75	10.35	10.20	10.15	8.56	0.98	0.318	0.913	0.884	0.192

[1] PC: positive control without AF and DON; NC: negative control with 175 µg/kg AF and 900 µg/kg DON; A: NC + 2 mg/kg of a clay additive; B: NC + 1.5 mg/kg of a clay and yeast additive; C: NC + 1.1 mg/kg of a clay and enzyme additive

[2] *P* value of negative control (NC) contrasted to each treatment

[3] MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; RBC: red blood cell count; WBC: white blood cell count

Table 5: Hematology of pigs fed diets containing mycotoxins and various feed additives measured on d 42

	Treatment[1]						NC vs.:[2]			
	PC	NC	A	B	C	SEM	PC	A	B	C
Hematocrit, %	34.67	37.49	36.49	34.85	35.36	0.88	0.024	0.427	0.035	0.092
Hemoglobin, g/dL	10.90	11.51	11.56	10.88	11.23	0.28	0.119	0.898	0.107	0.479
MCH[3], pg	16.93	17.80	17.84	17.21	18.10	0.25	0.015	0.904	0.091	0.397
MCHC[3], g/dL	31.44	30.66	31.79	31.22	31.92	0.36	0.129	0.032	0.274	0.017
MCV[3], fL	53.93	58.13	56.00	55.27	56.79	0.71	0.000	0.037	0.005	0.183
Platelet, 10^3/µL	430.6	409.7	370.1	328.8	369.1	31.1	0.636	0.379	0.070	0.366
RBC[3], 10^6/µL	6.44	6.46	6.47	6.32	6.24	0.15	0.893	0.961	0.473	0.279
WBC[3], 10^3/µL	18.61	21.40	19.64	17.97	20.10	1.36	0.148	0.365	0.076	0.504
Basophil, 10^3/µL	0.09	0.16	0.12	0.13	0.20	0.03	0.097	0.359	0.450	0.258
Eosinophil, 10^3/µL	0.61	0.57	0.83	0.46	0.60	0.13	0.848	0.162	0.540	0.892
Lymphocyte, 10^3/µL	9.56	9.18	10.10	8.83	9.49	0.53	0.617	0.229	0.632	0.692
Monocyte, 10^3/µL	0.97	1.43	1.05	0.95	1.26	0.13	0.011	0.040	0.009	0.341
Neutrophil, 10^3/µL	7.39	10.05	7.53	7.60	8.40	1.17	0.108	0.134	0.138	0.325

[1] PC: positive control without AF and DON; NC: negative control with 175 µg/kg AF and 900 µg/kg DON; A: NC + 2 mg/kg of a clay additive; B: NC + 1.5 mg/kg of a clay and yeast additive; C: NC + 1.1 mg/kg of a clay and enzyme additive

[2] *P* value of negative control (NC) contrasted to each treatment

[3] MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MCV: mean corpuscular volume; RBC: red blood cell count; WBC: white blood cell count

Table 6: Immunology of pigs fed diets containing mycotoxins and various feed additives

	Treatment[1]						NC vs.:[2]			
	PC	NC	A	B	C	SEM	PC	A	B	C
d 28										
IgG[3], mg/mL	25.28	18.00	19.95	28.73	18.68	3.65	0.163	0.707	0.041	0.896
IgM[3], mg/mL	2.46	2.69	2.54	2.86	2.70	0.31	0.594	0.734	0.705	0.989
TNFα[3], pg/mL	131.1	115.4	163.8	141.3	143.4	14.5	0.444	0.020	0.216	0.173
d 42										
IgG[3], mg/mL	10.46	15.12	8.62	13.54	11.07	1.60	0.043	0.005	0.487	0.078
IgM[3], mg/mL	3.02	4.39	2.71	3.19	3.23	0.43	0.026	0.007	0.050	0.062
TNFα[3], pg/mL	118.4	97.4	108.3	132.3	118.1	12.6	0.239	0.546	0.052	0.244

[1] PC: positive control without AF and DON; NC: negative control with 175 µg/kg AF and 900 µg/kg DON; A: NC + 2 mg/kg of a clay additive; B: NC + 1.5 mg/kg of a clay and yeast additive; C: NC + 1.1 mg/kg of a clay and enzyme additive

[2] *P* value of negative control (NC) contrasted to each treatment

[3] IgG: immunoglobulin G; IgM: immunoglobulin M; TNFα: tumor necrosis factor α

Table 7: Serum biochemistry of liver function of pigs fed diets containing mycotoxins and various feed additives measured on d 28

	Treatment[1]						NC vs.:[2]			
	PC	NC	A	B	C	SEM	PC	A	B	C
Albumin, g/dL	2.12	2.39	2.43	2.17	2.29	0.09	0.048	0.713	0.096	0.462
Albu:globulin[3]	0.62	0.70	0.77	0.59	0.71	0.05	0.289	0.340	0.129	0.924
Alk phos[3], U/L	227.2	231.9	239.8	204.5	261.5	18.9	0.863	0.764	0.301	0.263
ALT[3], U/L	27.08	29.93	30.27	27.53	30.13	2.61	0.453	0.923	0.512	0.956
AST[3], U/L	32.92	41.73	44.00	39.93	38.27	3.97	0.130	0.684	0.746	0.533
Bilirubin, mg/dL	0.108	0.107	0.100	0.100	0.100	0.004	0.870	0.273	0.273	0.273
UN:creatinine[3]	20.54	25.20	24.60	23.93	27.40	1.41	0.025	0.761	0.521	0.266
Calcium, mg/dL	9.92	10.16	10.29	9.79	9.88	0.16	0.284	0.544	0.098	0.204
Chloride, mEq/L	98.77	98.67	99.20	99.20	99.47	0.54	0.896	0.480	0.480	0.291
Chol[3], mg/dL	77.38	79.20	74.80	74.47	76.60	3.07	0.684	0.308	0.273	0.546
CPK[3], U/L	527.2	436.7	767.9	544.3	405.0	121.5	0.608	0.055	0.527	0.852
Creatinine, mg/dL	0.65	0.63	0.64	0.67	0.63	0.03	0.468	0.711	0.198	1.000
Globulin, g/dL	3.60	3.51	3.32	3.80	3.34	0.16	0.715	0.399	0.213	0.449
Glucose, mg/dL	94.0	91.3	97.9	93.9	100.2	4.45	0.670	0.290	0.666	0.153
Na:K[3]	23.92	23.80	22.47	22.93	22.93	0.71	0.904	0.180	0.381	0.381
P[3], mg/dL	9.02	9.55	9.80	9.29	9.75	0.24	0.126	0.464	0.428	0.565
Potassium, mEq/L	5.98	6.05	6.35	6.28	6.18	0.20	0.791	0.292	0.415	0.648
Protein, g/dL	5.72	5.90	5.75	5.97	5.63	0.13	0.352	0.423	0.688	0.147
Sodium, mEq/L	140.9	141.0	141.2	141.6	141.2	0.7	0.942	0.843	0.553	0.843
Urea N[3], mg/dL	13.23	15.53	15.47	15.67	16.80	0.60	0.010	0.937	0.874	0.136

[1] PC: positive control without AF and DON; NC: negative control with 175 µg/kg AF and 900 µg/kg DON; A: NC + 2 mg/kg of a clay additive; B: NC + 1.5 mg/kg of a clay and yeast additive; C: NC + 1.1 mg/kg of a clay and enzyme additive

[2] *P* value of negative control (NC) contrasted to each treatment

[3] Albu:globulin: albumin to globulin ratio; Alk phos: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN:creatinine: BUN to creatinine ratio: Chol: cholesterol; CPK: creatine phosphokinase; Na:K: sodium to potassium ratio; P: phosphorus; Urea N: urea nitrogen

Table 8: Serum biochemistry of liver function of pigs fed diets containing mycotoxins and various feed additives measured on d 42

	Treatment[1]						NC vs.:[2]			
	PC	NC	A	B	C	SEM	PC	A	B	C
Albumin, g/dL	2.49	2.80	2.94	2.63	2.76	0.11	0.047	0.370	0.268	0.797
Albu:globulin[3]	0.81	0.93	0.98	0.79	0.96	0.06	0.170	0.612	0.130	0.772
Alk phos[3], U/L	233.1	235.9	233.5	208.7	249.7	15.4	0.895	0.912	0.215	0.527
ALT[3], U/L	27.13	29.47	29.27	29.33	32.60	1.97	0.404	0.943	0.962	0.264
AST[3], U/L	31.53	38.80	36.67	46.87	42.33	4.54	0.262	0.741	0.214	0.584
Bilirubin, mg/dL	0.100	0.100	0.100	0.100	0.107	0.003	1.000	1.000	1.000	0.118
BUN:creatinine[3]	16.00	20.60	21.93	20.80	23.00	1.15	0.329	0.415	0.903	0.145
Calcium, mg/dL	9.87	10.29	10.53	9.93	10.12	0.14	0.033	0.239	0.071	0.381
Chloride, mEq/L	100.6	100.7	101.5	101.3	102.1	0.7	0.891	0.710	0.536	0.171
Chol[3], mg/dL	78.73	76.93	72.80	72.60	75.20	2.32	0.585	0.212	0.191	0.599
CPK[3], U/L	655.9	722.9	548.2	636.0	714.6	176.7	0.788	0.484	0.732	0.973
Creatinine, mg/dL	0.66	0.64	0.64	0.68	0.65	0.03	0.603	1.000	0.300	0.729
Globulin, g/dL	3.25	3.17	3.05	3.37	3.00	0.15	0.705	0.571	0.362	0.414
Glucose, mg/dL	111.3	109.9	113.8	105.4	107.7	3.00	0.752	0.361	0.285	0.602
Na:K[3]	26.00	25.33	24.93	26.00	25.27	0.72	0.517	0.697	0.517	0.948
P[3], mg/dL	8.95	9.63	9.51	9.02	9.37	0.27	0.075	0.753	0.111	0.496
Potassium, mEq/L	5.57	5.73	5.82	5.55	5.75	0.16	0.475	0.677	0.439	0.929
Protein, g/dL	5.74	5.97	5.99	5.99	5.76	0.12	0.161	0.904	0.904	0.199
Sodium, mEq/L	143.2	143.1	143.8	142.8	142.5	0.7	0.899	0.488	0.800	0.570
Urea N[3], mg/dL	12.27	13.00	13.87	13.67	14.60	0.51	0.310	0.231	0.355	0.029

[1] PC: positive control without AF and DON; NC: negative control with 175 µg/kg AF and 900 µg/kg DON; A: NC + 2 mg/kg of a clay additive; B: NC + 1.5 mg/kg of a clay and yeast additive; C: NC + 1.1 mg/kg of a clay and enzyme additive

[2] *P* value of negative control (NC) contrasted to each treatment

[3] Albu:globulin: albumin to globulin ratio; Alk phos: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN:creatinine: BUN to creatinine ratio: Chol: cholesterol; CPK: creatine phosphokinase; Na:K: sodium to potassium ratio; P: phosphorus; Urea N: urea nitrogen

Table 9: Tissue analysis of weight and color of pigs fed diets containing mycotoxins and various feed additives

	Treatment[1]						NC vs.:[2]			
	PC	NC	A	B	C	SEM	PC	A	B	C
Organ weight, g										
Liver	718.7	800.4	840.1	713.6	778.0	40.2	0.155	0.488	0.131	0.695
Kidney	148.6	146.0	159.3	140.8	146.8	9.4	0.848	0.321	0.700	0.951
Spleen	61.13	58.54	64.99	56.41	57.71	4.06	0.653	0.265	0.712	0.886
Organs, % of body weight										
Liver	2.71	3.29	3.31	2.80	3.14	0.10	<0.001	0.890	0.001	0.344
Kidney	0.56	0.60	0.62	0.55	0.59	0.02	0.169	0.438	0.137	0.839
Spleen	0.23	0.24	0.25	0.22	0.24	0.01	0.654	0.391	0.452	0.871
Color liver[3]										
Lightness (L*)	34.67	35.57	35.23	35.24	35.57	0.50	0.209	0.635	0.645	0.987
Redness (a*)	13.95	13.79	13.83	13.70	13.89	0.20	0.567	0.877	0.762	0.722
Yellowness (b*)	3.17	3.60	3.90	3.94	4.65	0.37	0.407	0.566	0.516	0.048
Color kidney										
Lightness (L*)	45.54	45.91	46.32	47.00	45.78	0.72	0.714	0.687	0.287	0.900
Redness (a*)	13.21	13.46	13.05	12.25	13.37	0.55	0.751	0.600	0.125	0.907
Yellowness (b*)	7.69	7.34	7.76	8.88	8.29	0.46	0.596	0.521	0.021	0.151
Color spleen										
Lightness (L*)	35.73	35.90	36.35	37.03	35.97	0.74	0.871	0.671	0.290	0.950
Redness (a*)	18.12	18.42	18.13	18.26	18.43	0.26	0.415	0.427	0.666	0.977
Yellowness (b*)	1.89	1.87	1.79	1.75	1.71	0.21	0.959	0.791	0.685	0.588

[1] PC: positive control without AF and DON; NC: negative control with 175 µg/kg AF and 900 µg/kg DON; A: NC + 2 mg/kg of a clay additive; B: NC + 1.5 mg/kg of a clay and yeast additive; C: NC + 1.1 mg/kg of a clay and enzyme additive

[2] *P* value of negative control (NC) contrasted to each treatment

[3] Tissue color measured via Minolta Colorimeter (Konica Minolta, Ramsey, NJ)

Table 10: Tissue damage analysis of pigs fed diets containing mycotoxins and various feed additives

	Treatment[1]						NC vs.:[2]			
	PC	NC	A	B	C	SEM	PC	A	B	C
Liver[3]										
Bile ductule hyp[4]	1.00	1.33	1.13	1.00	1.13	0.08	0.005	0.083	0.005	0.083
Fibrosis	1.13	1.07	1.27	1.13	1.13	0.09	0.614	0.133	0.614	0.614
Hydropic degen[4]	1.73	1.93	1.47	1.87	1.80	0.11	0.185	0.003	0.657	0.376
Inflammation	1.00	1.00	1.00	1.00	1.00	0.00	-	-	-	-
Megakaryosis	1.00	3.00	1.87	1.40	1.40	0.18	< 0.001	< 0.001	< 0.001	< 0.001
Necrosis	1.00	1.00	1.00	1.00	1.00	0.00	-	-	-	-
Vacuolation	1.13	1.53	1.40	1.20	1.40	0.12	0.021	0.434	0.053	0.434
Kidney[3]										
Fibrosis	1.00	1.00	1.00	1.00	1.00	0.00	-	-	-	-
Necrosis	1.00	1.00	1.00	1.00	1.00	0.00	-	-	-	-
Protein casts	1.00	1.00	1.00	1.00	1.00	0.00	-	-	-	-
Regeneration	1.00	1.00	1.00	1.00	1.00	0.00	-	-	-	-
Vacuolation	1.00	1.13	1.07	1.00	1.00	0.05	0.066	0.353	0.066	0.066

[1] PC: positive control without AF and DON; NC: negative control with 175 µg/kg AF and 900 µg/kg DON; A: NC + 2 mg/kg of a clay additive; B: NC + 1.5 mg/kg of a clay and yeast additive; C: NC + 1.1 mg/kg of a clay and enzyme additive

[2] *P* value of negative control (NC) contrasted to each treatment

[3] Liver and kidney tissue microscopic examinations measured on degrees of the change: 1. normal to minimal (0-5%); 2. mild (5-15%); 3. moderate (15-40%); 4. severe (>40%)

[4] Bile ductule hyp: bile ductule hyperplasia; Hydropic degen: hydropic degeneration

CHAPTER 4

FINAL CONCLUSIONS

Mycotoxin contamination of grains can be problematic for hog producers. Swine health and production may be altered due to decreased growth and feed intake, and increased organ damage and immune suppression. The two mycotoxins, AF and DON, are of particular concern as they are prevalent in the United States and throughout the world. Although the degree of grain contamination may change from year to year, these toxins continuously pose a problem for farmers. When animals ingest high concentrations of AF and DON above the FDA regulatory standards, the effects associated with the toxins are easily observable. However, it is rare for animals to consume these high levels of mycotoxins. It may in fact be the continuous consumption of feeds with low levels of mycotoxins that pose a greater problem, and in this situation, the effects are not well documented. Our study sought to quantify the effects of consuming low dietary mycotoxins on young swine over an extended period of time. This research also examined the ability of feed additives to ameliorate the problematic effects of feeding low mycotoxins levels.

To accomplish these objectives, two groups of pigs (BW 13.9 ± 0.2 kg and 8.8 ± 0.4 kg) were fed both AF and DON at dietary concentrations under the FDA regulations for these mycotoxins. The first group of pigs consumed diets which contained increasing levels of each mycotoxin together: 0, 60, 120, or 180 µg/kg AF and 0, 300, 600, or 900 µg/kg DON. The dietary mycotoxin concentration of was chosen due to the fact that it caused, needed for statistical difference determination. The second group of pigs was then fed 180 µg/kg AF and 900 µg/kg DON, the diet from the first experiment that resulted in at least a 15 % change to pig growth performance and immune status parameters. Diets in the second study

also contained different feed additives which have potential for reducing mycotoxin toxicity. These additives include clay, clay and yeast, or clay and enzymes. Below is a summary of results obtained from these two experiments.

Summary of the Effects of Low Dietary Aflatoxin and Deoxynivalenol on Pigs, and the Efficiency of Feed Additives to Reduce Toxicity

- Growth Performance
  - Average Daily Gain
    - Reduced up to 21%
    - Feed additives did not improve ADG
  - Average Daily Feed Intake
    - Reduced up to 15 %
    - Feed additives did not improve ADFI
- Immune System
  - Hematology
    - Increased white blood cell numbers by 27 %
    - Increase of 42 % for monocytes
    - Additive composed of clay and yeast reduced white blood cells
    - Feed additives composed of clay and yeast, or clay and enzymes, reduced monocytes
  - Immunoglobulin Subsets
    - Increased IgG and IgM by 45 %
    - All three feed additives reduced effect on IgG and IgM
- Organ Health
  - Liver Function
    - Albumin and globulin increased
    - Feed additives did not alter mycotoxin effects
  - Liver Damage
    - Bile ductule hyperplasia increased by 33 %
    - Fibrosis increased by 50 %
    - Megakaryosis increased by 200 %
    - Vacuolation increased by 35 %
    - All three feed additives reduced tissue damage
  - Kidney
    - Vacuolation increased by 13 %
    - Feed additives composed of clay and yeast, or clay and enzymes, reduced kidney damage

Implications

Our experiments show that concentrations of AF and DON can be detrimental to swine health and performance even at levels under FDA standards. Although the effects of mycotoxins are not as obvious at low dietary concentrations, growth performance, immune status, and organ health were still altered. Our results imply that regulatory levels for the mycotoxins AF and DON may be set too high. The results also demonstrate that farmers should be concerned of feeding grains contaminated by levels of mycotoxins previously thought safe.

As our second experiment shows, incorporating feed additives into the diets of swine can be a somewhat effective method for reducing mycotoxin toxicity. Although they did not statistically improve growth performance, additives composed of clay, clay and yeast, or clay and enzymes, showed potential to improve immune system and organ health of swine consuming AF and DON. Future research on the area of feed additives will be important to fully understand how they eliminate toxins, which mycotoxins each type of additive binds or transforms most efficiently, and how much additive is needed for sufficient detoxification. In order to combat the detriments of mycotoxins, further research is also needed to determine accurate and sustainable methods of preventing fungal growth, detecting mycotoxin presence, and eliminating toxins from grains containing both high and low contamination levels. A reconstruction of regulatory limits of mycotoxins allowed in foods and feeds may also be necessary. The importance of these future findings and changes can not only benefit the swine industry, but all animal and human industries impacted by mycotoxins.