EFFICACY OF ADSORBENTS AND ANTIOXIDANTS IN REDUCING THE EFFECTS OF MYCOTOXINS IN BROILERS AND PIGS

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CHAPTER I

INTRODUCTION

Feed is the most expensive aspect of livestock production, accounting for around 69% of total cost (Donohue and Cunningham, 2009). This high cost drives the incentive to offer a properly balanced diet, which supplies the ideal amount of nutrients to provide for optimal growth, at a decreased cost of production. The main ingredient used in feed, especially for monogastrics such as poultry and swine, is corn. There are few alternatives available to corn that provide a comparable energy to cost benefit.

Since corn makes up the majority of a monogastric diet, there is concern in the poultry and swine industries about grain quality. A chief concern of producers is contamination by fungi and associated mycotoxins. Mycotoxins are toxic secondary metabolites produced by organisms of the fungi kingdom, which are not directly essential for growth of the fungi. The term “mycotoxins” comes from “mykes” meaning fungi and “toxicon” meaning poison. There are over 200 species of molds that produce mycotoxins (Murugesan *et al., 2015).*

The modern era of mycotoxin study began in England in 1960, after over 100,000 turkeys died from ‘Turkey-X disease.’ The cause of this devastating disease was the consumption of peanut meal contaminated with aflatoxin; at the time this was a new group of mycotoxins produced by the fungus *Aspergillus flavus*. In the years since this massive mortality, other important mycotoxins including fumonisin, ochratoxin,

vomitoxin, the trichothecenes, and zearalenone have been discovered and described (Schmale *et al.*, 2009).

Today, mycotoxins are still a problem for producers since they lead to increased economic loss through: 1) lower crop yield due to disease; 2) reduced crop value from contamination; 3) losses in animal production from health problems; and 4) human health costs. In addition to these costs, there is a higher management expense at the level of crop production, storage, processing, and animal production. The estimated cost of mycotoxins in the United States varies, with reports from $0.5 billion to $5 billion per year for the U.S. and Canada (Schmale III *et al.*, 2013). Aflatoxins in corn are estimated to cost $225 million per year (Schmale III *et al.*, 2013).

Prevention of mold growth and mycotoxin contamination of feedstuffs is very important, but, if contamination cannot be prevented, decontamination of the materials is needed before they can be used in feed. Several approaches have been used including physical, chemical and biological treatments to detoxify affected feedstuffs. A successful detoxification process must be economical and capable of eliminating all traces of toxins without leaving harmful residues or impacting the nutritional quality of the grain or feed (Oguz, 2012). Strategies that have been used to combat the negative effects of mycotoxin contamination include: mold inhibitors; nutritional supplements; microbial and thermal inactivation; irradiation; ammoniation; ozonation; solvent extraction; mechanical separation; antioxidants; competitive exclusion; molecular biology techniques, and adsorbents (CAST, 2003). A common approach to the problem is to use non-nutritive, inert adsorbents in diets to bind mycotoxins and reduce absorption in the

GI tract, but high inclusion rates and possible potential interactions with nutrients are a cause for concern. Another common method is biological degradation through yeast and yeast components, which works best when contamination is low. Ledoux *et al.* (1999) added hydrated sodium calcium aluminosilicate clay (HSCAS) to a broiler diet containing 4 ppm aflatoxin (AF). The addition of this adsorbent to the diet improved AF dependent changes in organ weights, serum chemistry, gross pathology, and reduced hepatic and renal histopathology changes. Zhao *et al.* (2010) used a combined method of HSCAS and yeast cell well components in a diet with 1 and 2 ppm AF, and saw significant improvements in performance, serum biochemistry and histopathology associated with aflatoxicosis. This study showed a greater improvement from the addition of HSCAS than yeast cell well components (Oguz, 2012).

The objectives of the current studies are to determine if 1) two hydrated sodium calcium aluminosilicate clays can reduce aflatoxin B_1 effects on broiler chicks up to 21 days; 2) an adsorbent product can reduce or prevent the effects of a combination of mycotoxins on broiler chicks up to 21 days; 3) the efficacy of an adsorbent to prevent or reduce the toxic effects of a combination of aflatoxin and fumonisin in weanling pigs, and 4) two antioxidants (curcumin and theracurcumin) would reduce the effects of aflatoxin B_1 in weanling pigs.

CHAPTER II

LITERATURE REVIEW

BIOTRANSFORMATION

Xenobiotics are foreign chemical substances found within an organism. Biotransformation of xenobiotics is the body's process of converting the original molecules to more hydrophilic compounds that can be excreted in urine or bile, via the kidneys or liver. Traditionally, biotransformation is a process that occurs in two phases (phase I and phase II), although some authors would disagree and believe that this classification should be eliminated. Phase I metabolism consists of enzyme-mediated hydrolysis, reduction and oxidation reactions. Phase II metabolism involves conjugation reactions of either the original compound, or the subsequent metabolites from phase I reactions (Gonzalo, 2011).

In the first phase of biotransformation of aflatoxin, the majority of the reactions are catalyzed by cytochrome P450 (CYP450) enzymes. The CYP450s are membrane bound enzymes that can be isolated in the microsomal fraction of the endoplasmic reticulum. These enzymes participate in a variety of oxidative reactions, including hydroxylation, hydration, *O*-demethylation, and epoxidation of a double bond (Gonzalo, 2011).

Translocation of xenobiotics across cell membranes by specific proteins has been termed by some as 'phase III' of biotransformation. In this process though, there is no modification of the xenobiotic structure, so it cannot be termed as metabolism.

AFLATOXIN

Aflatoxins are a class of mycotoxins produced by various species of the fungi *Aspergillus,* most commonly *Aspergillus flavus, Aspergillus parasiticus.* There are at least 14 different forms of aflatoxins (AF) found in nature, including: aflatoxin B_1, B_2, G_1, G_2, M_1 and M_2 (Figure 2.1). Aflatoxin M_1 (AFM_1) and AFM_2 are hydroxylated metabolites of AFB_1 or AFB_2 found in milk or urine of animals fed aflatoxin-contaminated grain (Dersjant-Li *et al.*, 2003). Aflatoxins with the subscript 2 (AFB_2, AFG_2) are considered less toxic than aflatoxins with the subscript 1 (AFB_1, AFG_1) because of the absence of the 8,9 double bond in the structure, which keeps epoxidation from occurring as it does in structures with the double bond.

Aflatoxin biotransformation primarily occurs in the liver, but can also occur at the site of absorption or in the blood. These reactions decrease aflatoxins toxicity and increase its solubility in water to facilitate excretion in urine (and milk) and protect the animal from its adverse effects (Yiannikouris *et al.*, 2002). In phase I, AFB_1 can go through several different reactions, catalyzed by CYP450s. One reaction that occurs is hydroxylation, which leads to the monohydroxylated metabolites: AFM_1, AFB_2, and AFQ_1. Aflatoxin M_1 is a cytotoxic and carcinogenic metabolite of AFB_1. Aflatoxin M_1 is most commonly found in milk, but can also be excreted in the urine. The highest levels in the body are found in the liver and kidney, showing the role these organs play in biotransformation and elimination. This process cannot be considered a detoxification because the metabolite formed is toxic as well, but in the case of AFB_2 the double bond

is hydrated to create a non-toxic metabolite of AFB_1. Some species are much more efficient in converting AFB_1 to its hemiacetal AFB_2, which makes them more resistant to the toxin.

The most concerning reaction in biotransformation of AFB_1 is epoxidation. The ether double bond in the furanofuran ring is epoxidized to form AFB_1-exo-8-9-epoxide. This is an unstable and highly reactive molecule, which binds to cellular components. It can readily bind to DNA and RNA, which is responsible for the carcinogenic and mutagenic effects of AFB_1.

In phase two biotransformation, the most studied reaction is the nucleophilic trapping process, in which glutathione (GSH) reacts with the epoxide (Gonzalo *et al.*, 2011). This conjugate can be excreted via bile into the intestinal tract. This is the principal detoxification pathway of phase II of active AFB_1 in the body, and is essential in preventing or reducing the carcinogenic effects of AF (Yiannikouris *et al.*, 2002).

The main target of AFB_1 is the liver; high-level exposure produces an acute hepatic necrosis, resulting in cirrhosis, and/or carcinoma of the liver. Acute hepatic failure is characterized by hemorrhage, edema, alteration in digestion, and changes in nutrient absorption or metabolism (Marin *et al.*, 2002). The ability of the epoxide to intercalate into DNA is thought to cause mutations in the p53 gene, a gene that is important in preventing cell cycle progression when there are DNA mutations, or initiating apoptosis (Aguilar *et al.*, 1993).

Several studies have been conducted with poultry to determine tolerable levels of aflatoxins among the species. Murugesan *et al.* (2015) reviewed several studies and

concluded that aflatoxins can cause a large variety of effects in poultry including: decreased weight gain, poor feed efficiency, reduced egg production and egg weight, increased liver fat, changes in organ weights, reduction in serum protein levels, carcass bruising, poor pigmentation, liver damage, decreased enzyme activity, and immunosuppression. It is estimated that for each mg/kg of aflatoxins added to the diet, growth rate in broilers would decrease by 5% (Dersjant-Li *et al*, 2003). Several studies have shown that aflatoxin additions between 0.1 and 0.8 mg/kg has no effect on broilers, and Huff *et al.* (1992) observed no negative effects on male broilers below 2.5 mg AF/kg of diet. Ducks are reported to be the most sensitive poultry species to AF, followed by turkeys, broilers and laying hens; ducks are approximately 200 times more susceptible than chicks, especially for acute hepatotoxic effects (Chen *et al.*, 2013).

Swine are much more sensitive to aflatoxicosis than poultry, especially during the weanling phase as AF can cause a variety of issues depending on the concentration of AF and consumption of diets containing AF. Pigs have shown on average a 16% depression in growth rate for every mg/kg aflatoxins in the diet and a 5% reduction in growth was shown at 0.3 mg/kg aflatoxin (Dersjant-Li *et al.,* 2003). This reduced growth rate is related to the decreased feed intake and decreased gain:feed experienced with the addition of aflatoxin in the diet.

The effects of feeding aflatoxins to swine depend on the age and health of the pig, aflatoxin concentration, and duration of exposure. Effects that come from feeding aflatoxin include: decreased rate of weight gain, decreased feed efficiency, immune suppression, reduced reproductive capability, and systemic hemorrhages. Liver damage

caused by aflatoxins can be characterized by enlargement, enzyme release into the blood (e.g., aspartate aminotransferase, y-glutamyltransferase, and alkaline phosphatase), and impaired protein synthesis (Schell, 1993). Aflatoxins can also be passed from lactating sows to nursing piglets via the milk, consequently affecting the piglets. AFM_1 was found in the milk of nursing sows consuming diets containing 500 and 750 ppb AFB_1. The piglets consuming this milk had a higher death rate and slower growth rate, which also impacted them later in the grower/finisher period (Crenshaw, 2008). Aflatoxins have a great impact on animal production, and because of this regulations have been on the amount of toxin that can be present in animal feed.

The Food and Drug Administration (FDA, 2011) has established the following action levels for aflatoxins present in animal feed and feed ingredients:

Intended Animal	**Grain, Feed, or other product**	**Amount of Total AF** Parts per billion (ppb)
Dairy Animals	Corn, peanut products, cottonseed meal, and other animal feed and feed ingredients	20 ppb
Immature Animals	Corn, peanut products, and other animal feed and feed ingredients (excluding cottonseed meal)	20 ppb
Breeding beef cattle, breeding swine, mature poultry	Corn and peanut products	100 ppb
Finishing swine	Corn and peanut products	200 ppb
Beef cattle, swine or poultry (regardless of age or breeding status)	Cottonseed meal	300 ppb
Finishing beef cattle (e.g. feedlot cattle)	Corn and peanut products	300 ppb

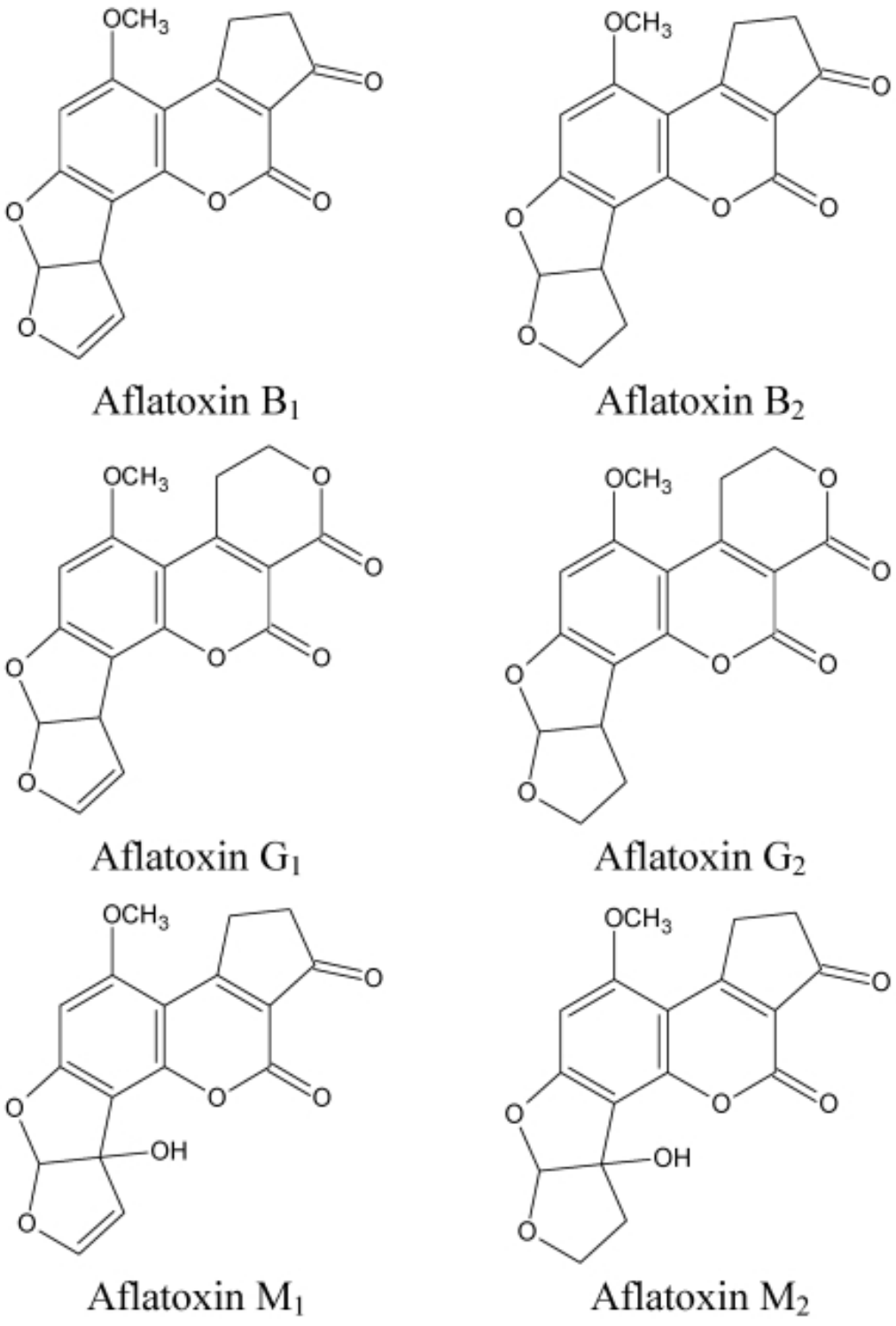

Figure 2.1 Structures of Aflatoxins (Leeson *et al.,* 1995)

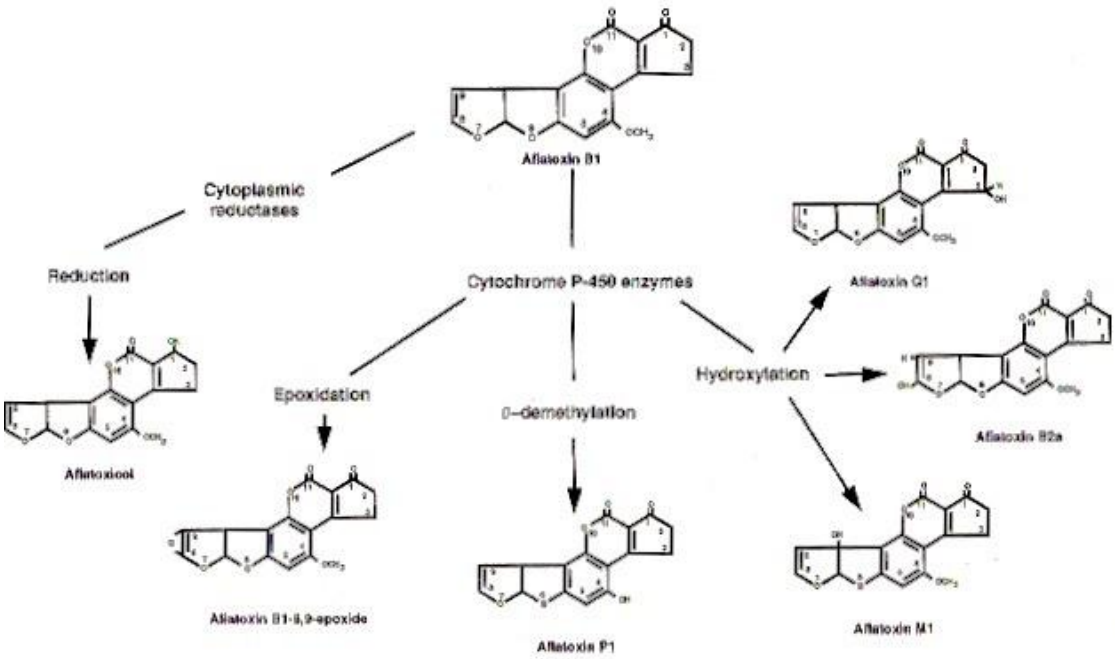

Figure 2.2 Biotransformation of Aflatoxin B_1 (Leeson *et al.,* 1995)

FUMONISIN

Fumonisins are a group of mycotoxins that are produced by *Fusarium* spp. of molds. This is a group of mycotoxins that are non-fluorescent, water-soluble, and polar. Although there are over 15 types of fumonisins only six have been identified and have known structures including: A1, A2, B1, B2, B3, and B4. FB_1 is the predominant form and the most significant form regarding toxic effects. FB_1 was first isolated and characterized by South African scientists in 1988 (Domijan *et al.*, 2012).

The symptoms of fumonisin toxicosis are very broad and affect several species. Ingesting these toxins can cause neural tube defects in newborns, brain lesions in horses, pulmonary edema in swine, and cancer. FB_1 itself is not mutagenic, but is cancer promoting. Though each species is affected differently in the body, the negative impact caused by these toxins is related to an alteration of sphingolipid metabolism.

The backbone FB_1 has a chemical structure that is similar to the sphingolipids, sphinganine and sphingosine (Figure 2.3). As a result of this similarity in structure, FB_1 competitively inhibits ceramide synthase, a key enzyme in sphingolipid metabolism. The inhibition of ceramide synthase reduces synthesis of sphingolipids, which increases free sphinganine and sphingosine in the body (Domijan *et al.*, 2012). These free sphingolipid bases are proapoptotic, cytoxic, and act as growth inhibitors. Sphingolipids and their metabolites also play key cellular roles both as structural components of membranes and as signaling molecules that mediate responses to stress. With such similar structures, and sphigolipid roles in membrane transport, these toxins can induce oxidative stress and cell damage through enhanced oxygen transport in cell membranes.

Accumulation of sphinganine and sphingosine in liver and kidney cells is the first microscopic evidence of the effects of FB_1 (Escriva *et al.*, 2011). An increase in the sphinganine:sphingosine ratio has been observed in tissues of broilers, turkeys, and ducklings fed FB_1.

In poultry species, the primary effects of feeding rations containing toxic amounts of FB_1 for 21 days include: decreased body weight gain and liver pathology. Chicks showed hepatic necrosis and biliary hyperplasia (Murugesan *et al., 2015*). Results from studies using between 10 and 474 mg/kg FB_1 were combined to get an overall look at the impact different levels have on broiler performance and health. This review showed that a 5% growth rate reduction was achieved at 251 mg/kg FB_1 in the diet of broilers (Dersjant-Li *et al.,* 2003). Poultry are much more effective at eliminated FB_1 than swine, and were shown to have eliminated 97% of the toxin in 24 hours, and that in laying hens fed 2 mg/kg BW only 1% of the oral dose was absorbed (Dersjant-Li *et al.,* 2003).

Fodor conducted an experiment in 2008, showing the toxic effects of FB_1 in swine. Pigs were fed 50 ppm FB_1 for 19 to 22 days. These high oral doses resulted in subacute toxicosis causing effects such as pathological alterations in the lungs and liver, and these effects occurred within 10 days of consuming toxin-free rations. In the second experiment, Fodor fed 45 ppm FB_1 for 10 days, which resulted in pulmonary edema in all animals. Other effects included pathological changes in liver, kidneys, and heart, and hyperplasia in the spleen (Escriva *et al.*, 2011). A review by Dersjant-Li *et al. (*2003) showed that a marginal growth reduction was observed at FB_1 levels as low as 0.42

mg/kg, and a 5% reduction was seen at 21 mg/kg diet. When pigs were fed levels of 105 and 155 mg/kg, they were unable to maintain a healthy body weight (Dersjant-Li *et al.,* 2003). Absorption in swine is much higher than in poultry, and elimination is slow. In 72 hours, pigs will absorb around 20% of the toxin fed (Dersjant-Li *et al.,* 2003).

OCHRATOXIN A

Ochratoxins are mycotoxins that are produced primarily during storage by *Aspergillus* fungus such as, *A Ochreus* in warmer climates and *Penicillium verrucosum* in more temperate areas. The most common and most toxic is ochratoxin A (OTA). Negative effects of OTA have been reported as early as the 1970's. Ochratoxin A consumption causes nephrotoxicity, hepatotoxicity, and immunotoxicity. The target organ of OTA is the kidney; the kidney has high susceptibility as a result of unfavorable elimination kinetics. The toxin is reabsorbed in the proximal and distal tubules of the kidney, which leads to accumulation in the renal tissue. Ochratoxin A can also impair protein metabolism, because OTA competes with phenylalanine and can bind to phenylalanine transfer-RNA-synthetase enzyme, thus inhibiting protein synthesis. This inhibition results in low protein levels in the blood (Duarte *et al.*, 2011). Battacore *et al.* (2010) lists the most relevant toxic effects of OTA as inhibition of protein synthesis, lipid peroxidation, DNA damage, and oxioreductive stress.

Swine are especially susceptible to OTA toxicity, due to the long half-life. This half-life is not only sustained by a high affinity for proteins, but also through enterohepatic circulation and bilary excretion. Enterohepatic circulation involves circulation of toxins from the liver to the bile, followed by entry into the small intestine,

absorption by the enterocyte and transport back to the liver (Duarte *et al.,* 2011). This allows for the toxin to accumulate and prolongs elimination. Ochratoxin A also increases swine susceptibility to infections, and decreases weight gain in growing barrows and pigs, as well as decreasing sperm production and quality (Duarte *et al.*, 2011).

Poultry are less affected by OTA because they can eliminate the toxins faster than mammals. The half-life in serum of swine is 20 to 30 times longer than in poultry. Even though poultry have a faster elimination time than swine, there are still negative effects of OTA such as nephropathy, increased mortality, reduced egg production in laying hens, poor growth, and reduced feed efficiency. A review by Duarte *et al.* (2011) described several episodes of ochratoxicosis in the United States affecting 970,000 turkeys, 70,000 laying hens, 120,000 broilers, and all showed significant negative effects, such as those listed previously.

T-2 TOXIN

T-2 toxin is a member of the trichothecenes family, a family of over 170 structurally related compounds produced by several *Fusarium* species of molds. Trichotheneces are the most potent small molecule inhibitors of protein synthesis. The main toxic effects of this family of toxins are primarily protein synthesis inhibition followed by secondary disruption of DNA and RNA synthesis (Murugesan *et al., 2015*). This family is classified into two groups: type A and type B toxins. T-2 is the most toxic and most common type A trichothenece mycotoxins (Biomin, 2010). T-2 is prominent in tropical and sub-tropical regions due to the warm, moist conditions that favor mold growth. This mycotoxin is a non-volatile, low-molecular weight compound that is highly

resistant to heat and UV light, making it hard to detoxify during feed production or processing (Sokolovic, 2008).

T-2 has several types of effects on animal health, such as genotoxic, cytotoxic and immune effects as well as affecting the skin and impairing performance (Sokolovic *et al.*, 2008). Murugesan *et al. (2015)* listed other toxic effects including oral lesions, reduced growth, abnormal feathering, decreased egg production and egg shell quality, regression of the bursa, peroxidative changes in the liver, abnormal blood coagulation, and proteinemia. The effects in poultry are dependent on time of exposure, dose, the presence of other toxins, and the animals age, sex, and overall health. The toxicity is a result of the presence of a 12,13 epoxide ring of T-2, with de-epoxidation there is a loss in toxicity. Broilers begin to show effects of T-2 toxin at concentrations of 3 to 4 mg/kg (Murugesan *et al., 2015*). The primary target of T-2 is the immune system, which results in changes in leukocyte counts or reduced antibody formation. It was found in poultry that the LD_{50} of T-2 in 7-day-old broilers was 4.97 mg/kg, which indicates it is more toxic than AF (LD_{50} = 6.8 mg/kg) (Sokolovic *et al.*, 2008).

ZEARALENONE

Zearalenone is a mycotoxin produced by numerous species of *Fusarium* on cereal grains (Biomin, 2010). Zearalenone (ZEN) has a very low acute toxicity; it is however, a powerful estrogenic metabolite with hormonal activity exceeding most naturally occurring non-steroidal estrogens (Metzler *et al.*, 2010). Zearalenone is metabolized in the liver by 3α and 3β hydroxysteroid dehydrogenase into α-zearalenol (α-ZOL) and β-zearalenol (β-ZOL). α-Zearlalenol has a binding affinity for estrogen receptors that is 92

times higher than β-ZOL, causing a much greater estrogenic effect in the body (D'Mello *et al.*, 1999).

Swine are very sensitive to ZEN, because in pigs the liver primarily metabolizes ZEN into α-ZOL. This causes hyperestrogenism and inflammation of the vagina, especially in prepubertal gilts, which are the most responsive. Hyperestrogenism includes symptoms such as prolonged estrus, anestrus, changes in libido, infertility, increased incidence of pseudopregnancy, increased udder or mammary development, and abnormal lactation.

Poultry are quite resistant to ZEN, for the reason that in poultry the liver primarily produces β-ZOL instead of α-ZOL. This causes less estrogenic effects in the body, but the consumption of ZEN can also cause liver and kidney damage. Though, in a study done there were no gross effects in broilers fed 50 mg ZEN/kg body weight daily for 7 days (Metzler *et al.*, 2010).

COMBINATION OF MYCOTOXINS

Mycotoxins can affect animals either individually or in combination with other mycotoxins present in feed. The additive effect of multiple mycotoxins can affect various organs such as the gastrointestinal tract, liver and immune system. There are over 200 species of molds that produce mycotoxins, and data have shown that contaminated grains typically contain more than just a single mycotoxin (Murugesan *et al., 2015*). When more than one fungal contaminant is present there is typically an additive or synergistic interaction, increasing the toxicity (Abidin *et al.*, 2011). When

both AF and OTA contaminate feed, they act in a synergistic manner. Ochratoxin A prevents the major effects of AF, changing the target organ from the liver to the kidney. A similar effect is seen with the combination of AF and T-2, showing a synergistic toxicity, meaning that the toxins in combination result in a greater impact than the addition of the two effects separately. Results range from synergistic to antagonistic, but it has been observed that a combination of mycotoxins can have a negative effect at concentrations that individually would not have an effect (Murugesan *et al., 2015*).

ADSORBENTS

The most effective and economic way to reduce the impact of AF is adding adsorbents to feed, effectively binding AF in the gut and reducing its bioavailability (Neef *et al.*, 2013). Substances used as adsorbents include indigestible adsorbent materials, such as silicates, activated carbons, complex carbohydrates, and others. These are recognized as safe feed additives and some are currently used as pellet binders or flow agents in diets. An ideal adsorbent would prevent mycotoxin absorption, form a stable complex in the GI tract, be safe, take up little space in the diet, and be biodegradable.

Some adsorbents, such as activated charcoal have shown high binding *in vitro*, but fail to have an effect on toxicity in the animal. The efficacy of each compound is based on a high affinity for aflatoxin. This is highly related to the chemical structure of the adsorbents, but also the properties of the adsorbed molecules (polarity, solubility, size, shape) (Grenier *et al.*, 2013). Hydrated sodium calcium aluminosilicates (HSCAS) include any clay material containing aluminum and silica, with exchangeable sodium and

calcium cations and waters of hydration. Hydrated sodium calcium aluminosilicates have a high affinity for aflatoxin specifically, resulting in the formation of a strong complex that reduces the possibility of absorption of the toxin (Neef *et al,* 2013).

Ledoux *et al.* (1999) found that a 1% addition of HSCAS to the diet was completely effective in preventing the toxic effects of 4 mg/kg AFB_1 in chicks. This adsorbent allowed the mycotoxin to pass harmlessly through the animal, bringing the animal's performance back to control values. These results are not consistent in all studies, as Neff *et al.* (2012) found that there were no improvements in performance with the addition of HSCAS to the diet at 0.5%. This variability can come from the amount of adsorbent in the diet, amount of toxin in the diet, or the adsorbent itself.

ANTIOXIDANTS

Oxidation is a chemical reaction that results in the loss of electrons from a substance to an oxidant agent; this can result in the production of free radicals. A free radical is an atom or group of atoms with one or more unpaired electrons. These are formed as intermediates of normal biochemical reactions, but in excess they can wreak havoc on macromolecules in the body due to their extremely high reactivity.

These free radicals belong to a group of molecules called reactive oxygen species (ROS). Reactive oxygen species are formed in mitochondria as oxygen is reduced via the electron transport chain. They are necessary intermediates for a variety of reactions, but can damage cells if the balance is off. The best-known toxic effect of ROS is causing damage to cell membranes, which is initiated by lipid peroxidation. This peroxidation most commonly targets unsaturated fatty acids in membrane phospholipids (Bowen,

2003). These harmful effects of free radicals are known as oxidative stress. Oxidative stress is defined as the imbalance of prooxidants and antioxidants. With oxidative stress, free radicals react with membrane lipids and proteins to induce cellular and tissue damage. Products of oxidation decrease absorption and utilization of fat-soluble vitamins and may react with other nutrients as well. Free radicals can impair animal health, growth and performance (Lu *et al.*, 2014).

Antioxidants are molecules or enzymes that inhibit the oxidation of other molecules. The preventive antioxidants, such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), are included in the first line of defense, which suppress the formation of free radicals. Superoxide dismutase protects cells from oxidative damage by breaking down a potentially hazardous free radical, superoxide (O_2-) to hydrogen peroxide (H_2O_2). Glutathione peroxidase is highly dependent on glutathione, the major thiol antioxidant. Due to its high concentration and central role in maintaining the cells redox state, gluthathione is one of the most important cellular antioxidants (Husain *et al.*, 2012). Aflatoxin B_1 causes cell damage due to the release of free radicals and lipid peroxidation involving cell membranes and fatty acids. Aflatoxin also induces oxidative stress, and reduced antioxidant activity (Gowda, 2009).

The decline of antioxidant activity caused by aflatoxin is due to decreased protein biosynthesis, inhibition of RNA synthesis and DNA dependent RNA polymerase activity. Antioxidants ameliorate oxidative stress during mycotoxicosis by reducing the level of free radicals in the body. Some plant compounds, such as turmeric (*Curcuma*

longa) have been shown to inhibit biotransformation of AF to its carcinogenic epoxide derivatives (Gonzalo, 2011).

Turmeric is a member of the ginger family, which is generally used in curry powder. The root is boiled, cleaned and sun-dried then ground into a powder. Crude turmeric though only contains a small amount of curcumin. Curcumin or curcuminoids are the compounds that give turmeric its yellow color. Curcumin inhibits biotransformation of AF to aflatoxicol in the liver. It effectively reduced liver damage in ducklings by reducing AFB_1-DNA adduct formation and modulation of cytochrome p-450 activity (Gowda, 2009). There are different forms of turmeric than can be used as an antioxidant, curcumin and theracurmin are two different products that come from turmeric. Theracurmin utilizes techniques to reduce the particle size of curcumin, which dramatically increases its solubility. Theracurmin is 30 times more bioavailable than curcumin (Kim, 2013).

Lu *et al.* (2014) found that pigs fed oxidative diets showed decreased G:F, increased liver weight, and increased lipid peroxidation when compared to pigs fed a control diet. An increase in liver weight shows oxidative stress in the animals. When the pigs were given antioxidants in the oxidative diets, growth rate improved and the pigs showed increased feed efficiency. In poultry, it was found that the addition of 0.5% turmeric powder, containing 1.4% curcuminoids, was effective in increasing superoxide dismutase and reducing peroxide levels in the liver of broiler chicks (Gowda, 2009).

CHAPTER III

EFFICACY OF ADSORBENTS TO REDUCE THE TOXICITY OF AFLATOXIN B_1 OR A COMBINATION OF MYCOTOXINS IN BROILERS FED TREATMENTS FROM HATCH TO DAY 21

ABSTRACT

The objective of this study was to evaluate the efficacy of two adsorbents in ameliorating the toxic effects of aflatoxin B_1, and the efficacy of a third adsorbent in ameliorating the toxic effects of a combination of mycotoxins (MM). Three hundred day-old straight run broiler chicks were purchased from a commercial hatchery, weighed, wing-banded, and assigned to chick batteries in a temperature-controlled room. A completely randomized design was used with six replicate pens of five chicks assigned to 10 dietary treatments from hatch to day 21. Dietary treatments included: 1) basal diet (BD) containing no mycotoxins or adsorbents; 2) BD plus 0.50% Raw Clay (RC); 3) BD plus 0.50% Concentrate clay (CC); 4) BD plus 0.50% Unike Plus (UP); 5) BD plus 2.0 mg/kg aflatoxin B_1 (AFB_1); 6) BD plus mycotoxin combination (MM) that included 1.00 mg/kg AF B_1; 1.0 mg/kg ochratoxin A (OA); 5 mg/kg fumonisin; 0.75 mg/kg T-2 toxin, and 0.5 mg/kg zearalenone; 7) BD plus 2.0 mg AFB_1 plus 0.50% RC 8) BD plus 2.0 mg AFB1 plus 0.50% CC; 9) BD plus MM plus 0.25% UP; and 10) BD plus plus MM plus 0.50% UP. The basal diet was a commercial corn soybean meal type diet formulated to meet or exceed the nutritional requirements of growing chicks as recommended by the National

Research Council (NRC, 1994). Pure T-2 toxin, and aflatoxin (1,100 mg/kg), ochratoxin A (1,100 mg/kg), fumonisin B_1 (1,800 mg/kg), and zearalenone (155 mg/kg) culture materials were incorporated into the diets to achieve the required dietary mycotoxin concentrations. Dietary mycotoxin concentrations were confirmed by analysis and all diets were screened for the presence of other mycotoxins prior to the start of the experiment. The addition of AF in the feed reduced ($P < 0.05$) feed intake (FI) and body weight gain (BWG) compared to control chicks. This reduction in FI was ameliorated ($P < 0.05$) with the addition of both RC and CC to the AF diet. Compared to chicks fed AF alone, BWG improved ($P < 0.05$) with the addition of RC and CC to the AF diet. Feed conversion (F:G) of chicks fed AF was poorer ($P < 0.05$) when compared to control chicks. Addition of RC and CC to the AF diet improved F:G ($P < 0.05$). Compared to controls, FI and BWG were significantly reduced ($P < .05$) in birds fed MM. Addition of UP at 0.25% and 0.5% to the MM diet ameliorated ($P < 0.05$) the growth depression caused by MM. Feed efficiency worsened in birds fed MM when compared to the controls, but UP addition at 0.25% and 0.5% to the MM diet improved ($P < 0.05$) F:G values that were comparable to the controls. There were no significant differences in mortality among control and AF birds. There was increased mortality ($P < 0.05$) in birds fed MM, and the addition of UP did not decrease mortality. Relative liver weight (RLW) increased ($P < 0.05$) in chicks fed AF. The addition of RC and CC to the AF diet ameliorated ($P < 0.05$) the increase in RLW caused by AF. Compared to controls, there was an increase ($P <0.05$) in RKW of chicks fed AF, however the addition of RC and CC reduced ($P < 0.05$) RKW. Relative liver weight was increased ($P < 0.05$) in birds fed MM

compared to the control birds. Adding UP at 0.25% to the MM diet reduced RLW to a value not significantly different from the controls, and adding UP at 0.5% reduced RLW to a value greater ($P < 0.05$) than that of the controls. Relative kidney was also increased when birds were fed MM, but the addition of UP at either concentration did not reduce or prevent ($P > 0.05$) the increase in RKW. There were no significant differences ($P > 0.05$) among treatments for glucose or globulin levels in the blood. Albumin levels decreased ($P < 0.05$) when the birds were fed AF, but RC and CC addition to the AF diet increased ($P < 0.05$) albumin levels to values that were comparable to control. Gamma glutamyl transferase levels increased ($P < 0.05$) in birds fed AF but decreased ($P < 0.05$) with the addition RC and CC to the AF diet. Gamma glutamyl transferase levels were also elevated ($P < 0.05$) in birds fed MM when compared to the controls. The addition of 0.25% or 0.50% UP to the MM diet returned values to control levels. Compared to controls, uric acid (UA) levels increased ($P < 0.05$) in birds fed AF alone. Addition of RC and CC to the AF diet reduced ($P < 0.05$) UA levels to values that were not different ($P > 0.05$) from control levels. Compared to controls, UA increased ($P < 0.05$) in chicks fed the MM treatment. Although the addition of UP to the MM diet reduced UA levels the values were not different ($P > 0.05$) from that of birds fed MM. Results Indicate that both RC and CC were effective in reducing the toxic effects of AF. Results also indicate that UP was partially effective in ameliorating the toxic effects of MM in broilers, with both levels of UP being equally effective.

INTRODUCTION

The presence of mycotoxins in feeds and foodstuffs is an important concern for human and animal health. *Aspergillus, Fusarium*, *Alternaria* and *Claviceps* species of fungi are ubiquitous in nature and under ideal conditions often infect economically important crops and forages in the field, during storage, shipment and processing. The most important mycotoxins found in the United States are aflatoxin B_1, fumonisin, ochratoxin A, vomitoxin, and the ergots. Many of these secondary fungal metabolites can cause serious health problems in animals and their presence in agricultural commodities may result in serious economic losses. Mycotoxins are also suspected of causing a variety of human diseases, including some forms of cancer. It has been estimated that mycotoxin-contaminated grains cost grain handlers and the livestock industry several hundred million dollars annually (CAST, 2003).

Many fungal species are capable of simultaneously producing several mycotoxins. Therefore, an individual grain source may be naturally contaminated with more than one mycotoxin, or the incorporation of numerous grain sources, which are each contaminated with a different mycotoxin, into a single feed may result in a feed that contains a number of different mycotoxins.

Recently, there has been a focus on the use of adsorbents in mycotoxin-contaminated feed to prevent the effects of mycotoxins on animal health. Adsorbents can be tested *in vitro* to determine binding capacity for aflatoxins (Ledoux *et al.*, 1999). The addition of adsorbent compounds to feed is one of the most economic and effective

methods to combat aflatoxins specifically. Due to the unique structure of AFB_1, adsorbents can readily bind AFB_1 in the gut, forming a strong bond and allowing the toxin to pass harmlessly through the gut of the animal (Neef *et al.,* 2013).

Therefore, the objectives of the current study were to determine the efficacy of two adsorbents to prevent or reduce the toxic effects of aflatoxin and the efficacy of a third adsorbent to prevent or reduce the toxic effects of a combination of mycotoxins in broilers fed dietary treatments from hatch to day 21.

MATERIALS AND METHODS

Three hundred day-old straight run broiler chicks were purchased from a commercial hatchery, weighed, wing-banded, and assigned to chick batteries in a temperature-controlled room. Chicks were maintained on a 24-hour constant-light schedule and allowed access to feed and water *ad libitum*.

A completely randomized design was used with six replicate pens of five chicks assigned to each of 10 dietary treatments from hatch to day 21. Dietary treatments included: 1) basal diet (BD) containing no mycotoxins or adsorbents; 2) BD plus 0.50% Raw Clay (RC); 3) BD plus 0.50% Concentrate Clay (CC); 4) BD plus 0.50% Unike Plus (UP); 5) BD plus 2.0 mg/kg aflatoxin B_1 (AFB_1); 6) BD plus mycotoxin combination (MM) that included 1.00 mg/kg aflatoxin B_1; 1.0 mg/kg ochratoxin A (OA); 5 mg/kg fumonisin; 0.75 mg/kg T-2 toxin, and 0.5 mg/kg zearalenone; 7) BD plus 2.0 mg AFB_1 plus 0.50% RC 8) BD plus 2.0 mg AFB_1 plus 0.50% CC; 9) BD plus MM plus 0.25% UP; and 10) BD plus (MM) plus 0.50% UP.

The basal diet was a commercial corn soybean meal type diet formulated to meet or exceed the nutritional requirements of growing chicks as recommended by the National Research Council (NRC, 1994). Aflatoxin (1,100 mg/kg), ochratoxin A (1,100 mg/kg), fumonisin B_1 (1,800 mg/kg), and zearalenone (180 mg/kg) culture materials and pure T-2 toxin were incorporated into the diets to achieve the required dietary concentrations. Dietary mycotoxin concentrations were confirmed by analysis and all diets were screened for the presence of other mycotoxins prior to the start of the experiment.

The raw clay used in this experiment was a natural bentonite clay from Bosnia. The raw clay was manufactured to create the concentrated, with an increased surface area and increased absorption. Unike Plus, is a mixture of additives, including hydrated sodium-calcium alumiosilicate clay (HSCAS), inactivated yeast and yeast extracts, calcium propionate, an antioxidant mixture, and botanicals.

Chicks were weighed at the beginning (day 1) and at the end of the experiment on day 21. Feed intake was also determined on day 21 and feed conversion was calculated. Mortality was recorded as it occurred and dead birds were necropsied. In addition, chicks were inspected daily and any health related problems were recorded.

On day 22, 18 birds per treatment (six replicates of three birds each) were anesthetized with carbon dioxide and blood samples (cardiac puncture) collected for determination of serum chemistries. Following collection of blood samples, the same 18 birds per treatment (six replicates of three birds each) were euthanized and livers and kidneys removed and weighed for determination of relative liver and kidney weights.

Following weighing, the color of each liver was scored using a Roche fan. Livers were harvested from 12 birds per treatment for gross and histopathologic evaluation.

Data were analyzed using the General Linear Models procedure of SAS (SAS Institute, 2008). Pen was the experimental unit. All statements of significance are based on the 0.05 level of probability. Means significantly different in ANOVA were analyzed with Fisher's LSD.

RESULTS

Dietary Analyses

A screen of the basal diet indicated that it was negative for aflatoxin, vomitoxin, zearalenone, ochratoxin A, and T-2 toxin, but contained 0.15 mg/kg fumonisin B_1. Diets of RC, CC, and UP in the basal diet all contained 0 mg/kg AFB_1. The diets containing AF had an average concentration 2.47 mg/kg AFB_1. In addition to AFB_1, these diets also contained an average of 87 µg/kg AFB_2, 899 µg/kg AFG_1, and 21 µg/kg AFG_2.

The diets containing MM diet were analyzed and contained an average of 1.19 mg/kg AFB_1, and 5.4 mg/kg FB_1. These diets were negative for vomitoxin, but contained 0.5 mg/kg zearalenone, 0.75 mg/kg T-2, and 1.0 mg/kg ochratoxin A. In addition to AFB_1, these diets contained an average of 40 µg/kg AFB_2, 379 µg/kg AFG_1, and 8 µg/kg AFG_2.

Growth Performance

Effects of dietary treatments on growth performance are summarized in Table 1. Feed intake (FI) was similar ($P > 0.05$) to control in chicks fed RC and CC alone (1,034 g

and 974 g vs. 987g, respectively). Feed intake of chicks fed 0.50% UP alone was also similar ($P > 0.05$) to that of control chicks (1,012 vs 987 g). The addition of AF to the basal diet decreased FI relative to control chicks ($P < 0.05$) from 987 to 806 g. When RC was added to the AF diet, FI increased ($P < 0.05$) from 806 g to 967g, and increased ($P < 0.05$) from 806 to 941 g with the addition of CC with both values similar to controls. Feed intake of chicks fed UP alone was similar ($P > 0.05$) to that of control chicks (1,012 vs. 987 g).

Chicks fed MM had a reduced FI ($P < 0.05$) compared to control chicks (670 vs. 987 g) and chicks fed 0.5% UP alone (670 vs. 1,012 g). Feed intake of chicks fed MM plus 0.25% UP and MM plus 0.50% UP was lower ($P < 0.05$) than that of control chicks (797 and 820 g, respectively vs 987 g) but higher ($P < 0.05$) than that of chicks fed MM alone (797 and 820 g, respectively vs 670 g). The addition of 0.25% and 0.50% UP to the MM diets lessened the reduction in FI caused by MM.

Body weight gain (BWG) in chicks fed RC and CC was similar ($P > 0.05$) to control chicks (760 and 706 g vs. 739 g). Body weight gain of chicks fed 0.50% UP was similar ($P > 0.05$) to that of control chicks (754 vs 739 g). When AF was added to the basal diet, BWG decreased ($P < 0.05$) from 739 g in controls to 579 g, this value was also significantly lower than chicks fed RC and CC alone (579 g vs 760 and 706 g). With the addition of RC and CC to the AF diet BWG increased ($P < 0.05$) and was similar to control values at 701 g and 693 g.

Body weight gain of chicks fed MM alone was lower ($P < 0.05$) than that of control chicks (456 vs 739 g) and chicks fed 0.50% UP alone (456 vs 754 g). Body weight

gain of chicks fed MM plus 0.25% UP and MM plus 0.50% UP was lower ($P < 0.05$) than that of control chicks. However, the addition of 0.25% and 0.50% UP to the MM diet improved ($P < 0.05$) BWG of chicks compared to chicks fed MM alone (558 and 584 vs 456 g).

When RC and CC were added to the basal diet, feed efficiency was similar to controls (1.36 and 1.39 g:g vs 1.34 g:g). Feed efficiency (FG) was poorer ($P < 0.05$) in chicks fed AF compared to controls (1.40 g:g vs 1.34 g:g). When RC and CC were added to the AF diet, FG improved to control values (1.36 g:g and 1.39 g:g, respectively vs. 1.34 g:g).

When UP was added to the basal diet at 0.50%, FG was similar ($P> 0.05$) to the controls (1.34 g:g vs 1.34 g:g). Feed efficiency, however, was poorer ($P < 0.05$) in chicks fed MM at 1.48 g:g compared to the control at 1.34 g:g. Feed efficiency of chicks fed MM plus 0.50% UP was similar ($P > 0.05$) to the controls at 1.41 g:g but was also similar ($P > 0.05$) to chicks fed MM alone.

Mortality was not significantly affected ($P > 0.05$) by the addition of RC and CC to the basal diet. There was also no difference in mortality ($P > 0.05$) between the controls and chicks fed 0.50% UP. When chicks were fed AF alone mortality increased numerically but was not different from controls ($P > 0.05$) at 10.0%. Mortality was not different ($P > 0.05$) among control chicks and chicks fed the AF diet supplemented with 0.50% RC or CC.

Compared to controls, chicks fed MM had increased ($P < 0.05$) mortality (23.33% vs. 0.0%). There was no difference in mortality ($P > 0.05$) among chicks fed MM and those fed MM plus 0.25 and 0.50% UP.

Organ Weights

Effects of dietary treatments on organ weights are summarized in Table 2. Relative liver weight (RLW) of chicks fed RC and CC alone was not different ($P > 0.05$) from the controls. The addition of AF increased RLW compared to controls ($P < 0.05$) from 2.85% to 4.32%. When RC and CC were added to the AF diet, RLW was reduced from 4.32% to 3.3% but this weight was still higher than control chicks b (3.3% vs 2.85%).

Relative liver weight of chicks fed 0.50% UP was the same as that of control chicks. Relative liver weight of chicks fed MM alone increased ($P < 0.05$) compared to control chicks (3.95 vs 2.85%) and chicks fed 0.50% UP alone (3.95 vs 2.85%). Relative liver weight of chicks fed MM plus 0.25% UP was similar ($P > 0.05$) to that of control chicks (3.24 vs 2.85%). The RLW of chicks fed 0.50% UP plus MM was similar ($P > 0.05$) to that of chicks fed 0.25% UP plus MM , but was higher ($P < 0.05$) compared to control birds (3.47 vs. 2.85%). The addition of 0.25% and 0.50% UP to the MM diet decreased ($P < 0.05$) RLW relative compared to birds fed MM alone.

Relative kidney weight (RKW) of chicks fed RC and CC were similar ($P < 0.05$) to controls (0.74, 0.73% vs 0.72%). When AF was added to basal diet, RKW weight increased ($P < 0.05$) relative to controls from 0.72% to 1.18%. This increase in RKW was reduced significantly ($P < 0.05$) with the addition of RC and CC, but was not brought to

control values at (0.94%, 0.96% vs 0.72%). Relative kidney weights of chicks fed 0.50% UP alone was similar ($P > 0.05$) to that of control chicks.

Relative kidney weight of chicks fed MM alone was greater ($P < 0.05$) than that of control chicks (1.18 vs 0.72%) and chicks fed 0.50% UP alone (1.18 vs 0.76%). Relative kidney weight of chicks fed MM plus 0.25% UP was greater ($P < 0.05$) than that of control chicks (1.07 vs 0.72%), and was not reduced ($P > 0.05$) relative to MM alone. When 0.50% UP was added to the MM diet, RKW was significantly reduced ($P < 0.05$) from 1.18% to 1.02%, but RKW was still greater ($P < 0.05$) than the controls (1.18% vs 0.72%).

Liver Color Scores

Liver color was determined using a Roche color fan and the results are summarized in Table 2. Liver color score of chicks fed RC and CC were similar ($P > 0.05$) to controls (13.6 and 13.17, respectively vs 14.33). The addition of AF reduced liver color score ($P < 0.05$) to 11.5, compared to controls at 14.33. Liver color score of chicks fed 0.50% UP was similar ($P > 0.05$) to that of control chicks. Liver color score of chicks fed RC and AF although numerically improved was not different ($P > 0.05$) from that of chicks fed AF only. Liver color score of chicks fed CC and AF was also not different ($P > 0.05$) from that of chicks fed AF alone but was also not different ($P > 0.05$) from control chicks.

Liver color score of chicks fed MM alone was lower ($P < 0.05$) than that of control chicks (10.67 vs 14.33) and chicks fed 0.50% UP alone (10.67 vs 14.33), but similar to that of chicks fed 0.25% UP plus MM (10.67 vs. 11.67). In contrast, chicks fed

the 0.50% UP plus MM diet had similar (P > 0.05) liver color scores to that of control chicks (12.83 vs 14.33).

Serum Chemistry

Table 3 contains a summary of the effects of dietary treatments on serum glucose, albumin, globulin, and total protein. Serum concentrations of glucose, albumin, globulin, and total protein of chicks fed RC and CC alone were similar (P > 0.05) to that of control chicks. There were no significant treatment differences (P > 0.05) in glucose or globulin levels.

Chicks fed AF had significantly lower (P < 0.05) albumin levels compared to control chicks (0.78 vs 0.99 g/dL). The addition of RC and CC to the AF diet brought albumin levels up to control values at 0.92 and 0.86 g/dL, respectively.

Chicks fed 0.50% UP alone had had similar (P > 0.05) serum glucose, albumin, total protein, and globulin to control chicks. Chicks fed MM alone had lower (P < 0.05) serum albumin values compared to control chicks (0.81 vs 0.99 g/dL) and chicks fed 0.50% UP alone (0.81 vs 1.05 g/dL). Serum albumin of birds fed MM plus 0.25% UP was similar (P > 0.05) to that of birds fed MM alone (0.79 vs 0.81 g/dL)). Also, birds fed MM plus 0.50% UP had serum albumin concentrations that were similar (P > 0.05) than that of birds fed MM alone (0.78 vs 0.81 g/dL) and those fed MM plus 0.25% UP (0.78 vs 0.79 g/dL).

Chicks fed AF had similar levels (P > 0.05) of total protein as control chicks (2.63 vs 2.70 g/dL). Chicks fed RC and CC alone also had similar (P > 0.05) total protein levels as control chicks (2.63 and 2.83 vs 2.70 g/dL). Chicks fed MM alone had similar (P > 0.05)

total serum protein to control chicks (2.61 vs 2.70 g/dL) and chicks fed 0.50% UP alone (2.61 vs 2.87 g/dL). The addition of up to 0.50% UP to the MM diet did not have any effect ($P > 0.05$) on serum total proteins when compared to the controls.

Table 4 contains a summary of the effects of dietary treatments on serum calcium (Ca), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT) activities, and uric acid (UA). Serum concentrations of Ca were not affected by dietary treatments ($P > 0.05$). There were no significant differences among treatments in aspartate aminotransferase (AST) levels, except for an increase ($P < 0.05$) in chicks fed AF + CC with a value of 331 IU/L compared to 209 IU/L in the control chicks.

The addition of RC and CC alone did not affect GGT activity in the blood when compared to controls ($P > 0.05$). Aflatoxin significantly ($P < 0.05$) increased GGT from 13.94 U/L in control birds to 18.08 U/L in birds fed AF. The addition of RC and CC to the AF diet brought levels back down to control levels at 14.00 and 15.17 U/L, respectively. The addition of UP alone did not affect gamma-glutamyl transferase (GGT) levels in the blood when compared to controls ($P > 0.05$). The addition of MM to the diet increased GGT levels ($P < 0.05$) to 17.61 vs controls 13.94 U/L. This increase was successfully reduced ($P < 0l.05$) by UP addition at both 0.25% and 0.50%, bringing levels to 14.06 and 13.94 U/L, respectively.

The addition of RC and CC alone did not affect UA levels in the blood when compared to controls ($P > 0.05$). Feeding AF alone increased uric acid levels ($P < 0.05$) in the blood compared to control chicks (8.7 vs. 5.35 mg/dL). Adding RC and CC to the AF

diet numerically reduced UA levels to 6.72 and 6.9 mg/dL, levels which were similar ($P > 0.05$) to both control levels and levels of chicks fed AF alone.

The addition of UP alone did not affect UA levels in the blood when compared to controls ($P > 0.05$). Chicks fed MM alone had higher ($P < 0.05$) serum UA compared to control chicks (9.36 vs 5.35 mg/dL) and chicks fed 0.50% UP alone (9.36 vs 5.25 mg/dL). Serum UA of birds fed MM plus 0.25% and 0.50% UP was similar ($P > 0.05$) to that of birds fed MM alone (8.31 and 8.87 vs. 9.36 mg/dL).

Histopathology

The effects of dietary treatments on liver lesion scores are summarized in Table 2. Liver lesions observed in the livers of birds fed diets containing AF and MM included infiltrations of heterophils, lymphocytes and macrophages. There was also mild fibrosis and bile duct proliferation in the portal tract areas, with some necrotic hepatocytes in the portal area. No liver lesions were observed ($P > 0.05$) in control birds or birds fed RC, CC, or UP alone. The highest liver lesion scores were observed in chicks fed AF with lesion scores increasing ($P < 0.05$) from 0 in the controls to 2.25 in chicks fed AF. The addition of both RC and CC to the AF diet decreased ($P < 0.05$) but did not prevent liver lesions (0.92 and 1.42, respectively).

Liver lesion score of chicks fed MM alone was greater ($P < 0.05$) than that of control chicks (1.08 vs 0.00). However, the addition of 0.25% UP to the MM diet was not effective ($P > 0.05$) in reducing the lesion score (1.33 vs. 1.08). The addition of 0.50% UP to the MM diet significantly reduced ($P > 0.05$) the lesion score (0.83 vs. 1.08). A detailed histopathology report is included in Appendix 1.

DISCUSSION

The raw clay used in this experiment was a natural bentonite clay from Bosnia. This raw clay was dried at 80°C and sieved to particles <74 µm in size, which were then dispersed in water to form a suspension. This suspension was dried and milled to particle sizes <63 µm, doing so created the concentrated clay from the raw clay. Unike Plus, is a mixture of additives, including hydrated sodium-calcium alumiosilicate clay (HSCAS), inactivated yeast and yeast extracts, calcium propionate, an antioxidant mixture, and botanicals.

It is not uncommon for mycotoxin-contaminated feed to contain more than one type of mycotoxin, and given the vast differences in structures of mycotoxins different strategies need to be combined in order to counteract their negative effects (Murugesan *et al.,* 2015). Unike Plus combines adsorbents to bind AF, yeast and yeast extracts to combat other mycotoxins, calcium propionate for gut health, and antioxidants/botanicals to combat oxidation caused by the absorption of mycotoxins.

Growth performance of birds fed raw clay (RC), concentrated clay (CC), or Unike Plus (UP) alone was not different from that of control birds, indicating that the adsorbents did not negatively affect the nutritional value of the diet, and that the concentration (0.50%) is safe for use in chicks. Similar observations were made by Gowda *et al.* (2009) and Neeff *et al*. (2013) who reported that FI and BWG of chicks fed 0.3% bentonite clay did not significantly differ from those of the control chicks.

In the present study, it was hypothesized that the addition of adsorbents, acting as binding agents will prevent or reduce the negative effects of AFB_1 or MM in chicks. In this study, AF inclusion reduced body weight gain (BWG) by 21%, feed intake (FI) by 18%, and feed efficiency (F:G) by 4%, consistent with results by Chen *et al.* (2014) who fed 1 and 2 mg/kg AF in broiler chickens and saw a significant reduction in performance compared to control. Inclusion of RC and CC in the diet brought growth, intake and feed conversion levels up to control values. Lopes *et al.* (2006) reported that the addition of 0.1% bentonite clay to a diet containing 3 mg/kg AF improved BWG by 9.5%, but values in that study were still lower than control. These results agree with the findings in the present study in which growth performance was improved by the addition of RC and CC.

The addition of MM to the diet decreased growth by 38%, FI by 32%, and F:G increased by 10%, compared to controls. Similar results were seen in a study by Zhu *et al.* (2014), where birds were fed a combination of mycotoxins including (AFB_1, 22.7 µg/kg, OTA, 2.4 µg/kg, T-2, 3.8 µg/kg), who saw a decrease in ADG of 23% and a 23% increase in F:G. Both studies showed improvement up to control values with the addition of an adsorbent and yeast combination.

Raw clay, CC, and UP alone did not have any significant effect on relative liver and kidney weight of chicks. Previous reports show no negative effects from the addition of a bentonite clay or yeast to the diet (Chen *et al.* 2014; Gowda *et al.,* 2009; Neeff *et al.* 2013). Previous studies have suggested that AF negatively affects relative organ weights in broiler chicks (Yunus *et al.,* 2011; Gowda *et al.,* 2009). In the present study, enlargement and discoloration of livers and kidneys, along with mild fibrosis, bile

duct proliferation, and hepatocellular vacuolation were observed in chicks fed AF and MM. These results agree with earlier reports by Gowda *et al. (*2009), and Neef *et al. (*2013) in which increased relative liver weights were observed in chicks fed 1 to 2.5 mg/kg AF. This is in contrast with a study done by Jiang *et al.* (2014), who fed a combination of mycotoxins including 102.08 μg/kg aflatoxin, 281.92 μg/kg zearalenone, 5,874.38 μg/kg fumonisin, and 2,038.96 μg/kg deoxynivalenol, but saw no histopathological differences in the liver. This could be due to differences in mycotoxin concentration fed. There was a reduction in organ weights with the addition of RC and CC to the AF diet, but this value was still greater than that of control birds. It was hypothesized that CC would have a better absorptive capacity, and therefore would have a greater ability to reduce or prevent the effects of AF. This was not proven, as the reduction in liver weight was the same for both clay products. The addition of both RC and CC to the AF diet reduced liver weight 24% and kidney weight 20% compared to chicks fed AF alone. Though this was not significant, the results are similar to a study by Rosa *et al.* (2001), who observed an 18% decrease in RLW and 27% reduction in RKW with the addition of 0.3% bentonite clay to chicks fed 5 mg/kg AF.

The addition of MM increased liver and kidney weights, and the addition of UP at 0.50% reduced this increase 18% in the liver and 14% in the kidney. These values were still significantly greater than control birds, but were lower than birds fed MM alone. Huff *et al.* (1992) fed a combination of mycotoxins and HSCAS to broiler chicks for 21 days, and also observed a significant increase in relative organ weights in birds fed MM compared to birds fed the control diet. Huff *et al.* (1992) did not see an improvement

with the addition of HSCAS alone, indicating that the other ingredients in UP helped to alleviate the impact multiple mycotoxins have on broiler health.

Adding RC, CC, or UP to the basal diet resulted in similar levels of glucose, globulin, protein, albumin, and calcium as in the controls suggesting that the adsorbents did not negatively affect the nutritional value of the diet. The addition of AF to the basal diet resulted in a decrease in albumin. This is an indicator of decreased protein synthesis, characteristically observed in aflatoxicosis (Rosa *et al.,* 2001). Compared to chicks fed AF alone, the addition of RC and CC to the AF diet increased the albumin levels to that of control chicks. When Rosa *et al.* (2001) fed 0.3% bentonite clay with 5 mg AF/kg diet to broilers; it was observed that serum changes could be ameliorated. The current study agrees with these results for albumin. Albumin reduction from AF was shown to be eliminated by the addition of HSCAS (0.5%) when AF was fed at levels from 0.5 to 2 mg/kg (Chen *et al.,* 2014). The addition of MM to the basal diet also resulted in a decrease in albumin. However, addition of UP to the AF diet was not effective in reducing or preventing the decrease in albumin.

The degree of liver damage can be assessed by specific enzyme tests; AST and GGT activities provide a specific measure of hepatic function or injury, as well as indicate liver or kidney toxicity (Boone *et al.* 2005). When AF was added to the diet, GGT increased compared to controls, confirming liver damage from the addition of AF. This was previously suggested by the increased liver weights and discoloration. Aspartate aminotransferase also increased 22%, indicating liver damage but was not statistically significant. Similar results were seen when Neeff *et al.* (2013) fed AFB_1 at 0.8 mg/kg and

2.5 mg/kg. In the current study, addition of RC and CC to the AF diet reduced GGT levels back to control values. Birds fed MM had significantly increased GGT and UA in the blood. This is consistent with findings by Jiang *et al.* (2014) who fed a diet contaminated by aflatoxin, 102.08 mg/kg; zearalenone, 281.92 mg/kg; fumonisin, 5,874.38 mg/kg; deoxynivalenol, 2,038.96 mg/kg) and observed an increase in GGT at 21 and 42 days. This increase was prevented the addition of HCSAS and yeast cell walls to the multiple mycotoxin diet. It has been reported that the increased enzyme activities caused by feeding naturally contaminated diets could be due to hepatic degeneration and subsequent leakage of enzymes into circulation.

CONCLUSIONS

The addition of RC, CC at 0.50%, and UP at up to 0.50% to the basal diet did not cause any negative effects in any of the measured response variables indicating that at this concentration these adsorbents are safe to feed to chicks of this age, and will not negatively impact performance. The addition of AF to the diet resulted in decreased performance, increased organ weights, reduced liver color, reduced albumin, increased GGT and UA, and increased liver lesions. The addition of both RC and CC were able to reduce the effects of AF and improve bird performance. There were no large differences between the two adsorbents in performance or other parameters measured. However, it would be more economical to use the raw clay, as the concentrated clay will be more expensive due to the additional cost of processing.

Compared to controls, addition of a combination of mycotoxins to the basal diet resulted in reduced growth performance, increased organ weights, reduced liver color scores, reduced serum proteins, increased serum UA, and increased liver lesion scores. Addition of 0.25% and 0.50% UP to the MM diet reduced the negative effects of MM on the affected response variables. The higher level of UP (0.50%) was more effective in reducing the effects of MM, and improved the performance the most.

The adsorbent UP at the level of 0.50% successfully ameliorated some of the effects of the combination of mycotoxins.

Table 3.1. Efficacy of Adsorbents to reduce the toxic effects of aflatoxin or a combination of mycotoxins on growth performance of broilers fed dietary treatments from hatch to day 21[1]

Treatments		Initial Body Weight (g)	Body Weight Gain (g)	Feed Intake (g)	Feed:Gain (g:g)	Mortality Percent (%)[3]
BD		37.50	739^{a}	987^{abc}	1.34^{c}	0.00^{c}
BD + 0.50% Raw Clay (RC)		37.43	760^{a}	1034^{a}	1.36^{bc}	3.33^{bc}
BD + 0.50% Concentrate Clay (CC)		37.43	706^{a}	974^{abc}	1.39^{bc}	3.33^{bc}
BD + 0.50% Unike Plus (UP)		37.33	754^{a}	1012^{ab}	1.34^{c}	0.00^{c}
BD + 2 mg/kg AF		37.53	579^{b}	806^{d}	1.40^{abc}	10.00^{abc}
BD + Mixed mycotoxins (MM)[2]		37.43	456^{c}	670^{e}	1.48^{a}	23.33^{a}
BD + 2 mg/kg AF + 0.50% RC		37.47	701^{a}	967^{bc}	1.39^{bc}	10.00^{abc}
BD + 2 mg/kg AF + 0.50% CC		37.53	693^{a}	941^{c}	1.36^{bc}	6.67^{bc}
BD + MM + 0.25% UP		37.50	558^{b}	797^{d}	1.43^{ab}	16.67^{ab}
BD + MM + 0.50% UP		37.37	584^{b}	820^{d}	1.41^{abc}	16.67^{ab}
ANOVA	S.E.M.:	0.12	23.60	22.66	2.83	0.09
	P-value:	0.025	<0.0001	<0.0001	0.06	0.005

[1]Data are means of six replicate pens of 5 chicks each.
[2]Mixed mycotoxins = aflatoxin (1 ppm); ochratoxin A (1 ppm); fumonisin B_1 (5 ppm); T-2 toxin (0.75 ppm); zearalenone (0.5 ppm).
[3]Data were transformed then subjected to analysis
[a-c] Means within columns with no common superscripts are significantly different ($P < 0.05$)

Table 3.2. Efficacy of Adsorbents to reduce the toxic effects of aflatoxin or a combination of mycotoxins on organ weight and skin color of broilers fed dietary treatments from hatch to day 21[1]

Treatments		Relative Liver Weight (%)	Relative Kidney Weight (%)	Color[3]	Liver Score
BD		2.85^{cd}	0.72^{c}	14.33^{a}	0.00^{d}
BD + 0.50% Raw Clay (RC)		2.74^{d}	0.74^{c}	13.60^{ab}	0.00^{d}
BD + 0.50% Concentrate Clay (CC)		2.78^{d}	0.73^{c}	13.17^{abc}	0.00^{d}
BD + 0.50% Unike Plus (UP)		2.85^{cd}	0.76^{c}	14.33^{a}	0.00^{d}
BD + 2 mg/kg AF		4.32^{a}	1.18^{a}	11.50^{cd}	2.25^{a}
BD + Mixed mycotoxins (MM)[2]		3.95^{a}	1.18^{a}	10.67^{d}	1.08^{bc}
BD + 2 mg/kg AF + 0.50% RC		3.30^{b}	0.94^{b}	12.00^{bcd}	0.92^{bc}
BD + 2 mg/kg AF + 0.50% CC		3.30^{b}	0.96^{b}	12.67^{abc}	1.42^{b}
BD + Mixed + 0.25% UP		3.24^{bc}	1.07^{ab}	11.67^{cd}	1.33^{bc}
BD + Mixed + 0.50% UP		3.47^{b}	1.02^{b}	12.83^{abc}	0.83^{c}
ANOVA	S.E.M.:	0.144	0.052	0.62	0.256
	P-value:	<0.0001	<0.0001	0.0009	<0.0001

[1]Data are means of six replicate pens of 3 chicks each.
[2]Mixed mycotoxins = aflatoxin (1 ppm); ochratoxin A (1 ppm); fumonisin B_1 (5 ppm); T-2 toxin (0.75 ppm); zearalenone (0.5 ppm).
[3]Color determined using a Roche color fan
[a-d] Means within columns with no common superscripts are significantly different ($P < 0.05$)
Aflatoxin B_1, ochratoxin A, fumonisin B_1 and zearalenone supplied from culture material, whereas as T-2 toxin was pure

Table 3.3. Efficacy of Adsorbents to reduce the toxic effects of aflatoxin or a combination of mycotoxins on serum of broilers fed dietary treatments from hatch to day 21[1]

Treatments		Glucose (mg/dL)	Albumin (g/dL)	Total Protein (g/dL)	Globulin (g/dL)
BD		248	0.99^{ab}	2.70^{ab}	1.71
BD + 0.50% Raw Clay (RC)		237	1.02^{a}	2.63^{abc}	1.66
BD + 0.50% Concentrate Clay (CC)		264	1.04^{a}	2.83^{a}	1.79
BD + 0.50% Unike Plus (UP)		228	1.05^{a}	2.87^{a}	1.81
BD + 2 mg/kg AF		230	0.78^{d}	2.63^{abc}	1.85
BD + Mixed mycotoxins (MM)[2]		271	0.81^{cd}	2.61^{abc}	1.81
BD + 2 mg/kg AF + 0.50% RC		257	0.92^{abc}	2.63^{abc}	1.68
BD + 2 mg/kg AF + 0.50% CC		235	0.86^{bcd}	2.59^{abc}	1.73
BD + Mixed + 0.25% UP		286	0.79^{b}	2.43^{bc}	1.63
BD + Mixed + 0.50% UP		261	0.78^{b}	2.34^{c}	1.56
ANOVA	S.E.M.:	14.037	0.047	0.111	0.075
	P-value:	0.07	<0.0001	0.05	0.17

[1]Data are means of six replicate pens of 5 chicks each.
[2]Mixed mycotoxins = aflatoxin (1 ppm); ochratoxin A (1 ppm); fumonisin B_1 (5 ppm); T-2 toxin (0.75 ppm); zearalenone (0.5 ppm).
[a-d] Means within columns with no common superscripts are significantly different ($P < 0.05$)
Aflatoxin B_1, ochratoxin A, fumonisin B_1, and zearlenone supplied from culture material, whereas T-2 was pure.

Table 3.4. Efficacy of Adsorbents to reduce the toxic effects of aflatoxin or a combination of mycotoxins on serum of broilers fed dietary treatments from hatch to day 21[1]

Treatments		Calcium (mg/dL)	AST (IU/L)	GGT (U/L)	UA (mg/dL)
BD		10.96	209^{bcd}	13.94^{c}	5.35^{d}
BD + 0.50% Raw Clay (RC)		10.95	204^{bcd}	13.22^{c}	6.01^{cd}
BD + 0.50% Concentrate Clay (CC)		10.86	225^{bcd}	13.67^{c}	6.26^{cd}
BD + 0.50% Unike Plus (UP)		10.83	257^{abc}	15.50^{abc}	5.25^{b}
BD + 2 mg/kg AF		10.83	257^{abc}	18.08^{a}	8.70^{ab}
BD + Mixed mycotoxins (MM)[2]		11.63	182^{d}	17.61^{ab}	9.36^{a}
BD + 2 mg/kg AF + 0.50% RC		11.13	191^{cd}	14.00^{c}	6.72^{bcd}
BD + 2 mg/kg AF + 0.50% CC		11.07	331^{a}	15.17^{bc}	6.90^{bcd}
BD + Mixed + 0.25% UP		11.12	228^{bcd}	14.06^{c}	8.31^{abc}
BD + Mixed + 0.50% UP		11.09	269^{ab}	13.94^{c}	8.87^{ab}
ANOVA	S.E.M.:	0.262	26.23	0.941	0.829
	P-value:	0.63	0.008	0.004	0.003

[1]Data are means of six replicate pens of 5 chicks each.
[2]Mixed mycotoxins = aflatoxin (1 ppm); ochratoxin A (1 ppm); fumonisin B_1 (5 ppm); T-2 toxin (0.75 ppm); zearalenone (0.5 ppm).
[a-d] Means within columns with no common superscripts are significantly different ($P < 0.05$)
Aflatoxin B_1, ochratoxin A, fumonisin B_1, and zearalenone supplied from culture material, whereas T-2 was pure.

Table 3.5. Grading of severity of changes in the livers

Treatment Group									
BD	RC	CC	UP	AF	MM	AF+RC	AF+CC	MM+0.25 UP	MM+ 0.50 UP
0	0	0	0	1	0	2	2	2	1
0	0	0	0	2	1	1	0	1	1
0	0	0	0	3	0	1	1	1	0
0	0	0	0	2	2	2	1	2	2
0	0	0	0	2	0	0	0	1	1
0	0	0	0	3	2	1	1	2	1
0	0	0	0	3	1	1	2	2	0
0	0	0	0	1	2	0	1	0	1
0	0	0	0	3	3	1	2	2	0
0	0	0	0	3	1	0	2	1	1
0	0	0	0	2	1	2	2	2	1
0	0	0	0	2	0	0	3	0	1

0 = Normal
1 = Mild changes
2 = Moderate changes
3 = Severe changes present

*See following histology report

Table 3.5 Continued - Histopathology Report

Twelve sections of formalin-fixed livers from chicks of each of five treatment groups were examined.

Treatment A (Basal diet) In the 12 examined sections of liver, each was within normal limits and contains a few scattered infiltrations of lymphocytes, macrophages, and heterophils.

Treatment B (Raw Clay) In the 12 examined sections of liver, each was within normal limits and contains a few scattered infiltrations of lymphocytes, macrophages, and heterophils.

Treatment C (Concentrated Clay) In the 12 examined sections of liver, each was within normal limits and contains a few scattered infiltrations of lymphocytes, macrophages, and heterophils.

Treatment D (0.50% UP) In the 12 examined sections of liver, each was within normal limits and contains a few scattered infiltrations of lymphocytes, macrophages, and heterophils.

Treatment E (2 ppm Aflatoxin) In the 12 examined sections of the liver, 10 of the sections had a few infiltrations of heterophils, lymphocytes, and macrophages. Many of the portal tracts are infiltrated by heterophils. There is mild fibrosis and bile duct proliferation in the portal tract areas. Many of the hepatocytes, especially in the portal tracts, are swollen and there is hepatacellular vacuolation. There are a few individual necrotic hepatocytes in the portal areas. Small numbers of hepatocytes contain mitotic figures. In two sections there are a few scattered infiltrations of lymphocytes, macrophages, and heterophils.

Treatment F (Mixed Mycotoxins) In four of the sections there are a few infiltrations of heterophils, lymphocytes, and macrophages. Many of the portal tracts are infiltrated by heterophils. There is mild fibrosis and bile duct proliferation in the portal tract areas. Many of the hepatocytes contain mitotic figures. In four sections there are a few scattered infiltrations of lymphocytes, macrophages and heterophils. No significant microscopic changes are detected in four of the sections.

Treatment G (2ppm AF + Raw Clay) In three of the sections there are a few infiltrations of heterophils, lymphocytes, and macrophages. Many of the portal tracts are infiltrated by heterophils. There is mild fibrosis and bile duct proliferation in the portal tract areas. Many of the hepatocytes, especially in the portal tracts, are swollen and there is hepatocellular vacuolation. There are a few individual necrotic hepatocytes in the portal areas. Small numbers of hepatocytes contain mitotic figures. In five sections there are a

few scattered infiltrations of lymphocytes, macrophages and heterophils. There were no significant microscopic changes detected in four of the sections.

Treatment H (2ppm AF + Concentrated Clay) In six of the sections there are a few infiltrations of heterophils, lymphocytes, and macrophages. Many of the portal tracts are infiltrated by heterophils. There is mild fibrosis and bile duct proliferation in the portal tract areas. Many of the hepatocytes, especially in the portal tracts, are swollen and there is hepatocellular vacuolation. There are a few individual necrotic hepatocytes in the portal areas. Small numbers of hepatocytes contain mitotic figures. In four sections there are a few scattered infiltrations of lymphocytes, macrophages and heterophils. There were no significant microscopic changes detected in two of the sections.

Treatment I (Mixed mycotoxins + 0.25% UP) In six of the sections there are a few infiltrations of heterophils, lymphocytes, and macrophages. Many of the portal tracts are infiltrated by heterophils. There is mild fibrosis and bile duct proliferation in the portal tract areas. Many of the hepatocytes, especially in the portal tracts, are swollen and there is hepatocellular vacuolation. There are a few individual necrotic hepatocytes in the portal areas. Small numbers of hepatocytes contain mitotic figures. In four sections there are a few scattered infiltrations of lymphocytes, macrophages, and heterophils. No significant microscopic changes are detected in two of the sections.

Treatment J (Mixed mycotoxins + 0.50% UP) In one of the sections there are a few infiltrations of heterophils, lymphocytes, and macrophages. Many of the portal tracts are infiltrated by heterophils. There is mild fibrosis and bile duct proliferation in the portal tract areas. Many of the hepatocytes, especially in the portal tracts, are swollen and there is hepatocellular vacuolation. There are a few individual necrotic hepatocytes in the portal areas. Small numbers of hepatocytes contain mitotic figures. In eight sections there are a few scattered infiltrations of lymphocytes, macrophages, and heterophils. No significant microscopic changes are detected in three of the sections.

Vacuolation, Ly in Portal Tracts = The hepatocytes contain fine clear cytoplasmic vacuoles and the portal tracts are infiltrated by small numbers of lymphocytes.

Lymphoid Nodules = There are a few lymphoid nodules scattered throughout the parenchyma.

Vacuolation + Heterophils = The hepatocytes contain fine clear cytoplasmic vacuoles and the portal tracts are infiltrated by moderate to large numbers of heterophils and lymphocytes. There is mild bile duct proliferation and fibrosis in the portal tracts. There are also a few lymphoid nodules scattered throughout the parenchyma.

Portal Fibrosis and Bile Duct Proliferation = The only changes are fibrosis and bile duct proliferation in the portal tracts.

CHAPTER IV

EFFICACY OF AN ADSORBENT 'UNIKE PLUS' AND ANTIOXIDANTS TO REDUCE THE TOXICITY OF AFLATOXIN (AF) AND A COMBINATION OF AF AND FUMONISIN B1 (FB) IN WEANLING PIGS FED DIETARY TREATMENTS FOR 26 days

ABSTRACT

The objective of this study was to determine the efficacy of two antioxidants and an adsorbent to reduce or prevent the effects of a combination of AF or a combination of AF and FB in weanling pigs fed dietary treatments for 26 days. Sixty weanling pigs (2 weeks post weaning) were purchased and assigned to dietary treatments. Each pig was individually housed with *ad libitum* access to feed and water. A completely randomized design was used with six 5-week-old barrows assigned to each of 10 dietary treatments for 26 days. Dietary treatments to be evaluated included: 1) basal industry type weanling diet (BD) containing no antioxidants, adsorbent or mycotoxins); 2) BD plus 200 mg/kg theracurmin (TCM); 3) BD plus 200 mg/kg curcumin (CMN); 4) BD plus 1 mg/kg AF; 5) BD plus 1 mg/kg AF and 200 mg/kg TCM; 6) BD plus 1 mg/kg AF and 200 mg/kg CMN; 7) BD plus 0.50% UP; 8) BD plus MM (1 mg/kg AF and 25 mg/kg FM) 9) BD plus MM plus 0.25% UP; and 10) BD plus MM plus 0.50% UP. Response variables measured included growth performance, relative liver and kidney weight, liver histopathology, lipid peroxides, aqueous peroxides, and serum concentrations of 8-OHdG, TNF-alpha, and IgG, and aflatoxin residues in liver and kidney.

There were no negative effects on pigs with the addition of TCM, CMN, or UP to the basal diet. The addition of AF and MM to the basal diet reduced feed intake and body weight gain in pigs. There was no improvement in FI with the addition of TCM or CMN to the AF diet, but 0.50% UP increased gain compared to MM alone. Relative liver weight increased with AF and MM in the diet, and was not improved with the addition of either antioxidant or the adsorbent to the contaminated diets. The addition of UP improved levels of TP, globulin, and Mg in serum and were comparable to control. The addition of UP to the contaminated diet did not improve urea nitrogen, albumin, or GGT levels. Aflatoxin M_1 and G_1 residues in the liver were numerically improved with the addition of TCM, CMN, and 0.50% UP to the toxin diets, and 0.50% UP improved AFB_1 residues in the liver. There were no negative effects from the addition of TCM, CMN, or UP in the diet. TCM and CMN showed no improvements in performance compared to AF alone, but improved serum characteristics and AF residues in liver and kidney. Pigs fed 0.50% UP in the MM diet had an improvement in growth, TP and Mg in serum, as well as reducing AFG_1 in the liver.

INTRODUCTION

Feed accounts for around 70% of animal production costs and because it is such a large proportion of total costs quality control is very important. Most pigs in North America are fed a corn and soybean meal based diet but in other parts of the world pigs are fed diets containing combinations of grain and other ingredients. Feeding a

combination of grains can lead to a mix of mycotoxins in the diet, negatively affecting production and profit. Mycotoxins are toxic secondary metabolites produced by several species of fungi. These are formed during growth, harvesting, drying or storage of fruits, grains, seeds, or their by-products (CAST, 2003). Among more than 300 known mycotoxins, aflatoxins and fumonisins are very important because of their frequent occurrence in feedstuffs and their high toxic potential demonstrated in domestic animals. The syndrome caused by mycotoxin contamination is called mycotoxicoses (Di Gregario *et al.*, 2014). Many fungal species are capable of simultaneously producing more than one mycotoxin. Therefore, an individual grain source may be naturally contaminated with more than one mycotoxin, or the incorporation of numerous grain sources, which are each contaminated with a different mycotoxin, into a single feed may result in a feed that contains a number of different mycotoxins.

In production animals, mycotoxins can cause a variety of effects, such as decreased performance, reduced feed intake, poor feed conversion, diminished body weight gain, immune suppression, reproductive disorders and residues in animal products (Kolosova and Stroka, 2011). These effects are impacted by a number of factors including intake level, species, sex, age, diet, physiological status, and duration of exposure. In 1980, it was reported that swine producers in 10 US states suffered losses estimated at $100 million. This loss was due to aflatoxin-infected corn from a local producer that was fed to 13.6 million animals; costs were broken down into mortality ($24 million) and inferior feed conversion ($76 million) compared to unaffected herds, with an average loss of $7.40 per pig (Kolosova and Stroka, 2011).

Lipid peroxidation plays a large role in the toxicity of aflatoxin. One alternative to protect against aflatoxicosis is to supplement feed with additives having antioxidant properties, such as turmeric (*Curcuma longa*). According to Rastogi *et al.* (2001), supplementation of antioxidants could ameliorate the effects of AFB_1 by preventing or reducing oxidation effects. Gowda *et al.* (2008) demonstrated an improved antioxidant status and partial protection against the adverse affects of AFB_1 when chicks were fed diets containing 1.0 mg/kg AFB_1 with 74 mg/kg of curcumin supplied by turmeric.

Recognition that mycotoxins affect health and productivity has led to extensive research on methods to counteract the effects of mycotoxins over the last few decades, including detection and elimination or detoxification. The most well known method for detoxification involves nutritionally inert adsorbents that will bind and immobilize mycotoxins in the GI tract, making them less bioavailable to the animal (Murugesan *et al.*, 2015). Therefore, the objective of the current study was to determine the efficacy of two antioxidants and an adsorbent to prevent or reduce the toxic effects of aflatoxin or a combination of AF and FB in weanling pigs fed dietary treatments for 26 days.

MATERIALS AND METHODS

All procedures were approved by the University of Missouri Institutional Animal Care and Use Committee. On day 14 post-weaning, 60 weanling pigs were purchased, weighed, ear tagged, and allotted by weight to dietary treatments. Each pig was individually housed with *ad libitum* access to feed and water. Pigs were housed in a

temperature controlled building with elevated 1.2 m^2 pens with plastic covered grate flooring over a flush system. Each pen had a stainless steel nipple waterer and a nursery feeder. A completely randomized design was used with six 5-week-old barrows assigned to each of 10 dietary treatments for 26 days. Diets were formulated to meet or exceed NRC requirements (NRC, 2012) for weanling pigs. Dietary treatments to be evaluated included: 1) basal industry type weanling diet (BD) containing no adsorbent or mycotoxins); 2) BD plus 200 mg/kg theracurmin (TCM); 3) BD plus 200 mg/kg curcumin (CMN); 4) BD plus 1 mg/kg AF; 5) BD plus 1 mg/kg AF and 200 mg/kg TCM; 6) BD plus 1 mg/kg AF and 200 mg/kg CMN; 7) BD plus 0.50% Unike Plus (UP); 8) BD plus MM (1 mg/kg AFB_1 and 25 mg/kg FB_1) 9) BD plus MM plus 0.25% UP; and 10) BD plus MM plus 0.50% UP. Dietary mycotoxin concentrations were confirmed by analysis and all diets were screened for the presence of other mycotoxins prior to the start of the experiment.

Pigs were weighed on day 26, euthanized and necropsied at the University of Missouri Veterinary Diagnostic Laboratory. Livers and kidneys were collected and weighed and samples of liver, kidney, and spleen were collected for histopathology. Blood and liver were collected from each pig for separate testing, including: lipid peroxides, aqueous peroxides; and analysis of serum concentrations of 8-OHdG, TNF-alpha, and IgG. Blood was centrifuged at 2500 RPM at 5°C for 10 minutes and a serum analysis was done for each treatment.

The activities of TNF-α in serum and aqueous peroxide levels in the liver were determined by assay kits (EP2TNFA and 23280, Thermo Scientific, Rockford, IL)

according to the manufacturer's instructions. Pig IgG activity was determined using an ELISA kit from Bethyl Laboratories (E101-104) following the manufacturer's instruction. Lipid peroxides assay kit was purchased from Sigma-Aldrich, and used to determine lipid peroxides in the liver following the manufacturer's instructions for tissue samples (Sigma-Aldrich, St. Louis, MO). Kits purchased from Cell Biolabs Inc. were used to determine oxidative RNA damage (STA-325) and total antioxidant capacity (STA-360) following instructions provided with the ELISA kits.

Data were analyzed using the General Linear Models procedure of SAS (SAS Institute, 2009). Pig was the experimental unit. All statements of significance are based on the 0.05 level of probability.

RESULTS

Dietary Analysis

A screen of the basal diet indicated that it was negative for vomitoxin, zearalenone, ochratoxin A, T-2 toxin, and fumonisin B_1, but contained 5 μg/kg AFB_1. Diets of TCM, CMN, and UP in the basal diet all contained 0 mg/kg AFB_1. The diets containing AF had an average concentration 0.96 mg/kg AFB_1. In addition to AFB_1, these diets also contained an average of 30 μg/kg AFB_2, 904 μg/kg AFG_1, and 7 μg/kg AFG_2.

The diets containing MM diet were analyzed and contained an average of 0.92 mg/kg AFB_1, and 24 mg/kg FB_1. In addition to AFB_1, these diets contained an average of 30 μg/kg AFB_2, 283 μg/kg AFG_1, and 6 μg/kg AFG_2.

Growth Performance

Effects of dietary treatments on growth performance are summarized in Table 4.1. There were no differences ($P > 0.05$) in feed intake (FI) among treatments at day 7. Compared to the controls, FI at day 14 was unaffected ($P > 0.05$) by the addition of TCM and CMN to the basal diet, (8.90 kg and 9.67 kg vs. 10.27 kg). Pigs fed AF had a numerical reduction in FI compared to controls, but it was not significant ($P > 0.05$) at 7.52 kg vs. 10.27 kg. The addition of TCM and CMN to the AF diet did not change ($P > 0.05$) FI compared to pigs fed only AF, and FI was similar to control pigs (6.93 kg and 7.81 kg vs. 10.27 kg).

The addition of UP to the basal diet did not affect ($P > 0.05$) FI relative to pigs fed the control diet (9.69 vs. 10.27 kg) at day 14. When pigs were fed MM, FI was reduced ($P < 0.05$) from 10.27 kg in control pigs to 7.03 kg at day 14. Compared to pigs fed MM alone, there was no improvement in FI ($P > 0.05$) when 0.25% UP was added to the MM diet, (7.21 kg vs. 7.03 kg). However, when 0.50% UP was added to the MM diet, FI improved ($P < 0.05$) from 7.03 kg to 7.94 kg, a value similar ($P > 0.05$) to control pigs.

Compared to control pigs, there was no effect ($P > 0.05$) on FI at day 21 from the addition of TCM or CMN to the basal diet (16.37 kg and 17.28 kg vs. 16.82 kg). The addition of AF to the basal diet reduced FI ($P < 0.05$) from 16.82 kg in the control pigs to 12.07 kg at day 21. There was no improvement in FI when TCM or CMN were added to the AF diet (11.40 kg and 12.89 kg vs. 12.07 kg) at day 21.

The addition of 0.50% UP to the basal diet did not affect FI ($P > 0.05$) relative to control pigs (16.78 kg vs. 16.82 kg) at day 21. Pigs fed MM had a reduced FI ($P < 0.05$)

compared to control (11.48 vs. 16.82 kg) at day 21. There was no improvement ($P > 0.05$) in FI with the addition of UP to the MM diet, at either level (11.78 kg and 11.84 kg. vs. 11.48) at day 21.

Feed intake at day 26 was unaffected ($P > 0.05$) by the addition of TCM or CMN to the basal diet relative to the control (23.22 kg and 23.48 kg vs. 23.57 kg). At day 26, pigs fed AF had a reduced FI ($P < 0.05$) compared to control pigs (16.14 kg vs 23.57 kg). The addition of TCM and CMN to AF diet did not improve ($P > 0.05$) FI relative to pigs fed AF alone at day 26 (15.38 kg and 16.33 kg vs. 16.14 kg).

At day 26, compared to control pigs, there was no effect ($P > 0.05$) on FI when UP was added to the basal diet, (22.57 kg vs. 23.57 kg). At day 26, the addition of MM to the basal diet reduced ($P < 0.05$) FI from 23.57 kg in control pigs to 13.91 kg. The addition of UP at either 0.25% or 0.50% to the MM diet did not significantly improve ($P > 0.05$) FI (15.59 kg and 16.41 kg vs. 13.91 kg) at day 26.

At day 7, body weight gain (BWG) of pigs fed TCM and CMN was similar ($P > 0.05$) to control pigs (2.83 kg and 3.10 kg vs. 3.24 kg). When AF was added to the basal diet, BWG decreased ($P < 0.05$) from 3.24 kg in controls to 2.23 kg. At day 7, the addition of TCM and CMN to the AF diet was not effective ($P > 0.5$) in preventing the decrease in BWG caused by AF (2.17 kg and 2.27 kg vs 2.23 kg).

At day 7, BWG of pigs fed 0.50% UP alone was similar to that of control pigs (3.38 kg vs. 3.25 kg). However, pigs fed MM had a reduced BWG ($P < 0.05$) compared to controls at 2.32 kg vs. 3.24 kg. At day 7, the addition of UP at 0.25% or 0.50% to the MM diet was not effective in preventing the reduction in BWG caused by MM alone.

Compared to control pigs, BWG at day 14 was reduced ($P < 0.05$) in pigs fed TCM alone (6.00 kg vs. 7.28 kg), whereas BWG of pigs fed CMN alone was similar to control pigs (6.92 vs 7.28 kg) but was not different from pigs fed TCM alone. At day 14, pigs fed AF had a reduced ($P < 0.05$) BWG compared to control pigs (4.79 kg vs. 7.28 kg). The addition of TCM and CMN did not prevent the reduction in caused by AF at day 14 (4.42 kg and 4.88 kg vs 4.79 kg).

There was no effect ($P > 0.05$) on BWG at day 14 with the addition of UP to the basal diet (6.97 vs. 7.28 kg). However, the addition of MM to the diet reduced ($P < 0.05$) BWG from control from 7.28 kg to 4.40 kg. There was no improvement ($P > 0.05$) in BWG with the addition of 0.25% UP to the MM diet (4.40 kg vs. 4.23 kg) at day 14. However, when UP was added at 0.50%, BWG improved ($P < 0.05$) from 4.40 kg to 5.61 kg, but this BWG was significantly lower ($P < 0.05$) than control pigs at 7.28 kg.

At day 21, there was no difference ($P > 0.05$) in BWG in pigs fed TCM and CMN diets compared to control pigs (12.48 kg and 13.03 kg vs. 13.84 kg). At day 21, pigs fed AF has significantly lower ($P < 0.05$) BWG than control pigs or pigs fed TCM or CMN alone (8.79 kg vs. 13.48 kg, 12.48 kg and 13.03 kg). The addition of TCM and CMN to AF diet was not effective in preventing the reduction BWG caused by AF. At day 21, BWG was unaffected by the addition of 0.50% UP to the diet (11.6 kg vs. 13.84 kg). The addition of MM to the basal diet reduced ($P < 0.05$) BWG from 13.84 kg to 5.66 kg, which was not improved ($P > 0.05$) by the addition of 0.25% or 0.50% UP (4.95 kg and 7.12 kg vs 13.84 kg).

At day 26, compared to control pigs, BWG was unaffected by the addition of TCM or CMN alone to the basal diet (15.41 kg and 16.49 kg vs. 16.31). At day 26, pigs fed AF had reduced ($P < 0.05$) BWG compared to control pigs (10.68 kg vs. 16.31 kg), and compared to pigs fed TCM or CMN alone (10.68 kg vs. 15.41 kg and 16.49 kg). The addition of TCM and CMN to the AF diet was not effective ($P > 0.05$) in preventing the reduction in BWG caused by AF.

Adding UP to the basal diet did not affect ($P > 0.05$) BWG at day 26 compared to control pigs (16.18 kg vs. 16.31 kg). However, pigs fed MM had a lower weight gain ($P < 0.05$) than control pigs (8.01 kg vs. 16.31 kg). This was not significantly improved ($P > 0.05$) with the addition of 0.25% UP to the MM diet, but was increased ($P < 0.05$) from 8.01 kg to 10.98 kg with the addition of 0.50% UP to the MM diet. However, pigs fed the combination of 0.50% UP and MM still had lower ($P < 0.05$) BWG than control pigs.

There were no differences ($P > 0.05$) among treatments in feed conversion (G:F) at day 7. At day 14, pigs fed TCM and CMN alone had similar ($P > 0.05$) G:F) to control pigs (0.67 and 0.72 kg:kg vs. 0.72 kg:kg). The G:F of pigs fed AF was similar to control pigs at 0.64 kg:kg compared to 0.73 kg:kg in the controls. The G:F of pigs fed combinations of TCM and AF and CMN and AF was similar ($P > 0.05$) to that of control pigs and pigs fed AF alone .

There was no effect on G:F at day 14 with the addition of UP to the basal diet, relative to the control diet (0.72 vs. 0.72 kg:kg). Pigs fed MM had a significant reduction ($P < 0.05$) in G:F compared to control pigs (0.58 kg:kg vs. 0.72 kg:kg). There was no improvement in G:F with the addition of 0.25% UP to the MM diet (0.58 kg:kg vs. 0.59

kg:kg). However, when 0.50% UP was added to the MM diet, G:F improved ($P < 0.05$) and was similar to control pigs (0.73 kg:kg vs. 0.72 kg:kg).

Pigs fed TCM and CMN alone had similar ($P > 0.05$) G:F to control pigs at day 21 (0.70 and 0.71 kg:kg vs. 0.74 kg:kg). There was a numerical reduction in G:F with the addition of AF to the basal diet, but it was not significant ($P > 0.05$) compared to controls (0.66 kg:kg vs. 0.74 kg:kg). There was a greater numerical reduction in G:F with the addition of TCM and CMN to the AF diet, and G:F was lower ($P < 0.05$) than control G:F values (0.63 and 0.60 kg:kg vs. 0.74 kg:kg).

At day 21, pigs fed UP alone had similar ($P > 0.05$) G:F to control pigs (0.70 kg:kg vs. 0.74 kg:kg). The addition of MM to the basal diet reduced ($P < 0.05$) G:F from 0.74 kg:kg in the controls to 0.52 kg:kg at day 21. There was no improvement ($P > 0.05$) in G:F when 0.25% UP was added to the MM diet (0.59 kg:kg vs. 0.52 kg:kg). The addition of 0.50% UP to the MM diet improved G:F ($P < 0.05$) relative to pigs fed MM alone (0.64 kg:kg vs. 0.52 kg:kg), but G:F was still lower ($P < 0.05$) than pigs fed the control diet (0.64 kg:kg vs. 0.74 kg:kg).

At day 26, pigs fed TCM and CMN alone had similar ($P > 0.05$) G:F to control pigs (0.67 and 0.70 kg:kg vs. 0.70 kg:kg). There was no difference ($P > 0.05$) in G:F with the addition of AF to the basal diet compared to controls (0.67 kg:kg vs. 0.70 kg:kg). There was a numerical reduction in G:F with the addition of TCM and CMN to the AF diet, but G:F was similar ($P > 0.05$) to controls (0.62 and 0.59 kg:kg vs. 0.70 kg:kg) and to pigs fed AF alone (0.62 and 0.59 kg:kg vs. 0.67 kg:kg).

Pigs fed UP alone had similar ($P > 0.05$) G:F to control pigs at day 26 (0.72 kg:kg vs. 0.70 kg:kg). The addition of MM to the basal diet reduced ($P < 0.05$) G:F from 0.70 kg:kg in the controls to 0.55 kg:kg at day 26. There was no improvement ($P > 0.05$) in G:F when 0.25% UP was added to the MM diet (0.58 kg:kg vs. 0.55 kg:kg). However, the addition of 0.50% UP to the MM diet improved ($P < 0.05$) G:F with a value similar ($P > 0.05$) to that of control pigs (0.67 kg:kg vs. 0.70 kg:kg).

Organ Weights

Effects of dietary treatments on liver and kidney weight are summarized in table 4.4. Relative liver weight (RLW) was similar ($P > 0.05$) to control pigs in pigs fed TCM or CMN alone (2.51% and 2.47% vs. 2.47%, respectively). The addition of AF to the basal diet increased RLW ($P < 0.05$) compared to control from 2.47% to 2.95%. The addition of TCM and CMN to the AF diet did not prevent ($P > 0.05$) the increase in RLW caused by AF but instead numerically increased RLW ($P > 0.05$) from 2.95% to 3.10% and 3.26%, respectively.

In pigs fed UP alone, RLW was also similar ($P > 0.05$) to that of control pigs (2.64% vs. 2.47%). The addition of MM to the diet did not increase RLW compared to control or UP alone ($P > 0.05$) (2.64% vs. 2.47% and 2.64%, respectively). When UP was added to the MM diet at 0.25% there was not a significant increase in RLW compared to control pigs or pigs fed MM alone (2.80% vs. 2.47% and 2.64%). However, compared to control pigs, there was an increase in RLW ($P < 0.05$) in pigs fed MM plus 0.50% UP, from 2.47% to 2.94%, but this value was not greater ($P > 0.05$) than that observed in pigs fed

MM alone. There were no differences ($P > 0.05$) in relative kidney weight among treatments.

Serum Chemistry

Effects of dietary treatments on serum concentrations of UN, ALB, P and GGT are summarized in Tables 4.5 and 4.6. Urea nitrogen (UN) was similar to control pigs in pigs fed TCM or CMN alone (15.33 mg/dL vs. 15.5 and 14.33 mg/dL. When AF was added to the basal diet, UN decreased ($P < 0.05$) from 15.33 to 10.33 mg/dL. This decrease was not prevented ($P > 0.05$) by the addition of TCM or CMN to the AF diet (10.67 and 11.83 mg/dL vs 10.33 mg/dL), and both levels of UN were significantly lower ($P < 0.05$) than those of control pigs and pigs fed TCM alone.

The addition of UP alone to the basal diet did not change UN levels in pigs when compared to control pigs ($P > 0.05$), but when pigs were fed MM, UN decreased ($P < 0.05$) from 15.33 mg/dL in control pigs to 9.17 mg/dL. The addition of UP at 0.25% or 0.50% did not prevent the decrease in ($P > 0.05$) UN levels in pigs caused by MM (9.33 and 11.67 mg/dL vs. 9.17 mg/dL).

Albumin levels of pigs fed TCM and CMN alone were similar to control values (3.61 and 3.76 g/dL vs. 4.03 g/dL). The addition of AF to the basal diet significantly reduced ($P < 0.05$) albumin levels compared to control pigs (3.10 g/dL vs 4.03 g/dL), and the level was less ($P < 0.05$) than that observed in pigs fed CMN alone (3.10 vs. 3.76 g/dL) but was similar to levels observed in pigs fed TCM alone. There was no improvement ($P > 0.05$) in albumin when TCM and CMN were added to the AF diet (3.09

and 2.74 g/dL vs. 4.03 g/dL) with all levels being lower than that of control pigs ($P < 0.05$).

Albumin levels of pigs fed UP alone was similar ($P > 0.05$) to that of control pigs. Albumin of pigs fed MM was lower ($P < 0.05$) than that of control pigs (2.97 vs. 4.03 g/dL), and was not improved by the addition of UP at 0.25% or 0.50% (2.97 g/dL vs. 2.92 and 3.20 g/dL, respectively).

Phosphorus levels were lower ($P < 0.05$) in pigs fed TCM and CMN compared to control pigs (9.30 and 9.49 mg/dL vs. 11.62 mg/dL). The addition of AF to the basal diet reduced P levels relative to control ($P < 0.05$) from 11.62 to 9.88 mg/dL, but the level was not different from that of pigs fed TCM or CMN alone (9.88 mg/dL vs. 9.30 and 9.49 mg/dL). When TCM was added to the AF diet, P levels increased and were similar ($P < 0.05$) to control levels (10.37 mg/dL vs. 11.62 mg/dL). There was no improvement in P levels ($P > 0.05$) from the addition of CMN to the diet compared to AF alone (8.53 mg/dL vs. 9.88 mg/dL).

The addition of UP alone did not affect P compared to control ($P > 0.05$) at 10.49 mg/dL vs. 11.62 mg/dL. The addition of MM to the basal diet reduced P levels from 11.62 mg/dL in the control to 8.53 mg/dL ($P < 0.05$). There was a numeric increase in serum P when MM were combined with 0.25% UP from 8.91 mg/dL to 9.68 mg/dL, but it was not significant ($P > 0.05$). The addition of 0.50% UP in the MM diet brought P levels up to control levels ($P > 0.05$) but this value was also similar to that observed in pigs MM alone (9.99 vs. 11.63 mg/dL).

Gamma-glutamyl transferase (GGT) activity in pigs fed TCM and CMN alone were similar ($P > 0.05$) to control levels (36.83 and 39.00 U/L vs. 44.83 U/L). AF addition to the basal diet numerically increased GGT levels (44%), but values were not different ($P > 0.05$) from control (65.17 U/L vs. 44.83 U/L). The addition of TCM and CMN to the AF diet did not prevent the numerical increase in GGT levels caused by AF, with values observed being similar ($P > 0.05$) to diets with AF alone and control diet (57.17 U/L and 64.17 U/L vs. 44.83 U/L).

There was no effect ($P > 0.05$) on GGT compared to control when UP was fed (28.33 vs. 44.83 U/L). The addition of MM to the basal diet increased ($P < 0.05$) GGT levels compared to control from 44.83 U/L to 113.83 U/L. The addition of UP at 0.25% was not effective ($P > 0.05$) in preventing the increase in GGT caused by MM alone (108.33 U/L vs. 113.83 U/L). The addition of 0.50% UP reduced ($P < 0.05$) GGT levels compared to MM alone (88.33 U/L vs. 113.83 U/L.\), but the value was still greater ($P < 0.05$) than that observed in control pigs.

There was no effect ($P > 0.05$) of dietary treatments on serum total protein or serum Ca concentrations. Effects of dietary treatments on serum concentrations of TP, globulin, Ca, and Mg are summarized in Table 4.6. Globulin levels were unaffected ($P > 0.05$) when TCM and CMN were fed, compared to control (1.82 g/dL and 1.70 g/dL vs. 1.85 g/dL). The addition of AF to the basal diet numerically increased globulin, but the value was similar ($P > 0.05$) to pigs fed the control diet (2.18 g/dL vs. 1.85 g/dL). This value was greater ($P < 0.05$) than when pigs were fed TCM or CMN alone (2.18 g/dL vs. 1.82 g/dL and 1.70 g/dL, respectively). When TCM was added to the AF diet, there was

no change in globulin levels (2.18 g/dL vs. 2.18 g/dL) so levels were similar to control, but greater than TCM alone. When CMN was included in the AF diet, globulin increased ($P > 0.05$) from 2.18 g/dL to 2.38 g/dL, which was similar to AF alone, but greater ($P < 0.05$) than control and CMN alone (2.18 g/dL vs. 1.85 g/dL and 1.70 g/dL).

Globulin levels of pigs fed UP alone was similar to control (2.15 g/dL vs. 1.25 g/dL), but when MM was added to the diet globulin increased ($P < 0.05$) from control at 1.85 g/dL to 2.37 g/dL. This globulin value was similar ($P > 0.05$) to UP alone (2.37 g/dL vs. 2.15 g/dL). This increase in globulin value continued with the addition of UP at 0.25% and 0.50% from 2.37 g/dL to 2.80 g/dL and 2.48 g/dL, respectively. The globulin value f pigs fed MM and UP at 0.25% was greater ($P < 0.05$) than MM alone, but at 0.50% the values were similar ($P > 0.05$) to that of pigs fed MM alone.

There were no effects of dietary treatment on calcium levels in the blood. Magnesium levels of pigs fed TCM and CMN were lower ($P < 0.05$) than control pigs (2.51 mg/dL and 2.53 mg/dL vs. 3.15 mg/dL). The addition of AF reduced ($P < 0.05$) Mg values relative to control (2.42 mg/dL vs. 3.15 mg/dL), but the value was similar ($P > 0.05$) to pigs fed the TCM and CMN diets (2.42 mg/dL vs. 2.51 mg/dL and 2.53 mg/dL). Magnesium levels increased when TCM and CMN were added to diets including AF, from 2.42 mg/dL to 2.78 mg/dL and 2.67 mg/dL, respectively, and was not different ($P > 0.05$) from control values.

There was no effect ($P > 0.05$) of UP alone on magnesium levels of pigs, compared to control (2.70 mg/dL vs. 3.15 mg/dL). The addition of MM to the diet, had no effect ($P > 0.05$) on Mg levels compared to control pigs (3.13 mg/dL vs. 3.15 mg/dL).

This value was reduced numerically, but not statistically ($P > 0.05$) when UP was added at 0.25% and 0.50% (3.13 mg/dL vs. 2.70 mg/dL and 2.89 mg/dL). These levels are not different ($P > 0.05$) from control values.

Aflatoxin Residues in Liver and Kidney

The effects of inclusion of 1 mg/kg AF and 25 mg/kg FB in the contaminated diets on liver and kidney residues are summarized in tables 4.7 and 4.8.

As expected, there were no liver residues of AFM_1, AFG_1, or AFB_1 in the liver of pigs fed the control, TCM, CMN, or UP diets alone. Pigs fed AF alone had AFM_1 residues of 2.05, which is greater ($P < 0.05$) than control pigs. The addition of TCM decreased ($P < 0.05$) AFM_1 residues from 2.05 to 0.88, which is significantly lower than AF alone, but greater ($P < 0.05$) than control or TCM alone (0.88 ppb vs. 0.00 ppb). When CMN was added to the AF contaminated diet, levels were reduced numerically from 2.05 ppb to 1.54 ppb, but were not significantly lower ($P > 0.05$) than pigs fed AF.

Pigs fed MM diet has AFM_1 liver residues of 1.44 ppb, this was an increase compared to control at 0.00 ppb ($P < 0.05$). When UP was added at 0.25% and 0.50%, liver residues were reduced numerically, but not statistically ($P > 0.05$) from 1.44 ppb to 1.11 ppb and 0.75 ppb, respectively. All values were greater ($P < 0.05$) than control or UP alone.

Pigs fed AF had similar ($P > 0.05$) AFG_1 levels to control pigs (0.26 ppb vs. 0.00 ppb). This level increased, but was not different with the addition of TCM to the AF diet (0.26 ppb vs. 0.39 ppb), and was also similar to control. The addition of CMN to the AF diet increased ($P < 0.05$) AFG_1 residue levels in the liver from 0.26 ppb to 0.71 ppb, and

this value was significantly greater ($P < 0.05$) than control. Pigs fed UP alone had no AFG_1 residues in the liver.

When MM was added to the diet, AFG_1 levels increased ($P > 0.05$) from 0.00 ppb to 0.24 ppb. There was no effect ($P > 0.05$) on AFG_1 levels with the addition of UP at 0.25% or 0.50% (0.24 ppb vs. 0.39 ppb and 0.13 ppb, respectively) and all levels were similar to control.

AFB_1 residue levels were 0.00 ppb in pigs fed the control, TCM, CMN, and UP alone diets. The addition of AF increased AFB_1 ($P < 0.05$) levels from 0.00 ppb to 2.81 ppb. The addition of TCM and CMN to the AF diet did not reduce ($P > 0.05$) residue levels (2.81 ppb vs. 2.39 ppb and 2.23 ppb). Residue levels of all pigs fed AF (alone or with antioxidants) were greater ($P < 0.05$) than control or TCM and CMN alone.

When MM were included in the diet, AFB_1 residue level increased ($P < 0.05$) from 0.00 ppb in the control to 3.55 ppb. The addition of 0.25% UP to the MM diet did not improve ($P > 0.05$) residue levels (3.55 ppb vs. 2.26 ppb). However, when 0.50% UP was added to the MM diet AFB_1 levels in the liver decreased ($P < 0.05$) from 3.55 ppb to 1.59 ppb, but this level was greater ($P > 0.05$) than that of control pigs or pigs fed UP alone (1.59 ppb vs. 0.00 ppb).

No AFM_1 residues were observed in the kidney of pigs fed the control, TCM, CMN and UP alone. When pigs were fed AF AFM_1 residue increased ($P < 0.05$) from 0.00 ppb to 1.08 ppb, this was numerically reduced with the addition of TCM from 1.08 ppb to 0.64 ppb, and numerically increased with the addition of CMN from 1.08 to 1.27 ppb.

Pigs fed MM had increased ($P < 0.05$) AFM_1 residue compared to control (1.16 ppb vs. 0.00 ppb). The addition of UP at 0.25% to the MM diet did not reduce AFM_1 ($P > 0.05$) levels (1.16 ppb vs. 1.79 ppb), and the addition of UP at 0.50% increased AFM_1 ($P < 0.05$) residue from 1.16 ppb to 3.27 ppb. No AFG_1 residues were in pigs fed the TCM, CMN, and UP diets. The addition of AF did not increase ($P > 0.05$) AFG_1 residue levels (0.06 ppb vs. 0.00 ppb). The addition of TCM and CMN to the AF diet increased levels from 0.06 ppb to 0.13 ppb and 0.10 ppb, respectively; this is higher than control ($P < 0.05$) at 0.00 ppb, but not higher than AF alone ($P > 0.05$).

The addition of MM increased ($P < 0.05$) AFG_1 residue from 0.00 ppb in control pigs to 0.11 ppb, and there was no reduction in residue levels with the addition of 0.25% or 0.50% UP to the MM diet (0.11 ppb vs. 0.12 ppb and 0.17 ppb).

No AFB_1 residues were observed in the kidneys of pigs fed TCM, CMN, and UP diets. Pigs fed AF had an increase ($P < 0.05$) in AFB_1 residue in the kidney from 0.00 ppb to 1.00 ppb. This was not reduced ($P > 0.05$) with the addition of TCM (1.00 ppb vs. 0.98 ppb) or CMN (1.00 ppb to 0.92 ppb).

Pigs fed MM had a larger increase ($P < 0.05$) in kidney AFB_1 residue levels, from 0.00 ppb in the control to 2.15 ppb. There was no change ($P > 0.05$) with the addition of 0.25% or 0.50% UP to the MM diet (2.15 ppb vs. 2.62 ppb and 1.66 ppb).

Tumor Necrosis Factor- α

The effect of dietary treatments on Tumor Necrosis Factor-α levels in blood in summarized in table 4.9. The addition of TCM and CMN to the basal diet did not affect TNF-α levels ($P > 0.05$) in pigs, compared to control (5.52 pg/ml and 3.94 pg/ml vs. 5.56

pg/ml). Pigs fed AF also had similar TNF-α levels to control pigs (4.84 pg/ml vs. 5.56 pg/ml). When CMN was added to the AF diet TNF-α levels were similar to control and pigs fed AF (4.59 pg/ml vs. 4.84 pg/ml and 5.56 pg/ml). However, TNF-α levels decreased ($P < 0.05$) when TCM was added to the diet to 2.85 pg/ml. The addition of UP to the diet decreased ($P < 0.05$) TNF-α levels from 5.56 pg/ml in control to 2.54 pg/ml. Pigs fed MM had similar TNF-α levels to pigs fed UP alone, but lower than control ($P < 0.05$) at 3.59 pg/ml vs. 5.56 pg/ml. There was no change in TNF-α with the addition of UP at 0.25% or 0.50% to the MM diet (3.93 pg/ml and 3.56 pg/ml vs. 3.59 pg/ml) but values were lower than control.

8- Hydroxyguanosine

The effect of dietary treatments on 8-hydroxyguanosine (8-OHG) levels in blood is summarized in table 4.9. The addition of TCM, CMN, and UP to the basal diet did not affect ($p > 0.05$) 8-OHG levels in the blood of pigs, compared to control. The addition of AF to the diet did not change ($P > 0.05$) 8-OHG levels compared to control (1.40 ng/ml vs. 1.65 ng/ml). When TCM was added to the AF diet, 8-OHG levels were reduced compared to control values ($P < 0.05$) from 1.65 ng/ml to 0.39 ng/ml, but were not different ($P > 0.05$) than 8-OHG levels of pigs fed AF alone (1.40 ng/ml vs. 0.39 ng/ml). Pigs fed AF and CMN had 8-OHG levels similar ($P > 0.05$) to control and AF alone (1.05 ng/ml vs. 1.65 ng/ml and 1.40 ng/ml, respectively). The addition of MM significantly increased ($P < 0.05$) 8-OHG levels from 1.65 ng/ml in control pigs to 3.30 ng/ml, but these levels were reduced with the addition of 0.25% and 0.50% UP to 1.65 ng/ml and 2.22 ng/ml respectively, which were similar to control ($P > 0.05$).

Immunoglobulin- G

The effect of dietary treatments on blood levels of IGG is summarized in table 4.9. The addition of CMN to the basal diet increased ($P < 0.05$) IGG levels in pigs from 35.81 ng/ml in control to 111.79 ng/ml. Pigs fed AF alone had similar levels of IGG to control (66.83 ng/ml vs. 35.81 ng/ml). The addition of TCM and CMN to the AF diet increased IGG levels ($P < 0.05$) compared to AF alone and control (130.56 ng/ml and 146.57 ng/ml vs. 66.83 ng/ml and 35.81 ng/ml). Pigs fed the MM diet had similar ($P > 0.05$) levels of IGG to control and pigs fed UP alone (67.62 ng/ml vs. 35.81 ng/ml and 69.39 ng/ml). These levels of IGG increased ($P < 0.05$) from 67.62 ng/ml to 118.27 ng/ml with the addition of UP at 0.50% to the MM diet.

Total Antioxidant Capacity

The effect of dietary treatments on total antioxidant capacity (TAC) is summarized in table 4.9. The addition of TCM and CMN to the basal diet did not affect ($P > 0.05$) TAC levels of pigs compared to control pigs (0.015 mM and 0.017 mM vs. 0.016 mM). Pigs fed AF alone had similar ($P > 0.05$) TAC levels to control pigs (0.017 mM vs. 0.016 mM), and there was no change ($P > 0.05$) in TAC levels when CMN was added to the AF diet (0.016 vs. 0.017 mM) similar to AF alone and the control pigs. The additon of TCM to the AF diet decreased ($P < 0.05$) TAC levels from 0.017 mM in pigs fed AF alone to 0.009 mM).

There was no effect ($P > 0.05$) on TAC levels of pigs fed UP alone relative to control pigs (0.012 mM vs. 0.016 mM). The addition of MM to the basal diet decreased

(P < 0.05) TAC levels from 0.016 mM to 0.009 mM. However, this reduction was increased with the addition of 0.25 and 0.50% UP to the MM contaminated diet and levels were similar (P > 0.05) to control values (0.017 and 0.016 mM vs. 0.016 mM, respectively).

Lipid and Aqueous Peroxides

There was no effect (P > 0.05) of dietary treatment on liver lipid or aqueous peroxides. (Table 4.10)

Histopathology

The effects of dietary treatments on liver lesion scores are summarized in Table 4.10. No liver lesions were observed (P > 0.05) in control pigs or pigs fed TCM, CMN, or 0.50% UP alone. Compared to controls, there was an increase (P < 0.05) in liver lesion score in pigs fed AF (0 vs 2.0). Pigs fed the combination of TCM and AF had a similar (P > 0.05) lesion score to that of pigs fed AF alone (2.0 vs. 2.0). In contrast, pigs fed the combination of CMN and AF had a higher (P < 0.05) lesion score than that of pigs fed AF alone (2.67 vs 2.0).

The highest liver lesion scores were observed in pigs fed MM with lesion scores increasing (P <0.05) from 0 in the controls to 3.50 in pigs fed MM alone. The addition of 0.25% UP to the MM did not prevent the increase (P > 0.05) in lesion score caused by MM, whereas the addition of 0.50% UP to the AF diet reduced (P < 0.05) the lesion score from 3.50 in pigs fed the MM diet to 2.50. Liver lesions observed in the livers of pigs fed diets containing AF and MM included mild to moderate hepatocellular degeneration to severe hepatic lobular collapse with cell necrosis and fibrosis. The most common

changes observed were severe vacuolar degeneration, often in the periportal zone (Zone 1) and sometimes in the mid-zone (Zone 2).

A detailed histopathology report is presented in table 4.11.

DISCUSSION

Aflatoxin and Antioxidants

Aflatoxin B_1 and fumonisin B_1 can be found as contaminants in feed ingredients, including corn. Corn is one of the main ingredients in swine diets, making these mycotoxins a concern for the swine industry. Ingesting contaminated feed causes decreased performance and poor health of pigs. In the present study, the efficacy of two antioxidants and an adsorbent to reduce or prevent the effects of AFB_1 or a combination of AFB_1 and FB (MM) in weanling pigs was evaluated. The two antioxidants used were theracurmin and curcumin; both were obtained after processing of turmeric (*Curcuma longa*), a deep orange-yellow powder common as a spice in curries. Turmeric has been shown to be effective at alleviating some of the effects of AF in broilers (Gowda *et al.,* 2008). Curcumin is found in the rhizomes of the plant (*Curcuma longa)*, but is not very bioavailable. Bioavailability of curcuminoids in turmeric can be increased through manufacturing methods. The curcumin used contained 95% curcuminoids and was prepared by concentrating the curcuminoids present in turmeric. Theracurmin is a product in which curcumin is dispersed with colloidal submicron-particles resulting in a

reduced particle size and increased bioavailability around 30x greater than curcumin. The theracurmin used in this study contained 10% curcuminoids.

Pig performance was not negatively affected by the addition of TCM or CMN to the basal diet, when compared to pigs fed the basal diet alone. This suggests that the additives did not negatively affect the nutritional value of the diet, and that concentrations used were safe for weanling pigs. In the current study, the addition of AF decreased performance and efficiency of weanling pigs. The addition of AF to the basal diet reduced gain 34% lower than control pigs. This reduction is much higher than reported by Dersjant-Li *et al.* (2003), who observed a 16% reduction in growth for every mg/kg of AF in the diet. The review put together by Dersjant-Li *et al.* (2003) was over pigs with a heavier initial weight than in the current study, which could have resulted in less of a reduction in growth. A reduction in performance of weanling pigs was also noted in a study done by Schell *et al.* (1993) where pigs had a reduction in growth and feed intake when fed diets contaminated with 800 ppb AF. A study in China showed a 13% reduction in gain and reduced efficiency when growing/finishing pigs were fed 0.1 mg/kg AF for 90 days (YingHua *et al.,* 2011). The addition of TCM and CMN to the AF contaminated diet showed no improvement in performance compared to pigs fed AF alone. This lack of improvement may have been a result of the levels of toxin used in this study. The level of 1 ppm AF may have caused oxidation problems that were too significant for this level of TCM and CMN to reduce the effects. Lu *et al.* (2014) fed a high oxidant diet with vitamin E and an antioxidant blend, and observed an increase in

growth with the addition of the antioxidants, which is contradictory to the present study.

There was no effect of dietary treatment on relative kidney weight. Relative kidney weights were comparable to studies done by Weaver *et al.* (2013) where no differences in RKW were observed when fed a combination of 150 µg/kg AF and 1100 µg/kg VOM. Aflatoxin primarily affects the liver, so it has a greater impact on liver weight than on kidney weight. Pigs fed AF had an increased relative liver weight compared to control, and the addition of TCM and CMN did not prevent the increase in RLW. YingHua *et al.* (2011) observed an increase in relative liver weight of 8.24%, which is lower than the increase observed in this study of 19%. This may have been because the pigs were in different stages of growth; the pigs in the current study were weanling pigs and the pigs used by YingHua were finishing pigs. The concentration of AF used by YingHua was 10x lower AF in the diet than in the current study (0.1 mg/kg vs. 1 mg/kg), which would have less of an effect on animal health. Weaver *et al.* (2013) observed an increase in liver weight of 21% in pigs fed 50 µg/kg AF and 1100 µg/kg VOM compared to control pigs, which is similar to the 19% increase observed in the current study. Lu *et al.* (2014) observed a significant decrease in RLW of pigs fed the oxidized diet supplemented with vitamin E and antioxidants, but RLW was still greater than control pigs.

Total antioxidant capacity (TAC) is a measure of the overall capability to counteract reactive oxygen species (ROS), resist oxidative damage and combat oxidative stress-related diseases. A decrease in TAC shows that the body is less capable to react to

oxidative damage, or that there has already been oxidative damage. This is the case with the reduction of TAC levels in pigs fed TCM and AF. This reduction is a result of TCM not being able to overcome the oxidative damage caused by AF. Sridhar *et al.* (2015) fed broiler chicks 1 mg/kg AF and observed a decrease in TAC levels. However, this decrease was eliminated with the addition of an antioxidant.

The addition of TCM and CMN reduced phosphorus (P) and magnesium (Mg) levels in serum, but had no effects on other serum chemistries. Phosphorus and Mg levels in pigs were slightly higher in controls than in other studies. Control levels of P at 11.6 mg/dL were greater than 8.64 mg/dL and 9.02 mg/dL as observed by Schell *et al.* (1993) and Weaver *et al.* (2013), respectively. Magnesium levels in control pigs were 3.15 mg/dL, compared to 1.86 mg/dL (Schell *et al.* 1993). Pigs fed AF had decreased levels of urea nitrogen (UN), albumin, Mg and P. This decrease in P and Mg brought P levels to a similar value of control pigs in the studies conducted by Schell *et al.* (1993) and Weaver *et al.* (2013). Schell *et al.* (1993) also observed decreased urea nitrogen and albumin when pigs were fed a diet contaminated with 800 ppb AF. A decrease in UN and albumin signifies that a decrease in protein metabolism is occurring because of the addition of AF to the diet; decreased protein synthesis is characteristic of aflatoxicosis. The addition of TCM to the AF contaminated diet improved levels of phosphorus to control values, but TCM was not effective in preventing the decrease in UN, albumin, or Mg. Glahn *et al.* (1991) reported that liver damage caused by AF leads to a reduction in vitamin D metabolism; vitamin D is required for the normal absorption and metabolism

of calcium and phosphorus. The addition of CMN did not significantly improve any of the effects of AF in serum.

There was a numeric increase in GGT of 45.3% when AF was added to the diet relative to control. Schell *et al.* (1993) reported an increase in GGT of 89% when pigs were fed 800 ppb AF compared to control pigs. This increase is numerically greater than what was observed in the present study, but GGT levels in pigs fed AF were similar between studies (65.17 U/L vs. 70 U/L). The control levels observed by Schell were lower than in the current study, resulting in a greater percentage increase. An increase in GGT signifies increased enzymes in the blood from liver damage (Boone *et al.* 2005).

The addition of AF to the diet increased AFM_1 and AFB_1 residue levels in the liver compared to control pigs. The addition of TCM to the AF diet decreased AFM_1 residues in the liver, but residue levels were higher than control, and there were no improvements in G_1 or B_1 levels with the addition of TCM or CMN. There was an increase in AFM_1 and B_1 residue levels in the kidney with the addition of AF to the diet, relative to control values. There was a numerical reduction of AFM_1 residue levels with the addition of TCM to the AF diet of 40%. There was no improvement in AFB_1 residue levels in the kidneys with the addition of TCM or CMN. The effects of AF on the body from adsorption cannot be completely prevented by the addition of antioxidants to the diet, because the antioxidants (TCM and CMN) cannot act against the toxins until after the animal has absorbed them. Unlike using a clay or adsorbent, which helps to prevent the uptake of toxins in the GI tract therefore preventing toxic effects before they begin.

Tumor necrosis factor–α (TNFα) is an inflammatory cytokine involved in several different functions such as lipid metabolism and regulating immune cells (Janeway *et al.* 1999). This cytokine was analyzed as a measure of systemic inflammation, occurring due to the mycotoxins, indicating a pro-inflammatory action of the immune system (Ali *et al.* 2013). Tumor necrosis factor–α is an inflammatory cytokine that can also stimulate free radical production, thus increasing oxidative damage (Tsutamoto *et al.* 2001). A decrease in TNFα reduces the body's inflammatory response, and can leave the animal more susceptible to infections (Ali *et al.* 2013). The addition of TCM and CMN as well as AF alone to the diet did not affect TNFα levels in pigs. Pigs fed CMN with the AF diet did not have a change in TNFα compared to control, however there was a decrease in TNFα with the addition of TCM to the AF diet. This decrease in TNFα levels when pigs were fed TCM and AF together could be a result of the high bioavailability of the antioxidant TCM reducing inflammation in the body. Weaver *et al.* 2013 fed a diet contaminated with 150 µg/kg AF and 1100 µg/kg VOM to pigs and saw no effect on TNFα.

In this study, we tested 8-hydroxyguanine (8-OHG) levels in the serum of pigs and saw few treatment effects. 8-hydroxyguanine is a good test for DNA oxidative damage in the body, which could be alleviated by the addition of antioxidants such as TCM and CMN. The higher the 8-OHG levels, the less antibody binds to the plate and hence the lower number. A decrease in number indicates an increase in 8-OHG levels, showing there is more oxidative damage occurring. The addition of TCM, CMN, and AF alone did not affect 8-OHG levels relative to control. Pigs fed CMN and AF also had similar levels to control. The addition of TCM to the AF contaminated diet caused a

reduction in the level of 8-OHG, which signals an increase in oxidative damage in the animal. Reactive oxygen species are a normal product of metabolism in the body, but when the balance gets out of line between ROS and antioxidants it causes more damage than good.

Proper function of the immune system is important for growing pigs. The adaptive immune system provides a specific immune response, which includes the production of antibodies such as immunoglobulin-G (IgG). Immunoglobulin-G was measured in serum, and an increase was seen when pigs were fed CMN alone and when TCM and CMN were added to the AF diet. Weaver *et al.* (2013) also saw an increase in IgG levels of pigs at day 42, after being fed a diet contaminated with 150 µg/kg AF and 1100 µg/kg VOM. Chaytor *et al.* (2010) fed three levels of mycotoxins (a diet with 60 µg of AF/kg and 300 µg of VOM/kg, a diet with 120 µg of AF/kg and 600 µg of VOM/kg, and a diet with 180 µg of AF/kg and 900 µg of VOM/kg) and only observed a change (12%) in IgG values at the highest level of toxins. This increase shows that antibody production was enhanced with the addition of antioxidants. This may be a result of an imbalance of prooxidants to antioxidants in the body, which can negatively affect overall health. Free radicals in excess are damaging but they are necessary for normal cellular function. An imbalance between free radicals and antioxidants can be just as damaging as excessive oxidation.

Mycotoxin Combination and Unike Plus

The adsorbent used in the present study was Unike Plus a commercial product that contains a mixture of additives, including hydrated sodium-calcium alumiosilicate

clay (HSCAS), inactivated yeast and yeast extracts, calcium propionate, an antioxidant mixture, and botanicals. Adding HSCAS to the diet can bind and immobilize toxins in the gastrointestinal tract of animals reducing their toxicity. Studies have shown that clays are effective against mycotoxins due to their binding properties (Weaver *et al.* 2013, Schell *et al.* 1993, Harper *et al.* 2010). The adsorption capacity for any clay is determined by the structure of the clay, surface properties, and exchangeable ions. The addition of clays, such as HSCAS, in the diet has been shown to influence growth, nutrient digestibility and the reproductive performance of swine through slowing the passage of food through the digestive tract, allowing more time for digestion (Subramaniam and Kim, 2015).

Pigs fed UP in the basal diet at 0.50% did not have a decreased performance relative to control, indicating that the adsorbent at this level is safe for use in weanling pigs without affecting performance. The addition of MM to the basal diet decreased performance of pigs, compared to control pigs. Body weight gain of pigs fed MM was 50% lower than gain in control pigs. It was reported that for every mg/kg of AF in the diet there would be a 16% reduction in gain, and a 5% reduction in growth with the addition of 21 mg/kg of FB (Dersjant-Li *et al.* 2003). The study conducted by Dersjant-Li *et al.* (2003) was done on pigs that were older and larger than the pigs used in this study, which would explain why there was a much greater reduction in growth than anticipated. The addition of 0.50% UP to the MM diet did not affect FI of pigs, UP improved weight gain at both 0.25% and 0.50%, but was still below control. The addition of UP at 0.50% numerically improved FI and significantly improved BWG and G:F in pigs

fed MM. These results are similar to a study by Weaver *et al.* (2013) who fed 150 µg/kg AF and 1100 µg/kg deoxynivalenol (VOM) with 2 mg/kg of a clay additive and a dried yeast additive, and saw no improvement in performance compared to AF alone.

There was no effect on relative organ weights with the addition of UP to the basal diet. Pigs fed MM had a relative liver weight similar to control, and when supplemented with 0.25% UP there was no change in organ weight. The addition of 0.50% UP to the diet increased RLW farther, and was greater than pigs fed the basal diet. Relative kidney weight of pigs was not affected by dietary treatments. The combination of AF and FB has been shown to have a greater impact on health than AF or FB alone. A study conducted by Gelderblom *et al.* (2002) on rat livers showed that when rats were treated with AF and FB together there was an increase in severity of liver lesions compared to either toxin alone.

Pigs fed a diet containing MM had reduced total protein, urea nitrogen, albumin, and phosphorus and increased GGT activity. A decrease in serum proteins is characteristic of animals fed AF, and indicated that mycotoxin inclusion has altered protein synthesis (Rosa *et al.* 2001). The addition of UP improved levels of TP, globulin, and Mg in serum and were comparable to control. The addition of UP to the contaminated diet did not improve urea nitrogen, albumin, or GG`T levels. This lack of improvement was in contrast to results of a study by Harper *et al.* (2010) where pigs were fed 0.5 mg/kg AB1 and supplemented with 0.5% HSCAS and an antioxidant preparation, and serum levels improved on all measurements.

Aflatoxin M_1, G_1, and B_1 residues in the liver and kidney were measured. There was no significant reduction in residue levels observed in diets with MM when the adsorbent was added. There was however, a numerical reduction in AFM_1 residues in the liver when pigs were fed MM with the addition of UP at 0.25 or 0.50% of 23 and 48%, respectively. The addition of 0.50% UP reduced AFG_1 residue levels in the liver by 45%, but this reduction was not statistically significant. The addition of UP at 0.50% to the MM diet significantly reduced AFB_1 residue levels in the liver, but values were still greater than control. There was no improvement in AFM_1, G_1, or B_1 residue levels in the kidneys with the addition of UP at either level. These results are similar to what was found in a study by Beaver *et al.* (1990) who fed pigs a diet contaminated with 500 to 600 ng/g AF in combination with 0.50% HSCAS, and saw an improvement in M_1 levels in the liver and kidney, but no improvements in residue levels of AFB_1 or AFB_2.

The addition of UP at 0.50% to the basal diet decreased TNFα relative to control. The addition of MM had similar TNFα levels to UP alone, as did the addition of UP at 0.25% and 0.50% to the MM diet. All values were lower than control, and this decrease shows that there was no immune response with the addition of MM or UP alone or in combination with each other at any level.

Oxidative damage was evaluated used 8-OHG as a marker of oxidative damage. The addition of MM to the basal diet increased 8-OHG levels, indicating sdecreased oxidative damage. When UP was added to the diet at 0.25% or 0.50% 8-OHG levels were returned to control values.

Total antioxidant capacity was decreased in pigs fed MM. This decrease is a result of excess ROS production in pigs fed MM, showing that there was oxidative damage. The addition of UP at 0.25% and 0.50% brought TAC back up to control values, showing that oxidative damage was prevented with the addition of the adsorbent.

Immunoglobulin-G was measured and there was no effect with the addition of UP or MM alone to the diet. When pigs were fed MM with either level of UP, there was an increase in IgG levels. Pigs fed MM plus UP had an increased immune response, similar to results seen in a study by Weaver *et al.* (2001) where IgG was increased in pigs fed 150 µg/kg AF and 1100 µg/kg VOM and decreased by the addition of clay and yeast additives, but values were still above control values.

CONCLUSION

The addition of TCM, CMN at 200 mg/kg and UP at up to 0.50% to the basal diet did not cause any negative effects in any of the measured response variables indicating that at this concentration these adsorbents are safe to feed to pigs of this age, and will not negatively impact performance. The addition of AF to the diet resulted in decreased performance, increased organ weights and liver lesions, reduced TP, Mg, UN, albumin, and phosphorus in serum, increased serum GGT, and increased residues in liver and kidney. The addition of both TCM and CMN was unable to improve performance of pigs fed AF. TCM improved serum levels of P, globulin and TAC, and both antioxidants improved GGT and Mg levels in the blood. TCM lowered AFG_1 levels in the liver, and

AFM_1 levels in the kidney. CMN reduced residue levels of AFB_1 in the kidney, as well as reducing oxidative RNA damage show by 8-OHG levels. The antioxidants cannot prevent absorption of AF, but were hypothesized to reduce the impact of AF on the animals' health. There was minimal improvement shown with either antioxidant.

Compared to controls, addition of a combination of mycotoxins to the basal diet resulted in reduced growth performance, increased organ weights, reduced serum proteins, reduced P, increased GGT, increased liver lesion scores, increased residue in organs, increased 8-OHG, and decreased TNF-α. The addition of MM to the diet decreased TAC, but this was ameliorated by the addition of UP at 0.25 and 0.50%. Addition of UP at 0.50% improved growth, but 0.25% UP did not improve performance compared to MM alone. The addition of UP at 0.25 and 0.50% improved TP and Mg in serum, as well as reducing AFG_1 in the liver. The levels of mycotoxins in the diet may have been too high for the adsorbent to bind and fully reduce the impact of AF and FB. The combination of AF and FB had a greater negative impact on pig health than the addition of AF alone. This shows that the combination of mycotoxins was more toxic than when pigs were fed AF. This makes it even more difficult for the adsorbent to reduce or prevent the effects of AF and FB.

Table 4.1 Efficacy of antioxidants and adsorbents to reduce the toxic effects of aflatoxin or a combination of mycotoxins on feed intake of weanling pigs fed dietary treatments for 26 days[1]

TREATMENT	Feed Intake (kg)			
	Feed Intake 7d	Feed Intake 14d	Feed Intake 21d	Feed Intake 26d
BD	4.43	10.27^{A}	16.82^{A}	23.57^{A}
BD + 200 mg/kg TCM	3.82	8.90^{AB}	16.37^{A}	23.22^{A}
BD + 200 mg/kg CMN	3.99	9.67^{A}	17.28^{A}	23.48^{A}
BD + 1 mg/kg AF	3.37	7.52^{AB}	12.07^{B}	16.14^{B}
BD + AF + TCM	3.24	6.93^{AB}	11.40^{B}	15.38^{B}
BD + AF + CMN	3.50	7.81^{AB}	12.89^{B}	16.33^{B}
BD+ 0.5% UP	4.52	9.69^{A}	16.78^{A}	22.57^{A}
BD + MM	3.34	7.03^{B}	11.48^{B}	13.91^{B}
BD + MM + 0.25% UP	3.22	7.21^{B}	11.78^{B}	15.59^{B}
BD + MM + 0.5% UP	3.60	7.94^{A}	11.84^{B}	16.41^{B}
ANOVA SEM:	0.31	0.54	0.72	1.11
ANOVA P-Value:	0.24	<0.0001	<0.0001	<0.0001

[1]Data are means of six replicate pens of one pig each.
[2]Mixed mycotoxins = Aflatoxin (1 mg/kg); Fumonisin B_1 (25 mg/kg)
[a-d] Means within columns with no common superscripts are significantly different ($P < 0.05$)

Table 4.2 Efficacy of antioxidants and adsorbents to reduce the toxic effects of aflatoxin or a combination of mycotoxins on body weight gain of weanling pigs fed dietary treatments for 26 days[1]

		Body Weight Gain (kg)			
TREATMENT		Weight Gain 7d	Weight Gain 14d	Weight Gain 21d	Weight Gain 26d
BD		3.24^{ab}	7.28^{a}	13.84^{a}	16.31^{a}
BD + 200 mg/kg TCM		2.83^{bc}	6.00^{bc}	12.48^{a}	15.41^{a}
BD + 200 mg/kg CMN		3.10^{ab}	6.92^{ab}	13.03^{a}	16.49^{a}
BD + 1 mg/kg AF		2.23^{d}	4.79^{de}	8.79^{b}	10.68^{b}
BD + AF + TCM		2.17^{d}	4.42^{e}	7.45^{bc}	9.6^{bc}
BD + AF + CMN		2.27^{d}	4.88^{de}	7.21^{bcd}	9.5^{bc}
BD+ 0.5% UP		3.38^{a}	6.97^{ab}	11.6^{a}	16.18^{a}
BD + MM		2.32^{cd}	4.40^{e}	5.66^{cd}	8.01^{c}
BD + MM + 0.25% UP		1.92^{d}	4.23^{e}	4.95^{d}	9.15^{bc}
BD + MM + 0.5% UP		2.44^{cd}	5.61^{cd}	7.12^{bcd}	10.98^{b}
ANOVA	SEM:	0.179	0.349	0.798	0.747
	P-Value:	<0.0001	<0.0001	<0.0001	<0.0001

[1]Data are means of six replicate pens of one pig each.
[2]Mixed mycotoxins = Aflatoxin (1 mg/kg); Fumonisin B_1 (25 mg/kg)
[a-e] Means within columns with no common superscripts are significantly different ($P < 0.05$)

Table 4.3 Efficacy of antioxidants and adsorbents to reduce the toxic effects of aflatoxin or a combination of mycotoxins on feed efficiency of weanling pigs fed dietary treatments for 26 days[1]

		Gain:Feed (kg:kg)			
TREATMENT		Gain:Feed 7d	Gain:Feed 14d	Gain:Feed 21d	Gain:Feed 26d
BD		0.75	0.72^{A}	0.74^{A}	0.70^{AB}
BD + 200 mg/kg TCM		0.71	0.67^{AB}	0.70^{ABC}	0.67^{ABC}
BD + 200 mg/kg CMN		0.78	0.72^{A}	0.71^{AB}	0.70^{A}
BD + 1 mg/kg AF		0.67	0.64^{AB}	0.66^{ABCD}	0.67^{ABC}
BD + AF + TCM		0.67	0.64^{AB}	0.63^{CDE}	0.62^{ABCD}
BD + AF + CMN		0.63	0.65^{AB}	0.60^{DEF}	0.59^{BCD}
BD+ 0.5% UP		0.68	0.72^{A}	0.70^{ABC}	0.72^{A}
BD + MM		0.57	0.58^{B}	0.52^{F}	0.55^{D}
BD + MM + 0.25% UP		0.60	0.59^{B}	0.59^{EF}	0.58^{CD}
BD + MM + 0.5% UP		0.70	0.73^{A}	0.64^{BCDE}	0.67^{ABC}
ANOVA	SEM:	0.04	0.04	0.03	0.04
	P-Value:	0.08	0.05	<0.0001	0.03

[1]Data are means of six replicate pens of one pig each.
[2]Mixed mycotoxins = Aflatoxin (1 mg/kg); Fumonisin B_1 (25 mg/kg)
[a-f] Means within columns with no common superscripts are significantly different ($P < 0.05$)

Table 4.4 Efficacy of antioxidants and adsorbents to reduce the toxic effects of aflatoxin or a combination of mycotoxins on relative organ weights of weanling pigs fed dietary treatments for 26 days[1]

Treatments		Relative Liver Weight (%)	Relative Kidney Weight (%)
BD		2.47^{D}	0.53
BD + 200 mg/kg TCM		2.51^{D}	0.55
BD + 200 mg/kg CMN		2.47^{D}	0.54
BD + 1 mg/kg AF		2.95^{ABC}	0.57
BD + AF + TCM		3.10^{AB}	0.57
BD + AF + CMN		3.26^{A}	0.58
BD+ 0.5% UP		2.64^{CD}	0.54
BD + MM		2.64^{CD}	0.58
BD + MM + 0.25% UP		2.80^{BCD}	0.63
BD + MM + 0.5% UP		2.94^{ABC}	0.48
ANOVA	SEM:	0.12	0.04
	P-Value:	<0.0001	0.63

[1]Data are means of six replicate pens of one pig each.
[2]Mixed mycotoxins = Aflatoxin (1 mg/kg); Fumonisin B_1 (25 mg/kg)
[a-d] Means within columns with no common superscripts are significantly different ($P < 0.05$)

Table 4.5 Efficacy of antioxidants and adsorbents to reduce the toxic effects of aflatoxin or a combination of mycotoxins on serum of weanling pigs fed dietary treatments for 26 days[1]

Treatments		Urea Nitrogen (mg/dL)	Albumin (g/dL)	Phosphorus (mg/dL)	GGT (U/L)
BD		15.33^{A}	4.03^{A}	11.62^{A}	44.83^{DEF}
BD + 200 mg/kg TCM		15.5^{A}	3.61^{AB}	9.30^{BC}	36.83^{EF}
BD + 200 mg/kg CMN		14.33^{AB}	3.76^{A}	9.49^{BC}	39.00^{EF}
BD + 1 mg/kg AF		10.33^{C}	3.10^{BC}	9.88^{BC}	65.17^{CD}
BD + AF + TCM		10.67^{C}	3.09^{BC}	10.37^{AB}	57.17^{DE}
BD + AF + CMN		11.83^{BC}	2.74^{C}	8.53^{C}	64.17^{CD}
BD+ 0.5% UP		15.50^{A}	3.56^{AB}	10.49^{AB}	28.33^{F}
BD + MM		9.17^{C}	2.97^{C}	8.91^{BC}	113.83^{A}
BD + MM + 0.25% UP		9.33^{C}	2.92^{C}	9.68^{BC}	108.33^{AB}
BD + MM + 0.5% UP		11.67^{BC}	3.20^{BC}	9.99^{ABC}	88.33^{BC}
ANOVA	S.E.M.:	1.03	0.19	0.59	8.71
	P-value:	<0.0001	<0.0001	0.03	<0.0001

[1]Data are means of six replicate pens of one pig each.

[2]Mixed mycotoxins = Aflatoxin (1 mg/kg); Fumonisin B_1 (25 mg/kg)

[a-f] Means within columns with no common superscripts are significantly different ($P < 0.05$)

Table 4.6 Efficacy of antioxidants and adsorbents to reduce the toxic effects of aflatoxin or a combination of mycotoxins on serum of weanling pigs fed dietary treatments for 26 days[1]

Treatments	Total Protein (g/dL)	Globulin (g/dL)	Calcium (mg/dL)	Magnesium (mg/dL)
BD	5.87^{A}	1.85^{CDE}	12.18	3.15^{A}
BD + 200 mg/kg TCM	5.43^{AB}	1.82^{DE}	11.58	2.51^{B}
BD + 200 mg/kg CMN	5.47^{AB}	1.70^{E}	11.38	2.53^{B}
BD + 1 mg/kg AF	5.27^{B}	2.18^{BC}	11.55	2.42^{B}
BD + AF + TCM	5.27^{B}	2.18^{BC}	11.28	2.78^{AB}
BD + AF + CMN	5.36^{B}	2.38^{B}	11.96	2.67^{AB}
BD+ 0.5% UP	5.72^{AB}	2.15^{BCD}	11.80	2.70^{AB}
BD + MM	5.33^{B}	2.37^{B}	11.37	3.13^{A}
BD + MM + 0.25% UP	5.70^{AB}	2.80^{A}	11.73	2.70^{AB}
BD + MM + 0.5% UP	5.73^{AB}	2.48^{AB}	12.28	2.89^{AB}
ANOVA S.E.M.:	0.17	0.12	0.36	0.17
P-value:	0.15	<0.0001	0.51	0.04

[1]Data are means of six replicate pens of one pig each.

[2]Mixed mycotoxins = Aflatoxin (1 mg/kg); Fumonisin B_1 (25 mg/kg)

[a-d] Means within columns with no common superscripts are significantly different ($P < 0.05$)

Table 4.7 Efficacy of antioxidants and adsorbents to reduce the toxic effects of aflatoxin or a combination of mycotoxins on AF residues in the liver of weanling pigs fed dietary treatments for 26 days[1]

Aflatoxin Residue in Liver				
Treatments		**AFM_1** (ppb)	**AFG_1** (ppb)	**AFB_1** (ppb)
BD		0.00^{D}	0.00^{B}	0.00^{C}
BD + 200 mg/kg TCM		0.00^{D}	0.00^{B}	0.00^{C}
BD + 200 mg/kg CMN		0.00^{D}	0.00^{B}	0.00^{C}
BD + 1 mg/kg AF		2.05^{A}	0.26^{B}	2.81^{AB}
BD + AF + TCM		0.88^{BC}	0.39^{AB}	2.39^{AB}
BD + AF + CMN		1.54^{AB}	0.71^{A}	2.23^{B}
BD+ 0.5% UP		0.00^{D}	0.00^{B}	0.00^{C}
BD + MM		1.44^{ABC}	0.24^{B}	3.55^{A}
BD + MM + 0.25% UP		1.11^{BC}	0.39^{AB}	2.26^{AB}
BD + MM + 0.5% UP		0.75^{C}	0.13^{B}	1.59^{B}
ANOVA	SEM:	0.26	0.15	0.45
	P-Value:	<0.0001	0.02	<0.0001

[1]Data are means of six replicate pens of one pig each.
[2]Mixed mycotoxins = Aflatoxin (1 mg/kg); Fumonisin B_1 (25 mg/kg)
[a-d] Means within columns with no common superscripts are significantly different ($P < 0.05$)

Table 4.8 Efficacy of antioxidants and adsorbents to reduce the toxic effects of aflatoxin or a combination of mycotoxins on AF residues in the kidney of weanling pigs fed dietary treatments for 26 days[1]

Aflatoxin Residue in Kidney				
Treatments		**AFM_1** (ppb)	**AFG_1** (ppb)	**AFB_1** (ppb)
BD		0.00^{D}	0.00^{C}	0.00^{C}
BD + 200 mg/kg TCM		0.00^{D}	0.00^{C}	0.00^{C}
BD + 200 mg/kg CMN		0.00^{D}	0.00^{C}	0.00^{C}
BD + 1 mg/kg AF		1.08^{BC}	0.06^{BC}	1.00^{B}
BD + AF + TCM		0.64^{CD}	0.13^{AB}	0.98^{B}
BD + AF + CMN		1.27^{BC}	0.10^{AB}	0.92^{BC}
BD+ 0.5% UP		0.00^{D}	0.00^{C}	0.00^{C}
BD + MM		1.16^{BC}	0.11^{AB}	2.15^{A}
BD + MM + 0.25% UP		1.79^{B}	0.12^{AB}	2.62^{A}
BD + MM + 0.5% UP		3.27^{a}	0.17^{A}	1.66^{AB}
ANOVA	SEM:	0.26	0.15	0.45
	P-Value:	<0.0001	0.02	<0.0001

[1]Data are means of six replicate pens of one pig each.
[2]Mixed mycotoxins = Aflatoxin (1 mg/kg); Fumonisin B_1 (25 mg/kg)
[a-d] Means within columns with no common superscripts are significantly different ($P < 0.05$)

Table 4.9 Efficacy of antioxidants and adsorbents to reduce the toxic effects of aflatoxin or a combination of mycotoxins on ELISAs of weanling pigs fed dietary treatments for 26 days[1]

Treatments		Tumor Necrosis Factor-α (pg/mL)	8-OHG (ng/mL)	Immunoglobulin-G (ng/mL)	Total Antioxidant Capacity (mM)
BD		5.56^{A}	1.65^{BC}	36^{D}	0.016^{A}
BD + 200 mg/kg TCM		5.52^{AB}	0.98^{CD}	38^{D}	0.015^{A}
BD + 200 mg/kg CMN		3.94^{ABCD}	0.93^{CD}	112^{AB}	0.017^{A}
BD + 1 mg/kg AF		4.84^{ABC}	1.40^{BCD}	67^{BCD}	0.017^{A}
BD + AF + TCM		2.85^{D}	0.39^{D}	131^{A}	0.009^{B}
BD + AF + CMN		4.59^{ABC}	1.05^{CD}	147^{A}	0.016^{A}
BD+ 0.5% UP		2.54^{D}	1.33^{BCD}	69^{BCD}	0.012^{AB}
BD + MM		3.59^{CD}	3.30^{A}	68^{BCD}	0.009^{B}
BD + MM + 0.25% UP		3.93^{BCD}	1.65^{BC}	110^{ABC}	0.017^{A}
BD + MM + 0.5% UP		3.56^{CD}	2.22^{B}	118^{A}	0.016^{A}
ANOVA	S.E.M.:	0.45	0.30	9.81	0.001
	P-value:	0.005	0.0004	<0.0001	0.01

[1]Data are means of six replicate pens of one pig each.

[2]Mixed mycotoxins = Aflatoxin (1 mg/kg); Fumonisin B_1 (25 mg/kg)

[a-d] Means within columns with no common superscripts are significantly different ($P < 0.05$)

Table 4.10 Efficacy of antioxidants and adsorbents to reduce the toxic effects of aflatoxin or a combination of mycotoxins on peroxidation in the liver of weanling pigs fed dietary treatments for 26 days[1]

Treatments		Lipid Peroxide (nm)	Aqueous Peroxide (µmol/L)	Liver Scores
BD		2.03	244	0.00^{E}
BD + 200 mg/kg TCM		2.21	241	0.00^{E}
BD + 200 mg/kg CMN		2.20	235	0.00^{E}
BD + 1 mg/kg AF		1.78	255	2.00^{D}
BD + AF + TCM		2.08	275	2.00^{D}
BD + AF + CMN		2.19	282	2.67^{BC}
BD+ 0.5% UP		2.13	224	0.00^{E}
BD + MM		2.29	252	3.50^{A}
BD + MM + 0.25% UP		1.30	225	3.17^{AB}
BD + MM + 0.5% UP		1.28	215	2.50^{CD}
ANOVA	S.E.M.:	0.20	9.81	0.08
	P-value:	0.13	0.10	<0.0001

[1]Data are means of six replicate pens of one pig each.
[2]Mixed mycotoxins = Aflatoxin (1 mg/kg); Fumonisin B_1 (25 mg/kg)
[a-d] Means within columns with no common superscripts are significantly different ($P < 0.05$)

Table 4.11 Efficacy of antioxidants and adsorbents to reduce the toxic effects of aflatoxin or a combination of mycotoxins on liver lesion scores of weanling pigs fed dietary treatments for 26 days[1]

0	Normal
1	Mild hepatocellular degeneration
2	Moderate to severe hepatocellular degeneration with severe vacuolation in Zone 1 (periportal area). Occasional apoptoses
3	Severe hepatocellular degeneration and cellular loss (small hepatic lobules). Severe vacuolation in Zone 1 and Zone 2 (mid-zone), common megalocytosis and megalokaryosis, occasional apoptoses and mitoses
4	Hepatic fibrosis with severe hepatocellular degeneration and necrosis, biliary hyperplasia, and inflammation.

	1	**2**	**3**	**4**	**5**	**6**	**Mean**
A	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0
D	3	1	2	2	3	1	2
E	3	1	2	2	2	2	2
F	2	3	2	3	3	3	2.67
G	0	0	0	0	0	0	0
H	3	4	3	4	3	4	3.50
I	3	4	3	3	3	3	3.17*
J	2	3	3	3	3	1	2.50**

* p=0.17 (compared to Group H)
* p=0.08 (compared to Group H)

Table 4.11 (continued)

After the necropsy, sections of the liver from each animal were fixed in 10% neutral buffered formalin. Fixed tissues from 60 pigs were trimmed, processed by routine tissue processing procedure, and embedded in paraffin. Tissues were sectioned by 5 um, placed on glass sides, and stained with haematoxylin and eosin.

Liver: The livers of the pigs from Group A, B, C, and G are normal and have no significant microscopic abnormalities. Yet, the livers of the pigs from Group D, E, F, H, I, and J have different degree of pathologic changes from mild to moderate hepatocellular degeneration to severe hepatic lobular collapse with cell necrosis and fibrosis. The most common changes are severe vacuolar degeneration, often in the periportal (Zone one) and sometimes in the mid-zone (Zone two). Each liver is scored separately.

The hepatic lesions of Group D, E, and F are not significantly different.

Although the hepatic lesions of Group I and J are not statistically significantly different from those of Group H, Group J lesions appear better than Group H ($p = 0.08$).

CHAPTER V

SUMMARY AND OVERALL CONCLUSIONS

Mycotoxins are a concern for producers, especially in non-ruminant production, because grains can be easily contaminated by fungi that produce mycotoxins. These toxins can have several negative effects on animal performance, including decreased performance, feed refusal, immune suppression, reproductive disorders, and residues in animal products. These effects are impacted by a number of factors including mycotoxin intake level, species, and duration of exposure.

Adsorbents, such as hydrated sodium calcium aluminosilicates (HSCAS) or clays, have been shown to bind aflatoxin (AF) in the gut, thus preventing or reducing absorption. The prevention/reduction in absorption of AF by adsorbents reduces the carcinogenic effects of AF and also the negative impact on animal health. Adsorbents such as HSCAS and other clays have been shown to only work on AF, but when paired with other additives including inactivated yeast cells, antioxidants, and calcium propionate, such as in Unike Plus, the adsorbents may have the ability to reduce the effects of other mycotoxins in the diet as well.

In the present chick study, use of bentonite clay (concentrated or raw) was successful in reducing the effects of AF and improving overall performance in broiler chicks. The use of a combination of clay and other additives (Unike Plus) was able to

reduce the effects of a combination of mycotoxins in broiler chicks when fed at 0.50% of the diet.

Curcuminoids are compounds present in Turmeric (*Curcuma longa*) powder have been shown to be potent antioxidants, and have also been shown to inhibit biotransformation of AFB_1 to its carcinogenic epoxide (AFB_1-8-9-epoxide). In the present pig study, 200 mg/kg curcuminoids supplied by theracurmin and curcumin was added to diets of weanling pigs to determine their efficacy in preventing or reducing the negative effects of AF. Both sources of curcuminoids have previously been shown to protect against the negative effects of AF in poultry.

In the current pig study, there were no improvements in performance of weanling pigs fed AF with the addition of either theracurmin or curcumin. There were some improvements in serum chemistry, as well as reductions kidney and liver AF residues. There also appeared to be a reduction in oxidative damage caused by AF. The levels of AF fed may have been too high for the antioxidants to improve performance.

In the current study with pigs, addition of a combination of mycotoxins to the basal diet resulted in a larger negative impact on both performance and health than when pigs were fed AF alone. Addition of UP to the MM diet, at 0.50% improved growth, but 0.25% UP did not improve performance compared to MM alone. The addition of UP at 0.25 and 0.50% improved serum chemistry and reduced AF residues in the liver and kidney, though not all improvements were significant. The combination of mycotoxins in the diet may have been too high for the adsorbent to bind and fully reduce the impact of AF and FB. The combination of AF and FB had a greater negative

impact on pig health than the addition of AF alone. This makes it even more difficult for the adsorbent to reduce or prevent the effects of AF and FB.

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