EFFECT OF MYCOTOXIN BINDERS ON GROWTH AND METABOLIC INDICATORS IN PIGS AND DUCKS FED MYCOTOXIN-CONTAMINATED DIETS

by

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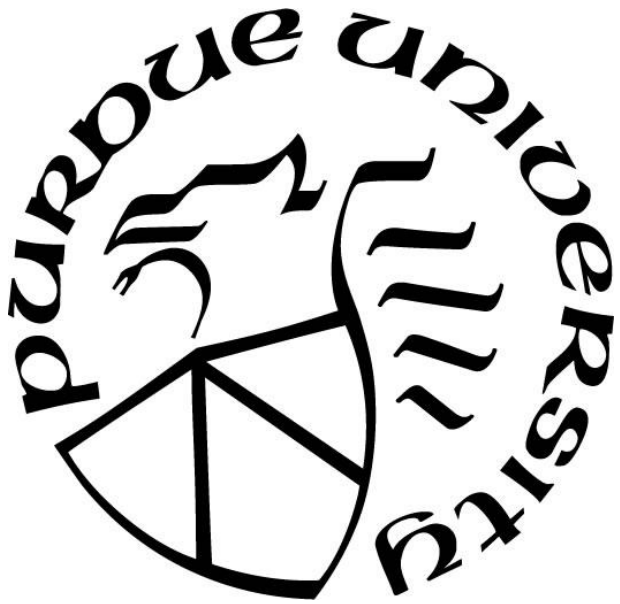

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Dedicated to my family and friends, without whose support I could do nothing.

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LIST OF ABBREVIATIONS

Abbreviation – Description

ADG – average daily gain

ALT – alanine aminotransferase

AST – aspartate aminotransferase

BCA – bicinchoninic acid assay

BW – body weight

CD14 – cluster of differentiation 14

CNS – central nervous system

DD – death domain

DNA – deoxyribonucleic acid

DON – deoxynivalenol

ELISA – enzyme-linked immunosorbent assay

ENS – enteric nervous system

FAO – Food and Agriculture Organization

FI – feed intake

G:F – gain:feed

GI - gastrointestinal

GLUT – glucose transporter

IFN – interferon

IgA – immunoglobulin A

IKK – IκB kinase

IL – interleukin

IRAK – IL-1 receptor associated kinases

IRF – interferon regulatory factor

IκB – inhibitor of κB

LDH – lactate dehydrogenase

LPS – lipopolysaccharide

ME – metabolizable enery

MyD88 – myeloid differentiation primary response 88

NADH – nicotinamide adenine dinucleotide

NC – negative control

NEMO – NF-κB essential modulator

NF- κB – nuclear factor κ-light-chain-enhancer of activated B cells

PACUC – Purdue University Animal Care and Use Committee

PAMP – Pathogen-associated molecular patterns

PC – positive control

PCV2 – porcine cirovirus type 2

PRR – pattern-recognition receptors

PRRSV – porcine reproductive and respiratory syndrome

RNA – ribonucleic acid

ROS – reactive oxygen species

SGLT-1 sodium-glucose transport

TAB – TGF-β activated kinase 1-binding protein

TAK1 – transforming growth factor-β-activated kinase 1TBK1 – TANK-binding kinase 1

TEER – trans epithelial electrical resistance

TG – triglycerides

TIRAP – Toll/IL-1 receptor associated protein

TLR – toll-like receptor

TNFα – tumor necrosis factor alpha

TRAF – TNF receptor-associated factor

TRAM – translocation associated membrane protein

TRIF – TIR-domain-containing adapter-inducing interferon-β

ZO-1 – zona occludens 1

ABSTRACT

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Title: Effect of Mycotoxin Binders on Growth and Metabolic Indicators in Pigs and Ducks Fed Mycotoxin-contaminated Diets
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Mycotoxins are feed contaminants that are a major problem in the livestock industry because of their prevalence in feedstuffs and the difficulty of removing them. They can cause a wide range of issues at varying levels of exposure. Each species is affected by different mycotoxins and at different levels. Pigs are more susceptible to deoxynivalenol (DON), whereas ducks are more susceptible to aflatoxin.

Effects of mycotoxin contamination on animal performance are not fully understood. Therefore, the two experiments described in this thesis were conducted to determine the response of pigs and ducks to consumption of feed contaminated with DON and aflatoxin, respectively. In the first experiment, the effect of a mycotoxin binder on duck feeds contaminated with aflatoxin was examined. One-day-old male Pekin ducks (n=360) were randomly divided into four groups; each group had 6 replicate pens with 15 ducks per replicate pen. The positive control (PC) group was fed a diet that was free of aflatoxin B_1, the negative control (NC) group was fed a diet that contained >75ppb of aflatoxin without a binder, the negative control with low binder (NC + 0.5) group was fed a diet that contained >75ppb of aflatoxin and 0.5 kg/ton of the binder, the negative control with high binder (NC + 1.0) group was fed a diet that contained >75ppb of aflatoxin and 1.0 kg/ton of the binder. The diets were fed in two phases, days 0-14 (phase 1) and 15-35 (phase 2). The results showed that during early phase 2, NC + 0.5 resulted in a higher rate of weight gain compared to NC ($P<0.05$); 2) NC + 0.5 ducks had higher feather quality than both NC and PC ($P<0.05$); 3) NC had higher relative liver weights ($P<0.05$); 4) blood glucose was higher in NC + 0.5 ducks ($P<0.05$); and 5) PC ducks had higher serum protein levels in the blood ($P<0.05$).

In the second study, effect of the same mycotoxin binder, used in the duck study, was examined in pigs fed diets contaminated with DON. A total of 128 pigs (Duroc × Landrace ×

Yorkshire, (1:1 barrows and gilts, aged 42 d) were randomly assigned to 4 treatments, 8 replicate pens with 4 pigs per. The treatments were DON, DON + liver protectant (1 kg/ton), DON + mycotoxin binder (0.5 kg/ton), or DON + liver protectant and mycotoxin binder. The study lasted 28 days and body weights (BW), feed intake (FI), and blood samples were taken on days 14 and 28. Body weights and feed intake were taken and used to calculate gain:feed (G:F). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured in the blood serum. BW, FI, and G:F were not significantly different at any point during the study. AST levels were significantly reduced ($P < 0.05$) on day 14 in pigs fed the liver protectant but were not significantly different day 28.

In summary, effects of the use of mycotoxin binders in feed can be highly variable. This depends on the type of mycotoxin present in the feed, the amount of mycotoxin, and the species fed the diet. In the present study, the mycotoxin binder did not have an impact on the feed efficiency of the ducks or pigs. Effects of additional binders need to be evaluated for their effectiveness in mitigating the negative effects of mycotoxins.

CHAPTER 1. LITERATURE REVIEW

1.1 Mycotoxins

Mycotoxins are secondary metabolites produced by various fungi when they are stressed. The mycotoxins are not necessary for the survival of the fungus. Even if the fungus is removed, the mycotoxins that it produced can still cause disease (Marin et al., 2013). There are nearly 400 different types of mycotoxins (Bezerra da Rocha et al., 2014). Mycotoxins are a big issue in food supplies around the world. Estimates have put mycotoxin contamination occurring in nearly 25% of all grains produced each year throughout the world (Chang, et al., 2016). In 2012, mycotoxins were the main cause of goods being rejected at the border in the European Union according to Rapid Alert System for Food and Feed (Marin et al., 2013). Mycotoxins can affect foods besides cereal grains. They have been found in nuts and seeds, fruits and vegetables, and spices (Bouhet and Oswald, 2005). The main groups of mycotoxins are the aflatoxins produced by *Aspergillus*, ochratoxin A produced by *Aspergillus* and *Penicillium*, tricothecenes, zearalenone, and fumonisins produced by *Fusarium*, and ergot alkaloids produced by *Claviceps* (Marin et al., 2013).

1.1.1 Aflatoxin

Aflatoxins are one of the most toxic mycotoxins known to exist (Chang et al., 2016). Aflatoxins are difuranocoumarins and are mainly produced by two different strains of *Aspergillus*. *Aspergillus flavus* produces B aflatoxins which are known to attack the leaves and flowers of plants. *Aspergillus parasiticus* produces both B and G aflatoxins (B=blue and G=green under ultraviolet light) and is generally found in the soil around plants. Aflatoxins accumulates mostly in hot, humid climates (Marin et al., 2013). Contamination of foodstuffs

with aflatoxins can occur at any point in the processing from growing to storage. Aflatoxins can accumulate in cereal grains with a moisture content of 18% and nuts/seeds with a moisture content of 9-10%. Aspergillus spp can grow in temperatures ranging from 12-42 °C (Dalvi, 1986).

Aflatoxins can have multiple negative impacts on the body when consumed. When consumed, aflatoxin attacks the liver of the host and changes the functions of enzymes located there (Han et al., 2008). Acute exposure to aflatoxins can lead to vomiting, depression in feed intake, diarrhea, and abdominal pain. Chronic exposure can lead to weight loss, liver cancer, decreased sperm levels, and an increase in secondary infections (Marin et al., 2013). Weight loss is caused by aflatoxin's ability to attach to DNA and disrupt protein synthesis (Bezerra da Rocha et al., 2014). Aflatoxins can increase the amount of leptin in the blood which helps to drive down feed intake (Chang et al., 2016). The structure of the intestine is changed when animals are fed aflatoxins because of the reduction in protein synthesis. Intestinal cells are constantly replicating to replace older cells, so they need plenty of protein. When there are insufficient proteins, the villi start to decrease in height and reduce absorption of nutrients. Aflatoxins can also reduce intestinal motility, causing increase in rate of gastric emptying (He et al., 2013). Ducks are the most susceptible to aflatoxin and they can lose 169 g of body weight for every 100 ppb increase in aflatoxin concentration in the feed (Chen et al., 2015).

Aflatoxins can also cause liver damage. This can be seen in many ways. Factor II and factor VII production is blocked by aflatoxins, causing a decrease in blood coagulation (Dalvi, 1986). Alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) activities are all increased in the blood serum. Total cholesterol and

triglyceride (TG) levels are decreased in the blood serum. Together, they show signs of liver damage and necrosis occurring after exposure to aflatoxins (He et al., 2013).

Secondary infections arise because aflatoxins are a strong suppressant of both innate and acquired immunity. Aflatoxins can disrupt the antigen-presenting capabilities of dendritic cells (Pierron et al., 2016). Low doses of aflatoxins can lead to a decrease in pro-inflammatory and increase in anti-inflammatory cytokines in weanling piglets. If aflatoxin is fed to pregnant sows, the piglets will have altered macrophage and neutrophil function as well as impaired lymphocyte activation (Pierron et al., 2016).

1.1.2 Fumonisin

Fumonisins are generally found in corn with high moisture content. Removing them from feed can be difficult because they are stable up to 150 °C. Fumonisins have a similar structure to sphinganine which is a precursor of sphingolipid. Shingolipids are found in the cell membrane. Fumonisins compete with sphinganine and blocks acylation of sphinganine. Therefore, reducing the levels of shingolipids which compromises the membrane integrity (Marin et al., 2013). Fumonisin B_1 is the most toxic of the fumonisins and is produced by *Fusarium verticillioides* and *Fusarium proliferatum* (Oswald et al., 2003).

The main targets of fumonisins are the liver, kidney, and intestine. High levels of fumonisin must be consumed to have an acute effect, so chronic exposure is the main concern. Chronic exposure can lead to decreases in feed intake, weakness, and death (Marin et al., 2013). In pigs, fumonisins can lead to pulmonary edema and hydrothorax (Bezerra da Rocha et al., 2014). In the intestine, fumonisin B_1 can decrease epithelial cell proliferation by arresting cells in the G0/G1 phase (Bouhet et al., 2004) and cause hyperplasia in goblet cells. This can lead to an increase in diarrhea (Bouhet and Oswald, 2005). Fumonisin B_1 can also decrease

transepithelial electrical resistance (TEER) in the intestine and increase the chances for infection. After a week of Fumonisin B_1 exposure, the intestinal colonization of pathogenic *E. coli* is increased (Bouhet et al., 2004). This increase in pathogenic *E. coli* and decrease in TEER is coupled with fumonisin's ability to affect the cytokine balance between T_H1 and T_H2 cells. T cell stimulation and MHC-II presenting abilities are also decreased. Combined these can increase the likelihood of an animal getting an infection in the gut (Pierron et al., 2016).

1.1.3 Trichothecenes

Trichothecenes are made up of Type A, HT-2 toxin and T-2 toxin, and Type B Deoxynivalenol. HT-2 and T-2 are produced by *Fusarium sporotrichioides*, *Fusarium langsethiae*, *Fusarium acuminatum*, and *Fusarium poae*. Deoxynivalenol is produced by *Fusarium graminearum*, *Fusarium culmorum*, and *Fusarium cerealis*. All trichothecenes contain a common tetracyclic sesquiterpenoid 12,13-epoxtrichothec-9-ene ring system. They can be found in the soil and growing on plants. They are very stable and almost impossible to remove from feed once contaminated. HT-2 and T-2 are generally found in oats and barley, while deoxynivalenol is found in corn and wheat (Marin et al., 2013).

1.1.4 Deoxynivalenol

Deoxynivalenol is the most common mycotoxin found in grains (Bezerra da Rocha et al., 2014). Deoxynivalenol can be toxic at very low levels. Just 1 ppm can lead to a drop in feed intake and 20 ppm can cause vomiting (Diesing et al., 2011). Deoxynivalenol exposure can cause weight loss through vomiting, diarrhea, and malabsorption of nutrients in the intestine (Bouhet and Oswald, 2005). Deoxynivalenol can also block protein synthesis and reduce peptidyl transferase activity (Bezerra da Rocha et al., 2014). Deoxynivalenol can increase the

size of the liver, decrease the size of the thyroid, and increase serotonin levels (Marin et al., 2013).

Pigs are the most susceptible to deoxynivalenol and it is rapidly absorbed in the intestine. Deoxynivalenol can decrease the levels of glucose transporters (GLUT), sodium-glucose transport 1 (SGLT-1), and amino acid transporters in the membranes of intestinal epithelial cells (Diesing et al., 2011). Deoxynivalenol can cause lipid peroxidation (Pacheco et al., 2012). Deoxynivalenol also causes the production of reactive oxygen species (Olegario da Silva et al., 2014). Phytic acid can help block lipid peroxidation (Pacheco et al., 2012) and reactive oxygen species production caused by deoxynivalenol (Olegario da Silva et al., 2014). Phytic acid is found in cereal grains and can decrease the formation of kidney stones, serum lipid levels, platelet clumping, cardiovascular diseases, and protect again neurodegenerative diseases (Olegario da Silva et al., 2014). Chronic deoxynivalenol exposure can lead to a suppressed immunity. It can disrupt leukocyte signaling and increase pro-inflammatory cytokine and immunoglobulin A (IgA) production. Deoxynivalenol can also increase the incidence of porcine circovirus type 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) infections. Deoxynivalenol increases pro-inflammatory cytokine production during bacterial infection (Pierron et al., 2016). Deoxynivalenol causes an increase in serum IgA levels (Bouhet and Oswald, 2005).

1.1.5 T-2

T-2 has multiple effects on the body including protein synthesis inhibition, immunity suppression, and changes to gut structure. T-2 blocks protein synthesis by blocking DNA and RNA synthesis. This prevents any new proteins from being made. Chronic exposure can lead to several complications including: diarrhea, vomiting, fever, destruction of bone marrow,

pneumonia, and death (Marin et al., 2013). T-2 causes gut structure to change by killing crypt cells in the jejunum and ileum (Bouhet and Oswald, 2005).

1.1.6 Ochratoxin A

Ochratoxin A is usually found in cacao, wine, rye, barley, wheat, spices, and dried fruits. The main targets of ochratoxin A are the kidney and immune system. It has a structure similar to phenylalanine and can inhibit protein synthesis by competitively binding with phenylalanine. It can survive most food preparation steps and is produced by members of the *Aspergillus* and *Penicillium* genera (Marin et al., 2013).

Ochratoxin A can interfere with mitochondria, causing damage, lipid peroxidation, inhibition of oxidative phosphorylation, and an oxidative burst. This can increase the chance of the cell undergoing apoptosis (Marin et al., 2013). Ochratoxin A causes a decrease transepithelial electrical resistance (TEER) by causing villus atrophy and decreasing the levels of claudin proteins (Bouhet and Oswald, 2005). Ochratoxin A can suppress the immune system leading to a decrease in cytokine response to lipopolysaccharide (LPS) and an increase in other infections like salmonella (Pierron et al., 2016). Ochratoxin A also reduces the size of the spleen, thymus, and lymph nodes and has been known to cross the placenta and cause damage to the fetus (Marin et al., 2013).

1.1.7 Zearalenone

Zearalenone has a structure similar to estrogen and can be found in corn, wheat bran, and vegetable oil. It is stable up to 150 °C and produced by *Fusarium graminearum*. Its main effects are on the endocrine and reproductive systems, due to its ability to competitively bind estrogen receptors. It can decrease embryo survival, lower testosterone in the male, and decrease milk production in the female (Marin et al., 2013). It can also lead to issues with conception and

increase the risk of abortion (Bezerra da Rocha et al., 2014). Zearalenone also increases the risk of prolapse of the vulva and damage to the follicles on the ovary (Pierron et al., 2016). Zearalenone can lead to oxidative stress in cells, which leads to oxidative damage to the DNA and eventually cell death (Gerez et al., 2015).

1.1.8 Patulin

Patulin is commonly found in fruits that have been damaged by handling and transport. It is produced by several different genera of fungi. Acutely, patulin can cause nausea and ulcers in the intestine. During chronic exposure, patulin can alter the intestinal barrier and slow the immune response. Patulin can alter macrophages and increase the number of T lymphocytes in the spleen helping to alter the immune response. Patulin has a strong affinity for sulfhydryl groups and can inhibit enzymes (Marin et al., 2013).

1.1.9 Ergot Alkaloids

Ergot alkaloids are one of the best-known mycotoxins and has been known since the Middle Ages. In the Middle Ages, it was referred to as "Holy Fire" or "St. Anthony's Fire". In 994, the south of France had a large infection of *Claviceps purpurea* in cereal grains leading to the death of several thousand people. Ergot alkaloids are usually found in rye breads but are often killed by the bread making process (Bezerra da Rocha et al., 2014). Ergot alkaloids are readily absorbed in the gastrointestinal tract and can act on neurotransmitter receptors (Marin et al., 2013). Ergot alkaloids usually affect the body in two ways: gangrene or convulsion. Gangrenous ergot alkaloids reduce the blood flow to the extremities causing cellular death. Convulsive ergot alkaloids affect the central nervous system and can cause uncontrolled convulsions in the body (Bezerra da Rocha et al., 2014).

1.2 Mycotoxin Occurrence

Most mycotoxins found in nature do not grow alone, but rather in a mixture of different genera (Broom, 2015). Mixtures of mycotoxins can be more harmful than any singular mycotoxin because they can work together to either amplify a specific issue or cause a wider range of issues. Mycotoxins also have a different range of toxicity. Deoxynivalenol is stronger than zearalenone, which is stronger than fumonisin (Wan et al., 2013). When aflatoxin and ochratoxin A are mixed together, they have a strong impact on poultry. Likewise, pigs are strongly affected by aflatoxin and deoxynivalenol (Broom, 2015). Mycotoxins can all cause oxidative stress and increase host susceptibility to secondary infections like *Salmonella* and *E. coli*.

1.3 Stresses on the Piglet

In addition to stress from eating feed contaminated by mycotoxins, the pig is exposed to multiple stressors. Some of the stresses are limited to the period immediately after weaning or some are chronic throughout the life of the pig. Weaning stress and maternal separation are some but the severest forms of stress for the piglet. The piglet can also experience stress from introduction to a new environment, transportation, new pen mates, and a new social hierarchy within the pen (Pohl et al., 2017).

1.3.1 Weaning Stress

Weaning is the act of removing the sow's milk from the piglet's diet. Pigs in the United States are typically weaned around twenty-ones days of age. The critical time for gut development occurs around the same time as weaning, and the stress from weaning can have a negative impact on gut development (Moeser et al., 2017). Once piglets are weaned, they are

transitioned onto a solid diet. Many pigs struggle to adapt to the new diet, with only 50% of piglets eating their first meal within 24hrs after weaning and 10% not eating within the first 48hrs (Lalles et al., 2004). This decrease in feed intake causes the gut to become anorexic (Montagne et al., 2007) and villi height can be reduced by up to 75% in 24hrs (Pluske et al., 1997). Within 24hrs piglets can lose 100-250g of body weight and lose 60-70% of metabolizable energy (ME) within a week (Campbell et al., 2013). When feed intake is reduced, the this adversely affects gut permeability, leaking to leaky gut, a higher chance of infection and inflammation (Spreeuwenberg et al., 2001). Providing freshly weaned piglets with sow's milk (Spreeuwenberg et al., 2001) or creep feed can reduce the villi atrophy and loss of gut function (Jayaraman and Nyachoti, 2017). Villi atrophy can result from a decrease in cell renewal or an increase in cell death. Higher cell death numbers cause the crypts to increase in depth (Pluske et al., 1997). Decreased villi height reduces the surface area for nutrient absorption. The increase in crypt depth allows for more secretion of electrolytes, causing water to leave via an osmotic gradient. This change in gut structure usually leads to a decrease in brush border enzyme activity like lactase and sucrase (Pluske et al., 1997).

1.3.2 Segregated Early Weaning

"Multisite swine production has become a widely applied strategy in commercial pig production" (Main et al., 2004). Raising pigs in multiple sites allows better control of what pathogens piglets are exposed to by segregating piglets after weaning. Piglets have no bacteria in their gut after birth and bacteria quickly colonize it. Those bacteria can have an impact on the way the gut develops after birth. Pigs that are maintained in a clean environment can have thinner intestinal walls, lamina propria, taller villi, and shallower crypts (Pluske et al., 1997). When piglets are segregated, they are exposed to less pathogens which leads to increased feed

intake and efficiency because bacteria in the gut lead to villi height reduction and increased crypt depth (Tang et al., 1999).

1.3.3 Stress from *Escherichia coli* Infection

One of the main pathogens that piglets are challenged with after weaning is *Escherichia coli* (McLamb et al., 2013). Exposure to enterotoxigenic *E. coli* alone usually does not cause diarrhea, but when infection overlaps with a stressor such as weaning, infection usually follows (Moeser et al., 2007). *E. coli* attaches to villi and releases enterotoxins that lead to diarrhea (Moeser and Blikslager, 2007).

1.4 LPS Recognition by TLR 4

Signaling by toll-like receptor (TLR) 4 is used by gram negative bacteria to cause inflammation and stress. Thus, an understanding of the mechanism of TRL4 signaling is needed for developing mitigation strategies against this form of stress. Toll-like receptors (TLR) are Pattern-Recognition Receptors (PRR) that can recognize Pathogen-Associated Molecular Patterns (PAMPs) from different bacteria, viruses, and other pathogens (Kumar et al., 2009). TLRs are made of three parts: the ectodomain, the transmembrane domain, and the cytoplasmic domain (Jiménez-Dalmaroni et al., 2016). TLR4 is located on the surface of epithelial cells in the gastrointestinal (GI) tract and recognizes lipopolysaccharide (LPS) from gram-negative bacteria (Kumar et al., 2009). When LPS binds to the ectodomain of TLR4, it causes a conformational change in the cytoplasmic domain leading to the production of a signal in the myeloid differentiation primary response 88 (MyD88) or TIR-domain-containing adapter-inducing interferon-β (TRIF) pathways (Jiménez-Dalmaroni et al., 2016).

1.4.1 MyD88 Dependent Pathway of TLR4 Signaling

When LPS (or any ligand) binds, TLR4 attracts Toll/IL-1 Receptor Associated Protein (TIRAP) to bind to the cytoplasmic domain. Once TIRAP is bound, MyD88 is free to bind to TLR4 and begin the signaling cascade that leads to the production of proinflammatory cytokines (Kumar et al., 2009). To continue the signal, MyD88 must attract the IL-1 receptor associated kinases (IRAK) complex and does so with the death domain (DD) on the N-terminus of MyD88 (Wesche et al., 1997). IRAK 4 binds to MyD88 and recruits IRAK 1 and 2 to complete the IRAK complex. The completed IRAK complex activates TNF receptor -associated factor (TRAF) 6 leading to two different paths in the signal. TRAF6 can directly interact with interferon regulatory factor 5 (IRF5) leading to the production of pro-inflammatory cytokines. TRAF6 can also interact with transforming growth factor-β-associated kinase 1 (TAK1), TGF-β activated kinase 1 binding protein (TAB) 1, 2, and 3 to activate the IκB kinase (IKK) complex. The IKK complex is made up of NF-κB essential modulator (NEMO), IKKα, and IKKβ, which together cause the degradation of inhibitor of κB (IκB). Once IκB is removed, nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) is free to activate the production of proinflammatory cytokines (De Nardo, 2015).

1.4.2 MyD88 Independent Pathway of TLR4 Signaling

After LPS is bound to TLR4, it can be engulfed in an endosome by cluster of differentiation 14 (CD14) which leads to the use of the MyD88 Independent Pathway to induce a signal. TRIF binds instead of MyD88 binding to the cytoplasmic domain of TLR4 (De Nardo, 2015). TRIF interacts with translocation associated membrane protein (TRAM) which can lead to activation of TRAF6, leading to the normal signal as in the MyD88 pathway, or TRAF3 which leads to the activation of TANK-binding kinase 1 (TBK1)/IKKi. The TBK1/IKKi complex

activates IRF3 and IRF7 leading to the production of Type I interferon (IFN) (Kumar et al., 2009).

1.5 Oxidative Stress

Multiple stressors and metabolic activities can lead to generation of reactive oxygen species (ROS). They are naturally occurring as by-products from cellular metabolism. ROS include molecules such as superoxide, hydroxyl radicals, and hydrogen peroxide. Most ROS are produced when not enough electrons are passed to oxygen during the electron transport chain in the mitochondria. The electron transport chain is made up of nicotinamide adenine dinucleotide (NADH)-ubiquinone oxidoreductase (Complex I), succinate dehydrogenase (Complex II), ubiquinol-cytochrome c oxidoreductase (Complex III), cytochrome c oxidase (Complex IV), coenzyme Q, and cytochrome c. Superoxide in particular is usually formed when only three electrons are passed to an oxygen molecule at Complex I or Complex III. Cytochrome c helps reduce some of the superoxide that is formed into an oxygen molecule (Bhattacharyya et al., 2014).

ROS can also be formed by exposure to environmental factors such as air pollution, radiation, chemicals, toxins, and foreign microorganisms (Lykkesfeldt and Svendsen, 2007). Microorganism infection can lead to an oxidative burst of superoxide from phagocytes to kill the invading pathogen. The oxidative burst is meant to kill pathogens and stimulate tissue repair (Gill et al., 2010). If ROS levels get too high, they can actually cause damage to proteins, lipids, carbohydrates, and nucleic acids (Bhattacharyya et al., 2014). Protein damage can lead to enzymes that are unable to function properly. Lipids can undergo lipid oxidation and affect the integrity of the cellular membrane (Kim et al., 2012). Hydrogen peroxide can cause colonic smooth muscle cells to lose contractility and increase in NF-Kb expression and activation (Shi et

al., 2003). DNA and RNA can be broken down by ROS. If the damage becomes severe, cells may undergo apoptosis to prevent the spread to surrounding areas (Lykkesfeldt and Svendsen, 2007).

1.6 Intestinal Epithelial Layer Structure

Mycotoxins and bacterial pathogens often compromise the integrity and functionality of the gut epithelial cells, leading to negative effects on digestive and absorptive functions. The small intestine contains millions of small fingerlike projections called villi that aid in the absorption of nutrients and water (Pluske et al., 1997). The small intestine also contains crypts that are responsible for secretion of ions and mucus to control the motility of the gut and aid in protection from pathogens. The lining of the small intestine is a single layer of columnar epithelial cells that have a basolateral and apical side separated by a series of tight junction proteins, which are found on the more apical side of the cell (Moeser and Blikslage, 2007). Controlling absorption and secretion is the enteric nervous system (ENS), which can itself be controlled by the central nervous system (CNS) or operate independently (Moeser and Blikslage, 2007). The ENS contains the myenteric plexus, which aids in motility and peristalsis, and the submucosal plexus, which aids in mucosal and epithelial functions (Moeser et al., 2017). On the apical side of the epithelial layer are channels and transporters that aid in secretion of Cl^- and HCO_3^- and the absorption of Na^+ to control the flow of water into the cell and buffer the lumen from stomach acid. On the basolateral side of the epithelial layer are channels and transporters to control the flow of Na^+ and K^+ into the cell to control the electrochemical gradient inside the cell (Moeser and Blikslage, 2007). At birth, the gut is not fully mature and continues to develop during the first few weeks of life (Tang et al., 1999).

1.6.1 Intestinal Epithelial Tight Junctions

Cells in the intestinal epithelial layer are held together by junctional proteins. These are made up of three parts: tight junctions, adherens junctions, and desmosomes. Together, the three form a seal between the basolateral and apical side of the epithelial cells. This seal controls the flow of nutrients, water, and pathogens between the intestine and the rest of the host. The junctions control the paracellular permeability of the epithelial cells (Groschwitz and Hogan, 2009). Tight junctions are mostly apical, followed by adherens junctions and desmosomes (Lechuga and Ivanov, 2017).

1.6.2 Intestinal Epithelial Tight Junction Proteins

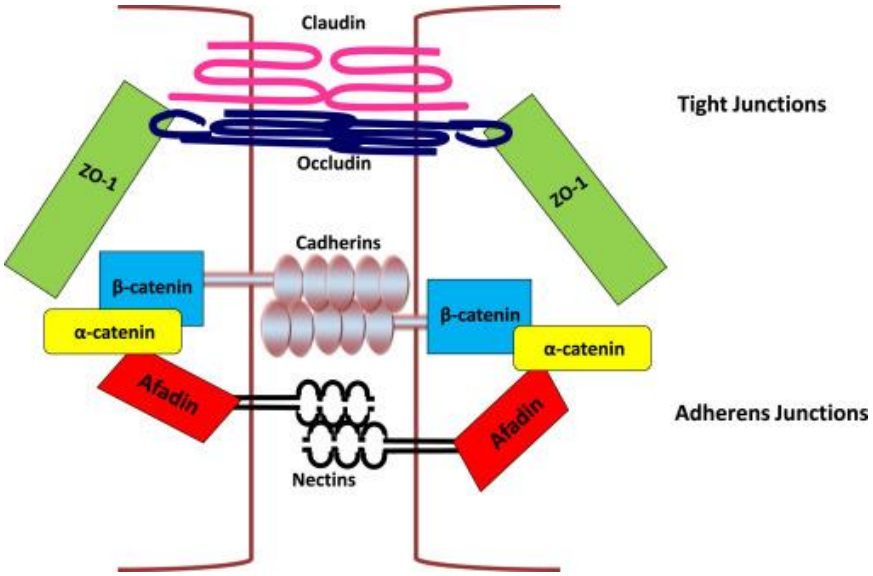

Figure 1-1: Overview of tight junction structure (Campbell et al., 2017)

The tight junctions are made up of three parts: transmembrane proteins, cytoplasmic scaffolding proteins, and cytoskeletal elements (Guttman and Finlay, 2009). Transmembrane proteins, like claudins and occludin stretch from one cell to the other and bind with like proteins to form dimers and seal the gap. Occludin also binds with proteins inside the cell like zona

occludens 1 (ZO-1). ZO-1 is a cytoplasmic scaffolding protein because it helps connect occludin and claudin to the cytoskeletal element actin. This allows for actin to control the contractility of the cell and the tight junction (Campbell et al., 2017). There are several types of claudins that can form the tight junction. They can either be sealing claudins (claudins 1, 4, 5, 8, 11, 14, or 19) or pore-forming claudins (claudins 2, 7, 10, 15, or 16). The level of either the sealing or pore-forming claudins will determine how restrictive the tight junction is (Capaldo and Nusrat, 2009).

1.6.3 Adherens Junctions

Adherens junctions are made up of cadherins and nectins. The most common cadherin in epithelial cells is E-cadherin. The interior portion of E-cadherin binds to actin while the exterior portion binds to E-cadherin from an adjacent cell. Nectins form similar bonds to those formed by cadherins but are weaker in strength (Campbell et al., 2017).

1.6.4 Formation of Intestinal Tight Junctions

Junctions are formed from the basolateral to the apical side. Nectins are the first proteins to form a bond with an adjacent cell. Once that bond is established, cadherins form their connections. After the entire adherens junction is formed, tight junctions begin to form. Occludin binds first with the help of ZO-1, followed lastly by claudin formation. ZO-1 is required for complete tight junction formation (Van Itallie and Anderson, 2014). Once the entire junction is formed, the permeability of the junction can be adjusted (Campbell et al., 2017). The transepithelial electrical resistance (TEER), a measure of cell permeability, can range from 5-1000 Ωcm^2. The TEER can be adjusted based on the amount of nutrients on the apical side of the cell (Schneeberger and Lynch, 1992). TEER increases logarithmically with increasing number of protein strands in the tight junction (Liang and Weber, 2014).

1.6.5 Cytokine Control of Tight Junctions During Stress in Pigs

Cytokines are often produced during inflammation of pathogenic invasion of the intestine. Young pigs are most susceptible to the infections and the negative effects of cytokines due to the immaturity of their intestinal immune system. These cytokines can have a huge negative impact on tight junction integrity and can increase or decrease their permeability. Cytokines that are pro-inflammatory like tumor necrosis factor alpha (TNFα), IL-1, and IL-6 cause the permeability of the tight junction to increase, which leads to an increase in nutrients and leakiness of water into the gut, as well as an increase in the chance for pathogens to cross into the basolateral side of the epithelial layer. INFγ in particular increases intestinal permeability by decreasing the levels of ZO-1. This causes the endocytosis of claudin and occluding, leading to a breakdown of the gut barrier. Cytokines that are anti-inflammatory like IL-10 work in the opposite fashion and decrease the permeability of the tight junction (Capaldo and Nusrat, 2009).

1.7 Summary

Animals undergo multiple stresses during their growth period. Pigs are especially vulnerable to stress early in their life. This stress can lead to a wide range of complications. Eating feed contaminated with aflatoxin represents a major risk and source of stress in growing pigs. Ducks are also extremely sensitive to aflatoxin. The use of mycotoxin binder can help mitigate the negative effects of the mycotoxins in feed for pigs and ducks. Therefore, two studies were conducted to determine the effectiveness of a mycotoxin binder to limit the negative effects of mycotoxins in pigs and ducks. The first study involved the use of a binder against aflatoxin contamination in ducks. In the second study, effects of a mycotoxin binder were investigated in pigs fed corn that was contaminated with DON.

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CHAPTER 2. EFFECT OF INCLUSION OF MYCOTOXIN BINDER TO AFLATOXIN B_1 CONTAMINATED FEED ON THE GROWTH PERFORMANCE, OVERALL APPEARANCE, AND BLOOD COMPONENTS IN PEKIN DUCKS

2.1 Abstract

The effects of a mycotoxin binder, when added to an aflatoxin B_1 contaminated diet, on growth and metabolic indicators was determined in ducks. One-day-old male Pekin ducks (n=360) were randomly divided into four groups; each group had 6 replicate pens with 15 ducks in each replicate pen. The positive control (PC) group was fed a diet that was free of aflatoxin B_1, the negative control (NC) group was fed a diet that contained >75ppb of aflatoxin and no clay binder, the negative control with low binder (NC + 0.5) group was fed a diet that contained >75ppb of aflatoxin and 0.5 kg/ton of binder, the negative control with high binder (NC + 1.0) group was fed a diet that contained >75ppb of aflatoxin and 1.0 kg/ton of binder. The diets were fed in two phases of 0 to 14 days (phase 1) and 15 to 35 days (phase 2). During the experiment, duck weights and feed consumption were calculated each week. At the end of the experiment, body condition was assessed through examination of feather quality, hemorrhaging in the toe webb, eye necrosis, and liver paleness. Relative weights of the heart, liver, and spleen were measured. Blood glucose, hemoglobin, hematocrit, and total protein levels were also assessed. The results showed that: 1) during early phase 2, NC + 0.5 had higher rates of weight gain compared to NC ducks ($P<0.05$); 2) NC + 0.5 ducks had higher feather quality than both NC and PC ducks ($P<0.05$); 3) NC ducks had significantly greater liver weights ($P<0.05$); 4) blood glucose was higher in NC + 0.5 ducks ($P<0.05$); and 5) PC ducks had higher serum protein levels in the blood ($P<0.05$). In summary, adding low levels of binder improved the health of ducks compared to feeding diets contaminated with aflatoxin B_1 without a binder.

2.2 Introduction

Mycotoxins are secondary metabolites produced by several genera of fungi. They can contaminate feedstuffs like grains at any time during the production process from the field to storage. Estimates have put mycotoxin contamination occurring in nearly 25% of all grains produced each year (Chang et al., 2016). Aflatoxins are a type of mycotoxin produced by *Aspergillus flavus* and come in four main forms: B_1, B_2, G_1, and G_2 (B are blue under UV light and G are green) (Bezerra da Rocha et al., 2014). Aflatoxin B_1 is one of the most toxic substances known to exist. Acute exposure to aflatoxin B_1 can lead to several health issues such as vomiting, diarrhea, and abdominal pain. Chronic exposure can cause weight loss, liver cancer, and increases in secondary infections (Marin et al., 2013). Aflatoxin B_1 affects different species in different ways. Mice are able to handle large doses of aflatoxin B_1 while birds are only able to withstand very small doses (Wogan, 1992). Ducks are very sensitive to aflatoxin contamination (Chen et al., 2013). Duck consumption has increased over the past decade, and with that so has duck production. In 2016, the United States slaughtered 27.3 million ducks (Agricultural Marketing Resource Center, 2017). The increase in duck production means that more feed, that is free of aflatoxin contamination, must be available for ducks to consume. Since clean feed is not always guaranteed, other means of controlling aflatoxin are needed. Mycotoxin binders have been shown to help offset the effects of aflatoxin B_1 on poultry (Phillips et al., 2002). This study looks at determining at what level is best for feeding to ducks so that they can be fed feed that is contaminated with aflatoxin B_1 while still being healthy enough produce.

2.3 Materials and Methods

All animal procedures were approved by the Purdue University Animal Care and Use Committee (PACUC).

2.3.1 Animals

The study took place at the Poultry Unit of the Purdue University Animal Science Research and Education Center. A total of 360, day-old, male Pekin ducks were obtained from Maple Leaf Farms in Leesburg, Indiana. The ducks had an initial weight of 52.9 g and were randomly assigned to one of four treatments. Each treatment had six replicate pens with fifteen ducks in each pen. The ducks were housed on the floor and were fed an ad libitum feed and water for five weeks.

2.3.2 Diet

The feed treatments were: Positive Control (0 aflatoxin + 0 mycotoxin binder), Negative Control (>75 ppb of aflatoxin + 0 mycotoxin binder), Low Binder (>75 ppb of aflatoxin + 0.5kg/ton of commercial binder A), High Binder (>75 ppb of aflatoxin + 1.0kg/ton of commercial binder A). The pure aflatoxin was obtained from Dr. George Rottinghaus, University of Missouri. Concentrations of aflatoxin in each diet were confirmed at the Animal Disease Diagnostic Laboratory at Purdue University. The diets were fed in two phases: d 0 to 14 and d 15 to 35. The diets were based on suggestions by Maple Leaf Farms. Complete diet compositions can be found in Table 2-1.

2.3.3 Weights

Feed was weighed each week to determine feed intake. Ducks were also weighed and the feed conversion ratio was calculated. Dead birds were tracked and feed efficiency was adjusted for mortality. At the end of five weeks, ducks were euthanized by asphyxiation with carbon dioxide.

2.3.4 Body Condition and Sample Collection

After euthanasia, the body condition of every duck was assessed and tissue and blood samples taken from five ducks per pen whose weights that matched the average pen weight. Samples of the liver, spleen, and heart were taken and weighed for determination of relative organ percentages. Body condition scoring was done through examination of parameters such as feather quality, web-toe hemorrhaging, eye necrosis, and liver paleness. Feather quality, web-toe hemorrhaging, eye necrosis, and liver paleness scores were based on sample pictures and ranking levels as shown in Table 2-2.

2.3.5 Blood

Blood samples were collected from the same five birds as the tissue samples. The blood was used to measure hemoglobin levels, hematocrit percentage, glucose levels, and total protein. Blood hemoglobin was measured using a hemoglobin enzyme-linked immunosorbent assay (ELISA) kit from Mybiosource (San Diego, CA) according to the manufacturer's instructions. Hematocrit was measured immediately after blood collection with a Damon/IEC micro hematocrit centrifuge and heparinized micro-hematocrit capillary tubes (Fisher Scientific, Chicago, IL). Blood was placed in the tubes and spun down at 16,800 $\times$ g for 15 minutes. Glucose levels were measured in fresh blood using a diabetes glucose meter (Freestyle, Abbott, Abbott Park, IL). Total blood protein was measured with the bicinchoninic acid assay (BCA) on TECAN plate reader at 540 nm.

2.3.6 Statistical Analysis

Data were analyzed with SAS 9.4 using PROC GLM. Results were considered significant when $P \leq 0.05$. Results are shown as least square means. For significant comparisons,

Tukey multiple comparison test was used to determine differences between means. Significant differences were designated using superscripts.

2.4 Results

Table 2-3 shows the gain, feed intake, and gain:feed ratio for each week, respectively. The gain per week for each treatment was similar during the first phase. During the first week of phase 2, NC ducks had a significant reduction in gain compared to the NC + 0.5 ducks ($P<0.05$). During the second week of phase two, the gain of NC + 0.5 birds had a tendency for a higher gain ($P=0.09$). For the final week of phase two, the gain of the NC + 0.5 ducks was significantly lower than the gain of the PC ducks ($P<0.05$). However, for the entire growth period, there was no significant impact of diet on the gain:feed ratio.

The overall appearance of the ducks is shown in Table 2-4. There was significant effect of diet on feather scores. The NC + 0.5 ducks had significantly better scoring feathers than both the PC and NC ($P<0.05$). Web-toe hemorrhaging was not different between diets. Eye necrosis score was also not between the diets.

Table 2-5 shows the organ weights as percent of total body weight. There was no difference between the diets in heart weight. The NC + 0.5 treatment had significantly heavier spleen weight compared to the PC ducks ($P<0.05$). The NC treatment had significantly higher liver weight compared to the PC ducks ($P<0.05$). The livers of NC + 1.0 ducks had a tendency ($P=0.08$) to be paler than those of other diets.

The effects of aflatoxin on the blood parameters of the ducks are shown in Table 2-6. Glucose concentration was significantly higher in NC + 0.5 ducks ($P<0.05$) compared to other treatments. There was no difference in the hematocrit or hemoglobin concentration between

diets. Total protein in the blood serum was significantly higher in the PC ducks compared to all other diets ($P<0.05$).

2.5 Discussion

There was a minimal effect of aflatoxin contamination on weight gain in this study. However, from d 14-21, birds on the NC+0.5 treatment had higher gain than the NC treatment. Lala et al. (2015) found that turkeys fed with clay adsorbents had increased weight gain compared to those fed with straight aflatoxin. This agrees with our study from d14-21. However, consistent effects of the binder on weight gain was not found. The reasons for the inconsistency and overall lack of performance response to aflatoxin could be the lower level of aflatoxin in the diets than anticipated (Table 2-7).

The increased feather loss in PC ducks was not expected. Feather growth involves rapid cellular growth inside the feather follicle (Prum, 1999). Aflatoxin exposure can cause a decrease in cellular growth due to its ability to inhibit protein synthesis (Bezerra da Rocha et al., 2014). The ducks that were exposed to aflatoxin would be expected to have more feather loss than those not exposed. The reason for the higher feather loss in the PC treatment is currently unknown. Forgacs et al. (1962) showed that exposure to aflatoxin led to an increase in hemorrhaging in multiple tissues. Therefore, we would expect that the level of hemorrhaging would be considerably higher in ducks exposed to aflatoxin compared to those of the control. However, this was not found in this study. The lower level of aflatoxin than expected could have prevented these events from happening. Additional studies where the level of aflatoxin is considerably higher will be warranted.

Grozeva et al. (2017) found that the weight of the spleen in turkey broilers decreased after exposure to aflatoxin. This is different from Ortatatli et al. (2005) who found no statistical

difference in the weight of spleens of broilers after aflatoxin consumption. The increase in the spleen weights of NC + 0.5 ducks compared to PC ducks in this study are most likely due to a species-specific response to the binder more than an effect of the aflatoxin. Aflatoxin exposure does not have any effect on the weight of the heart in ducks (Han et al., 2008). Aflatoxins are known to have a strong impact on the liver function of animals. Aflatoxin can cause the liver to become pale as the levels of toxin increase (Council for Agricultural Science and Technology, 2003). The level of liver paleness was not affected by the treatment in this experiment and could be due to the low level of aflatoxin contamination. The relative weight of the liver in ducks exposed to aflatoxin is inconsistent with those found by Ortatatli et al. (2005). They found no difference in liver weight from aflatoxin contamination. The increase in relative liver weight would be expected because aflatoxin exposure causes severe liver damage and necrosis. This should cause the liver weight to decrease, but the total body weight should also decrease due to a reduction in growth rate and protein synthesis. The reduction in total body weight should be more than the reduction in liver weight causing an increase in relative liver weight. There is also a possibility that low level of aflatoxin contamination used in this study led to increased compensatory liver growth as a result of the load on the liver to detoxify the aflatoxin from the body.

Tung et al. (1975) found that exposure to aflatoxin caused a reduction in both hematocrit and hemoglobin. The hemoglobin and hematocrit levels in this study were not reduced in response to aflatoxin as expected. Most likely this was because the ducks were not administered a sufficient amount of aflatoxin in the feed. At higher doses, one would expect to see a reduction in both hematocrit and hemoglobin levels. Total protein in the PC ducks were significantly higher which is similar to findings by Shabani et al. (2010) with broilers. Decreases in blood

protein could be an indication of a decrease in protein synthesis in the body. Thus, plasma protein concentration could be a sensitive marker of low-level aflatoxin contamination.

In conclusion, adding a binder to the feed can offset some of the negative effects of aflatoxin on weight gain. The binder increased the quality of the feathers, perhaps allowing for better protection against the elements. Although not significant, the binder caused an increasing numerical trend in total serum protein concentration compared to ducks exposed to aflatoxin only. Experiments are needed to determine the identity of proteins that may have been upregulated by aflatoxin. However, overall effects of aflatoxin were variable. Additional studies are needed using higher concentrations of aflatoxin to confirm observed effects of aflatoxin in ducks.

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Table 2-1: Ingredient composition of diets in the experiment

	Phase 1				Phase 2			
	PC	NC	NC + 0.5	NC + 1.0	PC	NC	NC + 0.5	NC + 1.0
Ingredient g/kg								
Corn	561.2	546.2	541.2	536.2	666.5	651.5	646.5	641.5
Soybean Meal (47.5%)	360	360	360	360	250	250	250	250
Soy Oil	30	30	30	30	30	30	30	30
Salt	4	4	4	4	4	4	4	4
Limestone (36% Ca)	21	21	21	21	23.5	23.5	23.5	23.5
Dicalcium Phosphate	17.5	17.5	17.5	17.5	14	14	14	14
Vitamin-Mineral Premix: DQ VIT/MIN-Turkey[2]	3	3	3	3	3	3	3	3
LYS-HCL (78% LYS)	0	0	0	0	5	5	5	5
DL-METHIONINE	3.30	3.30	3.30	3.30	4	4	4	4
Aflatoxin Corn	0	15	15	15	0	15	15	15
Binder Premix	0	0	5	10	0	0	5	10
Total	1000	1000	1000	1000	1000	1000	1000	1000

[1]Phase 1 was fed d0-14 and Phase 2 was fed d15-35.

[2]Supplied the following per kilogram of diet: vitamin A, 5,484 IU; vitamin D_3, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg; riboflavin, 5.49 mg; D-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B_{12}, 13.2 µg; biotin, 55.2 µg; thiamine mononitrate, 2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; and Se, 300 µg.

Table 2-2: Body condition score breakdown

Score	Feather	Web-toe Hemorrhagic	Eye Necrosis	Liver Paleness
0	N/A	Normal	N/A	N/A
1	Poor	Slight Hemorrhage	Normal	Normal
2	Moderate	Moderate Hemorrhage	Moderate	Moderate
3	Good	Severe Hemorrhage	Severe	Severe

Table 2-3: Gain, feed intake, gain:feed ratio

	Diet					
Item	PC	NC	NC + 0.5	NC + 1.0	SEM	P-value
Gain (g/wk)						
d 0-7	189	207	195	197	5.22	0.26
d 7-14	491	477	493	497	10.35	0.22
d 14-21	637[ab]	612[b]	671[a]	631[ab]	12.72	<0.01
d 21-28	797	782	831	791	15.47	0.09
d 28-35	757[a]	711[ab]	669[b]	720[ab]	17.35	<0.01
Overall	2871	2789	2859	2836	43.41	0.27
Feed Intake (g/wk)						
d 0-7	230	245	243	243	14.93	0.88
d 7-14	678	670	682	698	23.17	0.78
d 14-21	1134	1062	1139	1131	24.49	0.12
d 21-28	1532	1513	1626	1593	43.40	0.25
d 28-35	1839	1719	1722	1826	35.47	0.10
Overall	5413	5208	5413	5491	118.45	0.36
Feed:Gain (g/kg)						
d 0-7	821	844	801	810	17.21	0.35
d 7-14	726	713	722	713	9.11	0.67
d 14-21	561	577	589	558	10.87	0.19
d 21-28	522	518	511	497	9.29	0.30
d 28-35	412	414	383	393	15.64	0.45
Overall	530	536	527	516	6.12	0.22

[ab]Values with different superscripts in the same column are different ($P < 0.05$).

Table 2-4: Body condition scores

	Diet					
Item	PC	NC	NC + 0.5	NC + 1.0	SEM	P-value
Feather	2.29[b]	2.31[b]	2.62[a]	2.44[ab]	0.07	<0.01
Web-toe	0.63	0.85	0.90	0.93	0.10	0.11
Eye	1.14	1.13	1.21	1.21	0.04	0.40

[ab]Values with different superscripts in the same column are different ($P < 0.05$).

Table 2-5: Relative organ weights as percentages of body weights

Item	Diet				SEM	P-value
	PC	NC	NC + 0.5	NC + 1.0		
Heart Weight	0.60	0.65	0.64	0.62	0.01	0.13
Liver Weight	2.05[b]	2.33[a]	2.22[ab]	2.14[ab]	0.06	0.01
Spleen Weight	0.07[b]	0.08[ab]	0.09[a]	0.08[ab]	0.005	0.03
Liver Paleness	1.33	1.50	1.67	1.77	0.13	0.09

[ab]Values with different superscripts in the same column are different ($P < 0.05$).

Table 2-6: Blood components

Item	Diet				SEM	P-value
	PC	NC	NC + 0.5	NC + 1.0		
Glucose (g/L)	1.08	1.11	1.26	1.09	4.96	0.04
Hematocrit (%)	31.3	29.5	30.0	30.1	0.53	0.14
Total Protein (g/L)	177.0[a]	149.2[b]	153.2[b]	157.1[b]	0.49	<0.01
Hemoglobin Levels (g/L)	154.06	195.91	184.94	214.24	25.50	0.43

[ab]Values with different superscripts in the same column are different ($P < 0.05$).

Table 2-7: Aflatoxin levels in feed

Item	Diet			
	PC	NC	NC + 0.5	NC + 1.0
Phase 1	None Detected	71.60 ppb	84.16 ppb	52.55 ppb
Phase 2 Batch 1	Trace <5.50 ppb	54.39 ppb	46.18 ppb	41.70 ppb
Phase 2 Batch 2	Trace <5.50 ppb	50.64 ppb	40.19 ppb	33.03 ppb

CHAPTER 3. EFFECT OF ADDING A LIVER PROTECTANT OR MYCOTOXIN BINDER TO DIETS OF PIGS FED LOW LEVELS OF DEOXYNIVALENOL

3.1 Abstract

Effects of a feed additive that enhanced liver function and a mycotoxin binder was examined in pigs fed deoxynivalenol (DON) contaminated feed. A total of 128 pigs (Duroc × Landrace × Yorkshire, equal number of barrows and gilts, age 42 d) were randomly assigned to 4 treatments. There were 8 replicate pens with 4 pigs per pen and pigs were blocked based on their initial BW. Treatments were DON, DON + liver protectant (1 kg/ton), DON + mycotoxin binder (0.5 kg/ton), or DON + liver protectant and mycotoxin binder. The study lasted 28 days. Body weights (BW), feed intake (FI) and blood samples were taken on days 14 and 28. Weights and feed intake were used to calculate feed efficiency (G:F) and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured in the blood serum. BW, FI, and G:F were not significant at any point during the study. Concentrations of AST were significantly reduced ($P < 0.05$) at day 14 for pigs fed the liver protectant but were not significantly different on day 28. In summary, feeding a liver protectant, mycotoxin binder, or combination of the two does not have an effect on pigs fed low levels of DON.

3.2 Introduction

Fungal growth on crops and grains is common in a wide range of feedstuffs that are commonly consumed by livestock. Fungi produce secondary metabolites called mycotoxins as they grow. *Fusarium* strains produce several different mycotoxins, with deoxynivalenol being one of the most common (Bergsjo et al., 1992). Deoxynivalenol is found in grains such as wheat, oats, barley, and corn that are commonly fed to swine (Danicke et al., 2006).

Deoxynivalenol is highly stable at high temperatures and is extremely difficult to remove from feed during processing (Rotter, 1996). Exposure to DON affects different species in various ways, and pigs in particular are very susceptible to DON (Lessard et al., 2015). Deoxynivalenol can cause a wide range of issues in pigs depending on the level of exposure. At low levels, DON can cause decreased feed intake and weight loss. At higher levels, DON can lead to feed refusal and vomiting (Stanek et al., 2012).

3.3 Materials and Methods

All animal procedures were approved by the Purdue University Animal Care and Use Committee (PACUC).

3.3.1 Animals and Diet

The study took place at the Swine Unit of the Purdue University Animal Science Research and Education Center. A total of 128 pigs (Duroc × Landrace × Yorkshire, 64 gilts and 64 barrows, weaning age 21 d) with an initial average weight of 10.64 kg were used in a randomized complete block design with 8 replicates (4 pens of barrows and 4 pens of gilts) and 4 pigs per replicate for a 28-day study. Pigs were assigned to one of four diets at age 42 d: 1) Control (2.40 ppm Deoxynivalenol); 2) Control (2.60 ppm Deoxynivalenol) + Commercial Liver Protectant (1 kg/ton); 3) Control (2.70 ppm Deoxynivalenol) + Commercial Binder (0.5 kg/ton); 4) Control (2.90 ppm Deoxynivalenol) + Commercial Liver Protectant + Commercial Binder. Diet compositions can be seen in Table 3-1. The pigs were housed in floor pens and given ad libitum feed and water. Deoxynivalenol contaminated corn was obtained from a feed mill near Jonesboro, IN. Deoxynivalenol levels were confirmed at United Animal Health,

Sheridan, IN. Mycotoxin concentrations provided by United Animal Health (Table 3-2) were then used to make feed with the appropriate amount of deoxynivalenol.

3.3.2 Sample Collection

Pigs and feed were weighed every two weeks to determine G:F and body weights. Pigs were also bled to determine the activity levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Enzyme activity levels were measured using ELISA kits (Bioassay Systems, Hayward, CA).

3.3.3 Statistical Analysis

Proc GLM of SAS (SAS, Cary, NC) was used to determine effects of gender and diet on growth performance. Initial body weight was used as a covariate. Values were considered significant when $P \leq 0.05$ and are denoted with superscripts. Tukey's multiple comparison test was used to determine differences between means.

3.4 Results

Analysis of the contaminated corn revealed it had concentrations of deoxynivalenol between 2.4-2.9 ppm, and trace amounts of zearalenone, fumonisin, and aflatoxin mycotoxins in the final diets. Table 3-3 shows body weights for each diet on days 0, 14, and 28. Diet had no significant effect on overall BW. Table 3-4 shows average daily gain (ADG), feed intake (FI), and gain to feed ratio (G:F) during each phase of the study. Diet had no impact on ADG, FI, or G:F.

Enzyme activity levels for aspartate aminotransferase and alanine aminotransferase is presented in Table 3-5. Addition of the liver protectant significantly reduced ($P=0.03$) the activity of AST on day 14 compared to the control diet. Enzyme activities were not different at any other points.

3.5 Discussion

The analyzed contaminated feed had more than just DON. Although our main interest was in DON, possible effects of the mycotoxin contaminants could not be ruled out. However, although numerical trends were observed, there was no significant treatment effect on final BW. Rotter et al. (1994) found that voluntary feed intake was reduced in pigs fed DON, but without having a positive control that had no mycotoxin, it is hard to compare whether or not the DON in this study had an effect on feed intake. Although there was a tendency (P = 0.07) for a treatment effect on feed intake in the first two weeks, this disappeared in phase 2. There was a significant difference in weight gain and a tendency for increased feed intake overall between males and females. This is consistent with findings of Goyarts et al. (2005). The reason for the decreased level of aspartate aminotransferase on d 14 in the liver protectant diet group is unknown. However, these differences have disappeared on day 28, perhaps suggesting an adaptation to the mycotoxins by the pigs. Pigs have been known to adapt to dietary DON over time (Dersjant-Li et al., 2003). In conclusion, feeding a liver protectant, mycotoxin binder, or a combination of the two to pigs exposed to low levels of DON did not seem to have significant effect on the pig. Future experiments should involve feeding a higher level of DON to determine the effects of the binder and liver protectant.

3.6 References

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Table 3-1: Ingredient composition of diets in the experiment

	Control	Control + Liver Protectant	Control + Mycotoxin Binder	Control + Liver Protectant and Mycotoxin Binder
Ingredient g/kg				
Corn	378.09	368.09	368.09	358.09
Mycotoxin Contaminated Corn	160	160	160	160
Soybean Meal (47.5% CP)	326.5	326.50	326.50	326.50
DDGS	72.5	72.50	72.50	72.50
Soybean Oil	25	25	25	25
Limestone	6.25	6.25	6.25	6.25
Monocalcium Phosphate	11.2	11.20	11.20	11.20
L-lysine-HCl	4.66	4.66	4.66	4.66
DL methionine	1.66	1.66	1.66	1.66
L-Threonine	2.04	2.04	2.04	2.04
L-Tryptophan	0.1	0.10	0.10	0.10
Liver Protectant	0	10	0	10
Mycotoxin Binder	0	0	10	10
Salt	5	5	5	5
ZnO, 72% Zn	2.5	2.5	2.5	2.5
Vitamin Premix[1]	2.5	2.5	2.5	2.5
Mineral Premix[2]	1.5	1.5	1.5	1.5
Selenium[3]	0.5	0.5	0.5	0.5
Total	1000	1000	1000	1000

[1]Vitamin premix supplied per kilogram of diet: vitamin A, 3,630 IU; vitamin D_3, 363 IU; vitamin E, 36,4 IU; menadione, 1.3 mg; vitamin B_{12}, 23.1 µg; riboflavin, 5.28 mg; D-pantothenic acid, 13.1 mg; niacin, 19.8 mg.

[2]Mineral premix supplied per kilogram of diet: Cu (as copper chloride), 11.3 mg; I (as ethylenediamine dihydroiodide), 0.46 mg; Fe (as iron carbonate), 121 mg; Mn (as manganese oxide), 15 mg; and Zn (as zinc oxide), 121 mg.

[3]Supplied 300 µg of Se per kilogram of diet.

Table 3-2: Mycotoxins present in initial contaminated corn

Mycotoxin	Amount Present	Maximum Allowed
DON	9.30 ppm	1.0 ppm
Fumonisin	<0.25 ppm	5.0 ppm
Zearalenone	0.59 ppm	0.5 ppm
Aflatoxin	1.35 ppb	20 ppb

Table 3-3: Body weights in kg

	Diet					
Item	Control	Liver Protectant	Mycotoxin Binder	Liver Protectant + Mycotoxin Binder	SEM	P value
Day 0	10.60	10.70	10.62	10.65	0.16	0.97
Day 14	17.25	17.39	17.02	17.07	0.21	0.71
Day 28	26.24	26.61	26.05	26.07	0.36	0.83

Table 3-4: Gain, feed intake, and gain:feed ratio

	Diet					Sex			P Value	
Item	Control	Liver Protectant	Mycotoxin Binder	Liver Protectant + Mycotoxin Binder	SEM[1]	Barrows	Gilts	SEM[2]	Diet	Sex
Gain (g/d)										
Phase 1	474.85	477.89	457.14	458.65	15.02	480.75[a]	453.44[b]	10.74	0.71	0.04
Phase 2	641.91	658.11	644.95	642.93	17.69	660.69	633.25	12.65	0.96	0.06
Overall	558.38	568.00	551.04	550.79	12.78	570.56[a]	543.50[b]	9.14	0.84	0.01
Feed Intake (g/d)										
Phase 1	747.97	727.88	702.11	708.71	12.99	727.81	715.44	9.29	0.08	0.11
Phase 2	1104.91	1113.13	1063.11	1079.95	32.59	1103.94	1076.50	23.31	0.73	0.25
Overall	926.44	920.51	882.61	894.33	17.26	915.94	895.94	12.34	0.27	0.09
Gain:Feed (g/kg)										
Phase 1	639.22	659.63	650.58	653.21	20.58	662.75	638.56	14.72	0.93	0.23
Phase 2	583.97	592.21	617.25	601.16	15.53	605.38	592.06	11.11	0.49	0.36
Overall	606.16	618.31	628.05	620.96	10.20	626.75	609.94	7.29	0.52	0.09

Phase 1 represents d 0-14 and Phase 2 represents d 14-28.

[ab]Values with different superscripts in the same column are different ($P < 0.05$).

[1]Diet

[2]Sex

Table 3-5: Enzyme activity levels (U/L)

	Diet					
Item	Control	Liver Protectant	Mycotoxin Binder	Liver Protectant + Mycotoxin Binder	SEM	P Value
Aspartate Aminotransferase						
Day 14	64.11[a]	45.79[b]	52.02[ab]	58.47[ab]	0.03	0.03
Day 28	76.14	69.94	74.20	73.52	0.01	0.31
Alanine Aminotransferase						
Day 14	69.35	65.47	66.56	61.55	0.02	0.21
Day 28	82.27	80.94	81.65	76.60	0.02	0.39

[ab]Values with different superscripts in the same column are different ($P < 0.05$).

CHAPTER 4. SUMMARY

The early exposure of animals to mycotoxins can have an impact on their development. When animals are young, their gastrointestinal tracts are still developing, and mycotoxins can change the way they develop. Mycotoxins can cause a reduction in feed intake and decrease the immune response of animals (Rotter, 1996; Broom, 2015). Mycotoxin binders added to the feed offer a possibiltiy to reduce the effects of mycotoxins on the developing animal (Phillips et al., 2002). The experiments described were aimed at finding if a binder fed in the diet of ducks exposed to aflatoxin and pigs exposed to low levels of deoxynivalenol could help mitigate some of the side effects of mycotoxin exposure.

In chapter 2, weight gain, feed efficiency, overall physical appearance, and effects on organs from aflatoxin exposure was investigated. Results showed that ducks given low levels of binder had better feathering and higher blood glucose levels. They also had higher weight gains than ducks that were exposed to aflatoxin without a binder from d14-21. However, the levels of aflatoxin in the feed were not sufficiently high to cause an adverse effect on the ducks. Future studies using higher levels of aflatoxin are needed to confirm that the type of binder used can help ducks recover from exposure to aflatoxin.

In chapter 3, weight gain, feed efficiency, and liver enzyme activity levels were assessed after exposure to deoxynivalenol in pigs. Results showed that given low levels of a liver protectant, a mycotoxin binder, or a combination of the two had no effect on the weight gain or feed efficiency of the pigs. The activity levels of the liver enzymes AST and ALT were not significantly changed at the end of the study, although AST was significantly reduced in pigs fed only a liver protectant after two weeks. Future studies are needed to see if the protectant and binder are more useful at higher levels of deoxynivalenol.

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