THE EFFECTS OF FEEDING AFLATOXIN CONTAMINATED SWINE DIETS WITH CLAYS ON PERFORMANCE, MINERAL METABOLISM, IMMUNE RESPONSE AND LIVER FUNCTION IN WEANLING AND GROWING PIGS

by

Timothy C. Schell

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APPROVED:

E. T. Kornegay,
Co-Chairman

M. D. Lindemann,
Co-Chairman

C. M. Wood

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T. C. Schell

Committee Co-Chairmen: E.T. Kornegay and M.D. Lindemann
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(Abstract)

Two studies were conducted using pigs to determine the effects of feeding aflatoxin contaminated diets (AC) with and without various clays on performance, liver function, mineral metabolism and humoral immune response. In the nursery phase of Study I, weanling pigs (n=96) were fed 1% sodium bentonite (SB) with and without AC (922 ppb). In the grower phase, 48 pigs from the nursery phase were continued on SB treatments for 5 wk; AC was discontinued. In the metabolism phase, 24 barrows from the nursery phase were continued on SB and AC. In the nursery phase, average daily gain (ADG), average daily feed intake (ADFI), and feed per gain (F:G) were depressed ($P < .05$), while serum gamma-glutamyltransferase (GGT), alkaline phosphatase levels, and liver weights were increased ($P < .05$) in pigs fed AC. Feeding SB with AC increased ($P < .05$) ADG and ADFI, and lowered ($P < .05$) serum enzyme levels. In the grower phase, performance and serum chemistry did not differ across treatments, but livers of pigs previously fed AC remained larger ($P < .01$). Pigs fed AC had depressed ($P < .05$) Na

absorption and increased P ($P < .05$) and Zn ($P < .10$) absorption compared with controls. SB decreased Na, K and Mg ($P < .01$), and P and Zn ($P < .10$) absorption when compared with pigs fed no SB. Pigs fed SB had lower Mg ($P < .01$) and Zn, Mn, and Na ($P < .10$) retention when compared to controls. SB decreased Ca absorption and retention when fed with AC and increased absorption when fed with control corn ($P < .01$). AC and SB treatments had no effect on Cu absorption or retention. Liver mineral levels were not affected in any of the phases. In Study II, two trials were conducted to examine the effectiveness of eight types of clays in reducing the negative effects of AC. In trial I, seven types of clays (.5%) were fed with AC (500 ppb). ADG and ADFI, and urea N were reduced ($P < .05$), and serum aspartate aminotransferase and GGT were increased ($P < .05$) by feeding AC compared with NC. Feeding all the clays with the AC improved ADG and ADFI ($P < .05$) when compared with pigs fed the AC alone. Some clays were effective in recovering AC altered serum chemistry. In trial II, hydrated Na Al silicate or calcium bentonite (1%) were fed with AC (171 ppb). ADG and ADFI which were reduced for the first 4 wk by feeding AC ($P < .05$) were improved ($P < .05$). Humoral immune response was unaffected. In summary, feeding clay with AC can prevent some of the negative effects of aflatoxicosis with minimal influences on mineral metabolism.

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Chapter I

INTRODUCTION

The contamination of crops with mycotoxins can be a problem for grain producers and buyers alike. Mycotoxin contamination can result in reduced yields and reduced market prices for the grain producer (CAST, 1989). For the grain buyer, specifically the swine producer, there are the problems of lowered animal productivity and longevity when contaminated grains are fed (Friend and Trenholm, 1988). Mycotoxins such as aflatoxins are known to reduce feed consumption and growth rate (Lindemann and Blodgett, 1991) as well as impair liver (Harvey et al., 1990) and immune (Panangala et al., 1986) function.

Traditional methods of dealing with the mycotoxin problem have been through prevention and detoxification. Efforts to minimize conditions conducive to mycotoxin growth and contamination have not always been successful. Also, efforts to reduce toxic levels of contaminated grains by blending, screening and ammoniation have met with limited success (Lindenfelser and Ciegler, 1970; Brekke et al., 1975; Feuell, 1977; Conway et al., 1978). A new approach to handling mycotoxin contamination is to reduce the bioavailability of the toxin to the animal when ingested. The use of clays, already approved for addition to livestock diets as anti-caking agents, to bind aflatoxins in the

gastrointestinal tract has been very successful (Phillips et al., 1988). Feeding clays in combination with contaminated feeds to chickens (Kubena et al., 1990) and pigs (Lindemann and Blodgett, 1991) has improved performance and decreased toxic effects such as liver damage (Harvey et al., 1991) and reduced immune response (Lindemann et al., 1988). However, the mode of action of the clays has not yet been elucidated and many questions remain as to whether the clays have side effects.

This research was conducted to examine the effects of feeding clays in conjunction with aflatoxin contaminated diets to swine. Specifically, growth performance, mineral absorption and retention, immune response and liver function were investigated.

Chapter II

REVIEW OF LITERATURE

Mycotoxins

Mycotoxins are chemical substances, produced by fungi, which are toxic to animals. There are three major genera of fungi which produce mycotoxins: *Aspergillus*, *Fusarium* and *Penicillium*. These fungi produce mycotoxins which exert their effect directly or indirectly by producing a metabolite which can influence a biological process (Friend and Trenholm, 1988). The effects of mycotoxins range from decreased performance to death (CAST, 1989).

Types of Mycotoxins. Each of the three major genera of fungi produce a variety of mycotoxins. The most abundant mycotoxins are the aflatoxins produced by *Aspergillus flavus*, the ochratoxins produced by *Penicillium viridicatum*, and zearalonone produced by *Fusarium graminearum* (CAST, 1989). Each of these toxins forms several different metabolites, each of which has a different structure and different effects (Hsieh and Wong, 1982).

Production of Mycotoxins. The production of each mycotoxin is favored by different environmental conditions (Tuite, 1979), but temperature and moisture are the two primary influencing factors (Montani et al., 1988). Aflatoxin production is favored by high temperatures and

high humidity such as that found in the southeastern United States. Zearalonone production is favored by cool temperatures and high humidity, conditions found in the Midwest in late summer. Mycotoxins can be produced in the field and in storage as long as the conditions are favorable (Tuite, 1979). Other factors that can influence mycotoxin production are insects, birds, and humans. All of these can spread molds and fungi and damage crops so that they are more easily inoculated. Additionally, humans are able to control storage conditions which can influence toxin production.

Effects of Mycotoxins. The effects of mycotoxins vary with the type of toxin, species and age of animals, method of exposure, and other factors. Generally, younger animals are more susceptible to mycotoxins and ingestion is the primary mode of exposure (Heathcote and Hibbert, 1978). The effects range from very specifically identified reactions such as the suppression of DNA expression and cell membrane alterations (Wogan, 1966), to the general decline in animal performance such as reductions in feed intake (Harvey et al., 1988). Some of the other effects include the estrogenic responses of pigs exposed to zearalonone (CAST, 1989), the carcinogenic and hepatotoxic effects of aflatoxin exposure in poultry and cattle (Hsieh and Wong, 1982), and

the nephrotoxic reaction of humans and dogs exposed to Ochratoxin A (CAST, 1989).

Aflatoxins

The study of aflatoxins was initiated with the discovery of "Turkey X disease" in 1960 (Betina, 1989). Since that time scientists have solved many of the mysteries of the fate of aflatoxins and their metabolites. Aflatoxins are produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus* and exist in several structural forms. The main forms of the toxin are B_1, B_2, G_1, and G_2, and the metabolic forms of M_1 and M_2. The most studied aflatoxin is B_1 (AFB_1) because it is the predominant and most toxic of the structures (Hsieh and Wong, 1982). Unlike most other mycotoxins, AFB_1 requires biotransformation to exert its effects. AFB_1 is transformed endogenously to the epoxide form, which is recognized as the active metabolite form (Betina, 1989). Some of the underlying effects of AFB_1 include the inhibition of DNA replication, the inhibition of RNA and protein synthesis, the alteration of cell walls, and the disruption of oxidative phosphorylation (Betina, 1989).

The effects of aflatoxins on animals have been well investigated because they can have a significant adverse economic impact (CAST, 1989). Research on aflatoxins in meat animal species has been focused mainly on poultry and

swine, because these animals are commonly fed grains which are susceptible to aflatoxin contamination.

Effects on Performance. The adverse effects of aflatoxins on livestock and poultry performance are well documented (Hintz et al., 1967; Sisk and Carlton, 1972; Lindemann and Blodgett, 1991). Giambrone et al. (1985) reported a 55% decrease in daily gains of turkeys fed 800 ppb AFB_1 in a five week study. Broiler chicken growth was depressed 73% when birds were fed 500 ppb AFB_1 by Huff et al. (1986). Lindemann et al. (1988) reported a linear depression in the gains of weanling pigs fed graded levels of AFB_1. Several studies have also shown a reduction in performance of grower pigs fed 250 ppb AFB_1 (Armbrechtal, 1971; Harvey et al., 1988). Depressed gains of 55% with 250 ppb (Harvey et al., 1990) to 78% with 400 ppb AFB_1 (Harvey et al., 1988) have been reported for swine. Southern and Clawson (1979) reported a linear depression in gains of finishing swine given increasing levels (385 to 1480 ppb) of AFB_1 with 385 ppb being the lowest level to elicite a response.

The primary influence of AFB_1 on growth rate is reduced feed intake. Panangala et al. (1986) reported a reduction in feed intake among weanling pigs fed 500 ppb AFB_1 compared with control pigs. Similarly, Coffey et al. (1989) reported a 14% reduction in feed intake of weanling pigs fed levels

of AFB_1 as low as 182 ppb, compared to controls. Harvey et al. (1988) reported a 45% decrease in feed intake within the first 7 d and a 62% decrease over a 4 wk test for growing barrows given 400 ppb AFB_1 versus control pigs. Southern and Clawson (1979) reported a linear reduction in feed intake for finishing pigs as aflatoxin level (385 ppb to 1480 ppb) in the diet increased.

Contradictory results have been reported for the effects of dietary aflatoxin on feed conversion. Lindemann and Blodgett (1991) reported no change in feed efficiency when 800 ppb AFB_1 was fed to weanling pigs. However, Panangala et al. (1986) reported a reduction in feed efficiency when 300 ppb AFB_1 was fed to weanling pigs. Inclusion of 800 ppb AFB_1 in turkey diets reduced feed conversion efficiency by 21% (Giambrone et al., 1985). Southern and Clawson (1979) found that feed conversion of finishing pigs was adversely affected only when the dietary level of AFB_1 was very high (1480 ppb). Ambrecht et al. (1971) reported no change in feed efficiency for growing pigs with 400 ppb AFB_1 until after 6 wk of feeding; lower levels (100 and 200 ppb) took longer to reduce feed efficiency. Conversely, Harvey et al. (1988) noted a decrease in feed per gain ratio for growing barrows after 7 d of feeding and then no significant response thereafter.

Effects on Organs. Several organs are known to be affected by aflatoxicosis, with the liver showing the most notable effects. Relative weights of both livers and kidneys are known to be increased in chickens (Maurice et al, 1982; Huff et al., 1986) and pigs (Armbrecht et al., 1971; Murthy et al., 1975; Harvey et al, 1990) exposed to aflatoxins. The increase in relative liver size is attributed to an increase in liver lipid content (Sisk and Carlton, 1972; Maurice et al., 1982). Bryden et al. (1979), however, reported no increase in chicken liver lipogenic activity to account for the increase in lipid content. Instead, they reported a decrease in lipogenic enzyme activity. Thus, their work suggests that there is a negative feedback mechanism slowing lipogenesis and decreasing lipid transport from the liver during aflatoxicosis.

Bryden et al. (1979) also showed that the activity of other liver enzymes was decreased for chickens given 300 ppb AFB_1 for 3 wk, including hepatic ATP citrate lyase and NADP-malate dehydrogenase. Liver RNA and DNA concentrations were also reduced. Additional indicators of altered liver function can be seen in serum chemistry profiles. Increases in the serum levels of aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), and alkaline phosphatase (ALP), which are indicators of liver damage (Kaneko, 1989),

have been reported for several studies in which swine were fed aflatoxins (Harvey et al, 1989a, 1990; Lindemann and Blodgett, 1991). The aflatoxin effect of reduced liver protein synthesis (Yu, 1977) has resulted in significantly lowered levels of serum albumin, urea N and total protein in growing pigs fed 250 ppb AFB_1 (Harvey et al, 1988, 1989b; 1990). Southern and Clawson (1979), however, reported no significant differences in the serum levels of albumin and total protein in finishing pigs fed up to 1480 ppb AFB_1. They attributed the lack of an observeable effect to the larger size of their animals compared with those used in other studies.

Effects on Serum Glucose. Blood glucose levels have been shown to be decreased in chickens (Maurice et al., 1983), lambs (Harvey et al., 1991), and pigs (Harvey et al., 1989b) fed AFB_1. Shankaran et al. (1970) described the effects of aflatoxins on carbohydrate metabolism as a reduction in glycogenic enzyme activity. Decreases in enzymes such as glycogen phosphorylase and glucose-6-phosphate have been reported in chickens fed AFB_1 (Hsieh, 1979).

Effects on Mineral Metabolism. Serum mineral levels have also been shown to be affected by aflatoxin ingestion. Harvey et al. (1989b) reported depressed levels of Ca, Mg, K, Na, and P in serum of growing pigs fed AFB_1 (250 ppb)

for 28 d. Lanza et al. (1979) found that chickens exposed to aflatoxins had reduced iron absorption and retention when compared with birds on control diets. In further investigations, Lanza et al. (1981) suggested a condition of hypoproteinemia that could be influencing Fe absorption in birds fed AFB_1.

Maurice et al. (1983) found that hepatic Zn, Cu, and Mn levels were reduced as aflatoxin levels in the diets of chickens increased. Plasma albumin levels were also found to be reduced, which may account for a lower level of Zn and Cu transport in hepatocytes, because albumin is responsible for the transport of these minerals. The reduction in protein synthesis may also have an effect on proteins such as metallothionein, which have roles in mineral metabolism.

Effects on Immunity. The primary effect of aflatoxin on the immunity of animals is the depression of cell-mediated immunity (Thurston et al., 1980; Ray et al., 1991). There is also some evidence suggesting effects on humoral immunity (Richard et al., 1975). Cell mediated immunity in turkeys has been found to be sensitive to aflatoxin levels as low as 200 ppb (Giambrone et al., 1985). However, these same researchers found no evidence of reduced humoral response with dietary levels of AFB_1 as high as 800 ppb. Similarly, Panangala et al., (1986) found a decrease in cell-mediated

response in weanling pigs fed 500 ppb AFB_1, but no effect on humoral response.

Detoxification of contaminated grains

The best practice for the controlling aflatoxins is prevention. Certain management practices can help prevent the contamination of crops with aflatoxins. Efforts to control insects, reduce plant diseases, harvest on time, and minimize crop damage can significantly reduce chances for crop contamination (Goldblatt and Dollear, 1979). During storage, care must be taken to maintain proper temperature, and moisture levels and to minimize insects. Heating, physically separating, irradiating and fermenting all have been used to detoxify feeds with limited success (Lindenfelser and Ciegler, 1970; Brekke et al., 1975; Feuell, 1977; Conway et al., 1978). Ammoniation of feeds has proven to be one of the most effective methods of detoxification, but the caustic nature of ammonia makes it a dangerous procedure (Norred, 1979). The blending of feeds to lower the concentration of toxins is also a practical method of diluting and reducing contamination levels before feeding, however, equipment costs may be high (CAST, 1989). Technical methods other than ammoniation have proven effective but not practical (CAST, 1989).

Control of the Bioavailability of Toxins. A relatively new method of reducing the toxicity of mycotoxins is by

controlling the bioavailability of the toxins to animals fed contaminated feeds. The use of clays like zeolites, bentonites, and sepiolites has proven beneficial in reducing the toxic effects of mycotoxins. Presently, some of the uses of these clays are as sealants, cat litter, water filters, detergents, and roof coatings. Since the 1960's, these clays have also been used by the feed industry as anti-caking agents and in the manufacturing of pellets to increase pellet durability. Although Kurnick and Reid (1960) suggest bentonites may have beneficial effects on feed intake and gains, Taverner et al. (1984) reported that sodium bentonite is indigestible and inert when added to growing pig diets. Oliver (1989) found the addition of clays had no influence on weight gain performance or feed intake when added to layer chicken diets.

Clays and Aflatoxins. The sorbent properties of hydrated sodium calcium aluminosilicates (HSCA) and bentonites have proven beneficial in reducing the effects of aflatoxins when fed to livestock. Blodgett et al. (unpublished data) found that in aqueous solution, bentonites, sepiolites, and attapulgites remove essentially 100% of the AFB_1 placed in the solution. They also found that under pH and temperature conditions that mimic the pig's intestinal tract, the clays were still found to be 92 to 100% effective in removing the aflatoxin from solution.

Phillips et al. (1988) found that zeolites absorbed AFB_1 in several different solutions with up to 98% efficiency. These studies demonstrate a binding potential, but the degree of binding is dependent on both the concentration of aflatoxin and the concentration of the clay.

Performance Recovery. Zeolites were found to eliminate the detrimental effects of reduced body weights and reduced feed consumption caused by zearalonone fed to rats (Smith, 1980). By binding T-2 toxin in the gut and by reducing intestinal transit time, bentonites were found to prevent T-2 toxicosis in rats (Carson and Smith, 1983). Lambs fed 2% HSCA and 260 ppb AFB_1 showed performance levels equal to control animals, but lambs given AFB_1 alone had significantly depressed body weights (Harvey et al., 1991). Similarly, Kubena et al. (1990) reported a 50 to 67% recovery of a 21 to 38% growth depression of chickens due to the feeding of 750 ppb AFB_1 when .5% HSCA was added. Lindemann et al. (1988) reported an 85% recovery of growth losses due to feeding 840 ppb AFB_1 when .5% HSCA was fed to weanling and growing pigs. In further studies, Lindemann and Blodgett (1991) reported an 83% recovery of aflatoxin depressed growth with .5% HSCA; the use of .5% sodium bentonite recovered gains slightly more than the .5% HSCA. Similarly, growing pigs have recovered 94% of losses in daily gains associated with feeding AFB_1 (30% growth

depression) when .5% HSCA was mixed in the diet (Colvin et al., 1989). However, Harvey et al. (1989a) reported a significant depression in growth of growing barrows fed 300 ppb AFB_1 with no significant improvement in performance when .5% HSCA was fed. Depressions in feed consumption associated with aflatoxin intake have also been overcome with the addition of .5% HSCA in the diet (Colvin et al., 1989; Lindemann et al., 1989, 1990).

Immunity Recovery. Lindemann et al. (1988) reported a linear decline in cell-mediated immunity with increasing aflatoxin levels in the diet and a significant increase in cell-mediated response with the addition of .5% HSCA.

Liver Function Recovery. The addition of clays to growing swine diets with 500 ppb AFB_1 reduced the levels of AFB_1 in the liver (Beaver et al., 1990). The relative weights of livers of growing pigs and chickens fed AFB_1 were reduced to normal when .5 % HSCA was added to the diets (Harvey et al., 1989a; Kubena et al., 1990). Liver function was also apparently not damaged when HSCA was fed with aflatoxin contaminated diets. Lambs fed 2% HSCA with 260 ppb AFB_1 showed serum AST, GGT, and albumin levels equal to those of lambs on control diets (Harvey et al., 1991). Similarly, chicken serum GGT, albumin, and total protein levels were restored to normal when HSCA was fed (Kubena et al., 1990). Swine serum urea N, AST, ALP, GGT, albumin, and

total protein levels have all been reported to be restored to control levels in pigs given .5% HSCA and AFB_1 versus animals on AFB_1 alone (Lindemann et al., 1988, 1990; Beaver et al., 1990).

Mineral Metabolism and Clays. Because clays readily bind to aflatoxins, there is concern regarding the interaction of clay with minerals in the gastrointestinal tract. The addition of sodium zeolite-A to swine grower diets increased liver and bone Zn, and decreased serum Ca and P, with no effect on bone Ca, P, Mn, Fe, Na, Ni, and Al (Ward et al., 1991). Pond et al. (1988) reported that the addition of 2% clinoptilolite had no effect on liver Cu stores. Adding .5% HSCA to swine diets returned aflatoxin depressed serum levels of P and Ca to levels similar to those of the controls (Harvey et al., 1989a). Lindemann et al. (1988, 1990) found no effects on serum Ca, Mg, and K levels when HSCA was added to swine diets.

Summary

Mycotoxins have proven to be a problem for swine producers. Aflatoxins are of particular importance in the southeastern United States where conditions favor their production. The feeding of aflatoxin-contaminated diets causes significant depressions in daily gains, feed intake, and in some cases feed efficiency. Livers are enlarged and protein synthesis is greatly reduced during aflatoxicosis.

Immunity is also impaired by aflatoxin ingestion. Current methods of dealing with aflatoxins, including mixing, screening, and ammoniation, have had limited success. The recent use of clays to restrict the bioavailability of aflatoxins in the gastrointestinal tract has been very successful. Clays have been shown to recover about 80% of aflatoxin-induced performance losses. Additionally, liver function and immune response has been partially restored with the addition of clays to swine diets. However, questions still remain regarding other influences that the clays may have on the metabolism of pigs.

Chapter III

OBJECTIVES

The purpose of this research was to investigate the effectiveness of feeding clays in combination with aflatoxin contaminated diets to swine. The specific objectives addressed in two studies were:

Study I.

Nursery phase

a: to determine the performance (ADG, ADFI, F:G) of weanling pigs fed aflatoxin contaminated and non-contaminated diets with and without the addition of 1% sodium bentonite.

b: to examine the effects of these diets on relative kidney and liver weights.

c: to investigate the influence of these diets on blood serum levels of glucose, urea N, total protein, albumin, AST, GGT, ALP, Ca, P, Mg, Na, K, Fe, Cu, Zn and Mn.

d: to determine the effects of these diets on liver levels of Ca, P, Mg, Na, K, Zn, Fe, and Cu.

Grower phase

a: to investigate the effects of withdrawing aflatoxin from the diet at the end of the nursery phase but continuing clay treatments on performance (ADG, ADFI, F:G) in the grower stage (5 weeks).

b: to examine the effects of these treatments on the parameters listed in b through d of the nursery phase.

Metabolism phase

a: to investigate the continuous feeding of the diets described in the nursery phase on the absorption and retention of Ca, Mg, P, K, Na, Zn, Cu, Fe, and Mn in growing barrows.

b: to examine the effects of these diets on relative liver and kidney weights.

c: to determine the influence of these diets on liver levels of Ca, P, Mg, K, Na, Cu, Fe, and Zn.

Study II.

Trial I

a: to compare the use of different types of clays on the performance (ADG, ADFI, F:G) of weanling pigs fed aflatoxin contaminated diets (500 ppb).

b: to investigate the effects of these diets on blood serum levels of glucose, albumin, total protein, urea N, AST, GGT, ALP, Ca, P, Mg, K, and Na.

Trial II.

a: to compare the use of a hydrated sodium calcium aluminosilicate and a calcium bentonite on the performance of weanling pigs fed aflatoxin contaminated diets (171 ppb).

b: to examine the effects of these diets on blood serum levels of glucose, albumin, total protein, urea N, AST, GGT, ALP, Ca, Mg, P, K, and Na.

c: to investigate the influence of these diets on the humoral immune response of weanling pigs.

Chapter IV

EFFECT OF FEEDING AFLATOXIN CONTAMINATED SWINE DIETS WITH AND WITHOUT CLAY ON PERFORMANCE, LIVER FUNCTION, AND MINERAL METABOLISM OF WEANLING AND GROWING PIGS

T.C. Schell, M.D. Lindemann, E.T. Kornegay and D.J. Blodgett

Virginia Polytechnic Institute and State University, Blacksburg. 24061

ABSTRACT

Crossbred weanling pigs (n=96) with an average age of 36 d and average initial weight of 8.75 kg were used in a three phase study to determine the effects of feeding aflatoxin contaminated diets with and without 1% sodium bentonite. In the nursery phase, a control corn or an aflatoxin contaminated corn (diets contained 922 ppb) was fed in a corn-soybean meal diet with and without 1% clay for 6 wk. Relative to controls, aflatoxin depressed ADFI and ADG ($P < .01$), and increased feed to gain ratios ($P < .05$) for the total trial, whereas the addition of clay increased feed intake and ADG ($P < .01$). Serum gamma glutamyltransferase were increased ($P < .01$), and alkaline phosphatase, and Fe tended to be increased ($P < .10$) by the aflatoxin treatment, and serum urea N, Cu, and bilirubin were decreased ($P < .05$) when compared to controls. Relative weights of livers were larger in aflatoxin-fed pigs ($P < .05$) and tended to be smaller in pigs fed clay ($P < .10$) compared with controls, but kidney weights were unaffected by treatments. Serum

levels of Ca, Mg, P, Na, K, Zn, and Mn, and liver content of Ca, Mg, P, Na, K, Zn, Fe, and Cu were not affected by treatments. In the grower phase, 48 pigs from the nursery phase were continued on their respective clay treatments for 5 wk, but non-contaminated corn replaced the aflatoxin contaminated corn. Average daily gain, feed intake, and feed efficiency were not affected by previous treatment or clay treatment. Pigs previously fed aflatoxin had lower serum glucose, Ca and K ($P < .05$), but serum enzyme levels, albumin, total protein, urea N, and liver minerals were not affected by treatments. Pigs fed aflatoxin in the nursery phase continued to have enlarged livers ($P < .05$) and pigs given clay had smaller livers than controls ($P < .05$). In the metabolism phase, 24 barrows (from the original 96) were continued on the same treatments as fed in the nursery for two total urine and feces collection periods of 4 d each. Aflatoxin increased serum GGT ($P <.05$), while clay tended to increase serum Ca, Na, Cl, Zn, ($P < .10$), and increase P and Cu ($P < .05$). Feeding aflatoxin depressed ($P < .05$) Na absorption and increased P absorption ($P < .05$) and retention ($P < .10$). Zn absorption tended to be higher ($P < .10$) in pigs consuming aflatoxin diets. The addition of clay to the diets decreased ($P < .01$) Na, K and Mg absorption and tended to decrease ($P < .10$) P and Zn absorption and when compared with control corn fed pigs.

Pigs fed clay had lower ($P < .01$) Mg retention and tended to have lower ($P < .10$) Zn, Mn, and Na retention when compared to pigs not fed clay. Clay decreased Ca absorption and retention when fed with aflatoxin and increased absorption when fed with control corn ($P < .01$). Aflatoxin and clay treatments had no effect on Cu absorption or retention. No treatment effects were found on the liver levels of Ca, P, Na, K, Fe or Cu. Weights of kidneys were smaller ($P < .05$) in pigs fed aflatoxin diets compared with pigs fed control corn diets, but relative weights of livers were larger ($P < .01$) for pigs treated with aflatoxin and were reduced ($P < .05$) when pigs were fed aflatoxin-diets treated with clay. Feeding clay with aflatoxin contaminated corn results in a partial recovery of performance and liver function without greatly influencing mineral absorption or retention.
Key Words: Pigs, Aflatoxin, Bentonite, Minerals

Introduction

Aflatoxin-contaminated feed has proven detrimental to the swine industry (CAST, 1989). Swine fed contaminated feed experience reduced feed intake, lowered daily gains, and in some cases poorer feed efficiency (Harvey et al., 1988). Physiological effects of aflatoxin feeding include liver damage characterized by enlargement, release of enzymes into the blood [Aspartate aminotransferase (AST), gamma glutamyltransferase (GGT), alkaline phosphatase (ALP)]

and impaired protein synthesis (NRC, 1979). Traditional methods of dealing with aflatoxin-contaminated grains, including blending, ammoniation, and screening, have been aimed at reducing toxin levels, and have met with limited success. Recent studies have shown that the addition of several clays to contaminated diets can reduce the bioavailability of toxins in the gastrointestinal tract. The clays, which are mined throughout the United States, have very high adsorptive properties and have been found to have the ablitity to absorb up to 100% of aflatoxins in solution with 10 ppm aflatoxin (AFB_1) (Blodgett et al., unpublished data). Up to 85% of performance losses due to aflatoxins has been recovered by the addition of clay to the contaminated diets, and liver damage due to aflatoxins has also been prevented (Lindemann and Blodgett, 1991). However, little is known as to whether the clays have negative effects on the availability of other nutrients.

This study was conducted to examine the effects of feeding clay (sodium bentonite) on performance, liver function, and mineral absorption and retention of weanling and growing pigs fed control and aflatoxin contaminated diets.

Materials and Methods

A total of 96 crossbred weanling pigs with an average initial weight of 8.75 kg and age of 36 ± 9 d were used in a three phase study (nursery, grower and metabolism).

Nursery Phase. Weanling barrows and gilts were randomly assigned from outcome groups based on gender, litter, and body weight to two levels of aflatoxin, (0 and 922 ppb) and two levels of clay[1] (0 and 1%) in a 2 X 2 factorial arrangement of treatments. An 18.9% crude protein corn-soybean meal basal diet was formulated to meet or exceed NRC (1988) levels of vitamins and minerals for 10 to 20 kg pigs (Table 1). All diets were sampled and later analyzed for mineral content.

Pigs (n=96) were housed eight pigs per pen in plastic-coated welded wire floored pens (1.22 m X 1.83 m) with three replicate pens per treatment. Pigs were weighed and pen feed intake was determined weekly. Blood samples were taken by anterior vena cava venipuncture initially and on d 42 of the 6 wk trial.

At the conclusion of the nursery trial, two barrows representative of the performance average for their respective treatments were selected from each nursery pen for the metabolism phase. From the remaining pigs, two pigs

[1]Volclay-90, a product of American Colloid Co., Arlington Heights, IL 60006.

from each pen were randomly selected for sacrifice and livers and kidneys were removed and frozen for later analysis. The remaining pigs were continued on treatments for the grower phase as outlined below.

Grower Phase. At the conclusion of the nursery phase pigs (n=48) were moved to 1.52 m x 3.05 m pens with partially slotted concrete floors (60% slotted and 40% solid area). All pigs were maintained with penmates from the nursery phase. Crude protein content of the diets was lowered to 15.9% and only non-contaminated corn was used (Diets I and III, Table 1). Pigs on diets I or II in the nursery phase were fed diet I in the grower phase and pigs on diets III or IV in the nursery phase were fed diet III in the grower phase. Clay level (1%) was continued as in the nursery phase. Diets were again sampled for later analysis of mineral content.

Pigs were weighed weekly and pen feed consumption was determined at that time. After 34 d on test, blood samples were taken from all animals by venipuncture. At the conclusion of the trial (d 35), one male and one female randomly selected from each pen was sacrificed by electro-immobilization and exsanguination. Livers and kidneys were removed, weighed, and frozen for later mineral determination.

Metabolism Phase. After the completion of the nursery phase, two barrows from each pen were randomly assigned to one of two groups, A or B.

Group A (n=12) animals were immediately placed in stainless steel metabolism cages. These barrows were continued on the same treatments they received in the nursery phase, except that the CP was lowered to 15.9%; they were fed 8% of metabolic body weight ($kg^{\cdot.75}$) per day divided into two equal feedings (0700 and 1500). Animals were given 1 h for eating, after which the ort was removed, weighed and recorded. Deionized water was available for 1.5 h, beginning at the start of feeding.

Barrows were given 4 d to adjust to the cages. On d 4, at the 0700 feeding, .05% Cr_2O_7 was added to all diets and fed. On d 8, barrows were again fed diets to which .05% Cr_2O_7 was added at the 0700 feeding. Total feces were collected during all feeding periods from the time the first marker was seen in the feces until the second marker was seen. Daily fecal collections were weighed and recorded, and the total fecal collection from each pig was stored at -10 °C. Total urine was collected starting 2 h after the feeding of the first marker until 2 h after the feeding of the second marker. Urine was collected through glass wool into 20 l plastic jugs containing 25 ml of 25% H_2SO_4. The pH of the urine was kept below 5 at all times to prevent

loss of ammonia. Collection jugs were emptied into storage containers, and volumes were determined and recorded daily.

At the end of the collection period, the total volume of urine per pig was mixed and representative urine samples (500 ml) were taken for each pig and frozen for later mineral analysis. After thawing at room temperature, total fecal samples for each barrow were oven dried at 60°C and ground to pass a 1 mm screen, then frozen for later mineral analysis.

At the begining of the metabolism phase, group B pigs (n=12) from the same dietary treatments were placed in 1.22 m x 1.83 m plastic-coated welded-wire floored pens (three pigs/pen). The crude protein level was lowered to 15.9%, but aflatoxin and clay treatments were continued as in the nursery phase. Group B barrows were given access to feed and water ad libitum.

Following the collection period, the group A barrows were removed, weighed, and placed in pens (three pigs per pen from the same dietary treatment) similar to those occupied by the group B pigs. Group A animals were then given free access to feed and water; dietary treatments were continued. The metabolism cages were then cleaned and the Group B barrows were weighed and placed in the cages. Group B pigs were given 2 d to adjust to the cages, and then a 4 d collection was made using the same procedures as for Group

A. At the conclusion of this collection period, Group B pigs were replaced by Group A pigs. After 1 d of adjustment, a 4-d collection was made. Following the collection, Group A pigs were weighed, blood samples were taken by venipuncture, and then they were sacrificed by electro-immobilization and exsanguination. Livers and kidneys were removed, weighed, and frozen for later mineral analysis. Group B pigs were then placed in the cages, and a 1 d adjustment and 4 d collection procedure was followed. At the end of the collection, Group B barrows were weighed, bled, sacrificed, and tissues were collected following the same procedures as for Group A.

Tissue and feed analysis. All minerals except P were analyzed using flame atomic absorption spectrophotometry on a Perkin-Elmer Zeeman 5100 (Norwalk, CT). Feed, feces, and livers were prepared using nitric-percholic acid digestion (Perkin-Elmer, 1982). Livers were thawed at 7°C for 24 h, then homogenized using a Waring blender (Fischer, #14-509-101) before nitric-perchloric digestion. When necessary urine was diluted with deionized water prior to analysis. For all Ca and Mg analyses, samples were diluted in 1% lanthanum oxide. Phosphorus determination was done using the Fiske and Sabbarow (1925) procedure.

Blood Samples. Immediately after collection, all blood samples were placed on ice prior to being centrifuged for 15

min. Serum was removed and samples were frozen for later analysis. Serum levels of Zn, Cu, Fe, and Mn were determined using flame atomic absorption spectophotometry. All other serum chemistry analyses were performed at the Virginia-Maryland Regional College of Veterinary Medicine using a Kodak Ektachem 700 (Eastman Kodak, Rochester, New York).

Statistical Analysis. Performance data were analyzed using the General Linear Models (GLM) procedure of SAS (1988), with pen as the experimental unit. Orthogonal contrasts were used to determine clay and aflatoxin effects. Mineral levels in serum, livers, urine, and feces, as well as organ weights were also analyzed for statistical differences using the GLM procedure, with pig as the experimental unit. Orthogonal contrasts were again used to determine diet effects. Models for all analysis included effects for diet and replicate.

Results

Nursery Phase. The diet mineral content on an as fed basis was as follows: Ca, .75%; P, .58%; Mg, .14%; Na, .16%; K, .79%; Zn, 120 ppm; Mn, 40 ppm; and Cu, 22 ppm. The Fe content of diets with clay was 446 ppm; while the Fe content of diets without clay was 292 ppm.

Average daily gains (ADG) and daily feed intake (ADFI) were depressed ($P < .01$) for pigs fed diets containing

aflatoxin contaminated corn compared with those fed non-contaminated diets (Table 2). Pigs fed diets containing clay consumed more and grew faster ($P < .01$) than pigs fed diets without clay. The aflatoxin * clay interactions ($P < .01$) indicated that the magnitude of the increase in ADG and ADFI was greater for pigs fed contaminated corn diets compared with pigs fed non-contaminated corn diets. Pigs fed aflatoxin diets had larger feed to gain ratios ($P < .05$) than pigs fed the control corn diets; however, clay treatments and the aflatoxin * clay interaction were not significant. When performance values were adjusted for final weight (comparing pigs at a similar weight), feed to gain ratios continued to be higher ($P < .05$) for pigs consuming the aflatoxin diet, and the clay had no effect.

Serum GGT was increased ($P < .01$) and serum ALP tended to be increased ($P < .10$) in pigs fed the aflatoxin diets compared to pigs fed control corn diets (Table 3). The aflatoxin main effect for aspartate aminotransferase (AST) was not significant, but the aflatoxin * clay interaction was ($P < .05$). AST levels were elevated in pigs fed diets containing aflatoxin corn and no clay, but were reduced in pigs fed contaminated diets with clay. The addition of clay also reduced ($P < .05$) ALP levels for non-contaminated and contaminated diets. The aflatoxin * clay interaction indicated that clay tended to reduced ($P < .10$) GGT levels

in the presence of contaminated corn, but increased levels when fed in combination with non-contaminated corn.

Pigs fed aflatoxin diets had depressed ($P < .01$) serum Cu and tended to have elevated ($P < .10$) serum Fe compared with pigs fed non-contaminated diets (Table 3), but these minerals were unaffected by the addition of clay. Serum Ca (9.6 mg/dl), Mg (1.9 mg/dl), P (7.5 mg/dl), Na (141.6 mmol/L), K (6.6 mmol/L), and Zn (.6 ug/L) were not affected by the treatments.

Serum urea N tended to be depresssed ($P < .10$) with aflatoxin treatments, and clay had no effect on urea N. The aflatoxin * clay interaction was significant for serum glucose ($P < .01$); the addition of clay depressed glucose levels when added to the control corn diets, but increased glucose levels when added to the aflatoxin diets. Serum albumin levels were higher ($P < .05$) when clay was fed with the aflatoxin contaminated diets than when added to control corn diets. The clay and aflatoxin treatments did not have an effect on serum total protein. However, the interaction of aflatoxin and clay did tend to be significant ($P < .10$); the clay increased total protein in aflatoxin fed pigs compared to control fed pigs but had no effect on pigs fed control corn. Total serum bilirubin levels tended ($P < .10$) to be lower in pigs fed clay compared with pigs not fed clay.

Relative weights of livers of weanling pigs were larger ($P < .05$) in aflatoxin fed pigs, whereas clay fed pigs tended to have smaller ($P < .10$) livers than pigs fed no clay (Table 4). Weights of kidneys were not affected by aflatoxin or clay treatment. The dry matter liver contents of Ca (.4 ppm), P (7.9 ppm), Mg (1.0 ppm), Na (7.3 ppm), K (16.4 ppm), Cu (4.1 ppm), Fe (69.3 ppm), and Zn (19.8 ppm) were also unaffected by the aflatoxin or clay treatment (Appendix Table 5).

Grower Phase. As seen in Table 2, overall ADG, ADFI and feed to gain ratio were not affected by aflatoxin in the nursery or by clay throughout.

Pigs fed clay tended to have lower ($P < .10$) ALP levels (Table 5). Serum ALP levels were reduced more in pigs previously fed aflatoxin than pigs fed control corn ($P < .05$). GGT and AST levels were not different for clay treatment or previous aflatoxin treatment.

Serum Ca levels were lower ($P < .05$) in pigs previously fed aflatoxin compared to pigs not fed aflatoxin, and the aflatoxin * clay interaction showed a larger ($P < .05$) increase in Ca levels for pigs fed clay and previously fed aflatoxin over those fed clay with control corn. The clay treatment tended to increase ($P < .10$) serum Cu levels over the levels of pigs not fed clay. Previous aflatoxin treatment had no effect on serum Cu levels. Serum K was

lower ($P < .01$) in pigs previously fed the aflatoxin diet with serum Na and Cl values numerically lower but not significantly lower. The aflatoxin * clay interaction shows that serum levels of K, Na, and Cl were all higher in pigs fed clay and aflatoxin in the nursery phase and decreased when clay was fed with the control diet ($P < .05$, .10, .01 respectively).

Glucose levels in the serum of pigs previously fed aflatoxin were lower ($P < .01$) than for pigs fed no aflatoxin. Clay had no effect on serum glucose. Serum values for urea N (9.85 mg/dl), albumin (3.6 g/dl), bilirubin (.17 mg/dl) and total protein (6.2 g/dl) did not differ across treatments (Appendix Table 8).

Pigs fed aflatoxin in the nursery phase continued to have enlarged livers in the grower phase ($P < .05$), and pigs fed clay had smaller livers ($P <.05$) compared with pigs not fed clay (Table 4). Kidney weights of pigs previously fed aflatoxin were also larger ($P < .05$) than pigs not fed aflatoxin. Liver dry matter content of Ca (.41 ppm), P (10.4 ppm), Mg (1.4 ppm), Na (6.6 ppm), K (12.7 ppm), Fe (104.5 ppm), Zn (38.1 ppm) and Cu (4.0 ppm) levels were not affected by previous aflatoxin or clay treatment (Appendix Table 9).

Metabolism Phase. The mean gain was 2.5 kg per pig for each collection-adjustment period with no differences between treatments.

Serum chemistry profiles showed pigs fed aflatoxin tended to have higher ($P < .10$) levels of GGT in their serum than pigs not fed aflatoxin (Table 6). There was no effect of clay treatment on serum GGT levels. Serum ALP levels were normal and unaffected by both treatments. Serum Ca, Na, Cl, and Zn tended to be higher for those pigs fed clay ($P < .10$). Serum P and Cu were also higher ($P < .05$) in pigs fed clay. Aflatoxin had no effect on these minerals. Additionally, clay was more effective in raising serum Cu levels when fed with aflatoxin ($P < .05$) compared with when it was fed with control corn. Serum Fe (2.6 ug/L), Mg (2.1 mg/dl), and K (8.0 mmol/L) levels were unaffected by either treatment (Appendix Tables 10 and 12).

Addition of clay to the diets increased serum albumin ($P < .01$) and tended to increase serum glucose ($P < .10$), but aflatoxin had no effect. Total protein (6.5 g/dl) and urea N (9.85 mg/dl) in the serum were not affected by any treatment (Appendix Table 12).

Pigs fed aflatoxin diets had larger relative liver weights than pigs fed control diets (Table 4). The clay was effective in preventing increased liver sizes when aflatoxin was fed ($P < .05$). Relative weights of kidneys were smaller

($P < .01$) in pigs fed aflatoxin diets than pigs fed control corn diets. The clay prevented the lower kidney weights when fed with aflatoxin contaminated diets ($P < .01$). Liver minerals, including Ca (.38 ppm), P (8.7 ppm), Mg (1.1 ppm), Na (6.7 ppm), K (13.8 ppm), Fe (66.3 ppm), Zn (31.2 ppm), and Cu (4.2 ppm), were also unaffected by clay or aflatoxin treatment (Appendix Table 13).

As shown in Table 7, Ca absorption and retention were depressed ($P < .01$) when clay was fed with the aflatoxin diets, but were increased when clay was fed with control corn diets. Phosphorus absorption was increased ($P < .05$) and P retention tended to be increased ($P < .10$) for pigs fed aflatoxin diets compared with pigs fed control corn diets. Pigs fed clay had lower P retention ($P < .05$) and tended to have lower ($P < .10$) P absorption than pigs not fed clay. Aflatoxin had no effect on absorption or retention of Mg, but Mg absorption and retention were reduced ($P < .01$) by feeding clay. Mn absorption was not influenced by feeding aflatoxin diets, but Mn retention tended to be lower for pigs fed clay when compared with pigs not fed clay.

As shown in Table 8, feeding aflatoxin diets increased Na absorption ($P < .05$), but clay additions decreased Na absorption ($P < .01$). Na retention tended to be lower ($P < .10$) when clay was fed with aflatoxin. Absorption of K was

only affected by feeding clay; pigs fed clay diets had depressed ($P < .05$) K absorption. There were no effects of treatments on K retention. The percentage of Fe absorbed and retained was lower in pigs fed clay, but was not influenced by feeding aflatoxin corn. The absorption and retention of Zn tended to be higher in pigs fed aflatoxin diets ($P < .10$). Clay treatment tended to reduce Zn absorption and retention ($P < .10$). Cu absorption (20.0 $\pm$ 5.1) and retention (18.3 $\pm$ 5.1)were unaffected by aflatoxin of clay treatment.

Discussion

Performance. In agreement with results of Lindemann and Blodgett (1991) and Colvin et al. (1989), these studies indicate that the performance of weanling and growing pigs fed aflatoxin contaminated diets were improved with the addition of the sodium bentonite to the diets.

In the nursery phase, aflatoxin depressed ADG by 22% and ADFI by 20%, which was similar to responses reported by Lindemann and Blodgett (1991). As reported by Panangala et al. (1986), feed to gain ratios were also increased by aflatoxin compared to pigs not fed aflatoxin. The results for performance after adjustment for final weight indicated that the differences in feed to gain ratios were due to aflatoxin and not to differences in body weights.

When clay was added to the diets contaminated with aflatoxin, ADG and ADFI were improved by 20% and 24%, respectively, compared with pigs fed aflatoxin alone. However, as reported by Lindemann et al. (1990), the clay was ineffective in lowering feed to gain ratios when fed with aflatoxin.

Removal of the aflatoxin-contaminated corn during the grower phase resulted in a recovery of performance as ADFI and ADG were similar for all pigs regardless of previous aflatoxin treatment or current clay treatment. This suggests no apparent long term negative effects on performance of feeding clay. Similar results have been reported for chickens (Oliver, 1989).

The fact that the pigs that continued on the aflatoxin treatments in the metabolism study had gains similar to pigs fed control corn suggests that the limit feeding neutralized any differences in performance due to treatments.

Mineral Metabolism. In the grower phase, the lower serum Ca levels for pigs previously fed aflatoxin compared to pigs not previously fed aflatoxin could be attributed to the rapid increase in growth rate after removal from the aflatoxin. NRC (1988) has reported an increased need of Ca for faster growing pigs. The lack of aflatoxin response in serum Ca levels in the nursery phase probably reflects the slow growth rate of pigs with body stores that were able to

keep levels normal (Lepine et al., 1985). In the metabolism phase, the decrease in Ca absorption and retention when clay was fed with the aflatoxin corn is proably due to the relative increase in Ca absorption and retention of pigs fed aflatoxin with no clay compared with pigs fed control corn with no clay.

The increase in P absorption and retention in aflatoxin-fed pigs may be related to a possible increased need for P during aflatoxicosis. The increased need for P may be related to an increased energy need as P is associated with ATPase and energy utilization (Georgievskii, 1982).

Serum levels of Cu appeared to be related more to performance than to aflatoxin treatment. In the nursery phase, Cu serum levels were lower in the aflatoxin-fed pigs, which is consistent with their slower growth rate. However, in the metabolism phase, when feed intake was standardized and performance was not different between aflatoxin-fed pigs and control corn-fed pigs, serum Cu levels were also not different and Cu absorption and retention were unaffected by treatments. The correlation between serum Cu and performance is further supported by the grower study in which there was no treatment effects on performance or serum Cu levels.

The higher level of Fe in the serum of nursery pigs fed aflatoxin compared with pigs fed control corn is consistent

with higher serum Fe levels during hemolytic anemia (Lanza et al., 1979), which has been reported in chickens fed aflatoxins (CAST, 1989). The lack of significant differences among treatments in pigs removed from aflatoxin in the grower phase suggests a recovery from the anemic condition found in the nursery phase. The lack of anemic response to aflatoxins in the metabolism study is in agreement with Lanza et al. (1978), who found that the anemic response in chickens is reduced over time. The absorption of Fe was not increased in this study as reported by Lanza et al. (1979) in chickens. However, Lanza reported a return to normal absorption after 3 wk. The pigs in this study were fed aflatoxin for at least 6 wk before Fe absorption was measured.

The increase in Zn absorption and retention of aflatoxin fed pigs reflects an increased demand for Zn during aflatoxicosis. Pigs fed aflatoxins have been reported to have enlarged adrenal glands (Armbrecht et al., 1972), which have been reported to be associated with increased corticoid hormone production (Eskeland, 1976). An increase in glucocorticoid production has been reported to increase Zn demands (Kaneko, 1989). The increase in Zn demands is related to the numerous enzyme reactions mediated by Zn including protein synthesis. This increased demand is normally accompanied by a decrease in serum Zn levels. In

this study, however, Zn serum levels were not lowered, possibly due to the increase in Zn absorption and retention maintaining serum Zn levels.

The increase in Na absorption and alterations in K and Cl metabolism during aflatoxicosis suggests an acid-base imbalance not previously reported. In the grower phase, the lower serum K in pigs previously fed aflatoxin suggests an increased demand for K, possibly because of the role of K in the energy releasing mechanism of ATPase activation (Georgieviskii, 1982). The energy demands for the pigs removed from aflatoxin should be high for their increased growth rate.

The failure of aflatoxin to invoke a response in the mineral levels of the liver suggests that the alterations in mineral metabolism due to aflatoxins are not mediated by the liver. This may be due to either damage done to the liver, making it unresponsive, or the liver may not be the primary site of response.

The feeding of clay resulted in several effects on mineral metabolism. In the metabolism phase, feeding clay lowered K absorption, Mn retention, and P, Na, Zn and Mg absorption and retention. These result suggest the possibility that the clays bind with these minerals gastrointestinally making them unavailable for absorption.

Anion exchange capacity has been shown to effect the abilities of clays to bind to toxins (Carson and Smith, 1983). Therefore, it is not surprising that the one of the strongest electrolytes was found to be less absorbed when the clay was fed. The lower percentage of Fe absorption and retention when the clays were fed may reflect the higher level of clay in the diets with the clays. When the mineral level in the diet exceeds the level of requirement by the animal the efficiency of absorption is reduced (Georgieviskii, 1982).

In the grower phase, the addition of clay had the effect of elevating serum levels of Ca, Na, Cl, and Cu that were depressed by feeding aflatoxin corn diets. Because the metabolism of these minerals was not affected as much when clay was fed with aflatoxin corn diets compared with the effects observed when aflatoxin corn diets were fed without clay, it is apparent that supplemental clay was moderating some of the effects of feeding aflatoxin corn. This supports the hypothesis that the clays in some way reduce the effect of the aflatoxin in the gastrointestinal tract (Phillips et al. 1988). Blodgett et al. (unpublished data) observed that clays are capable of binding aflatoxin in vitro in solutions which mimic the conditions in the gastrointestinal tract of the pig. With the binding of aflatoxin in the intestinal tract and the resulting

deactivation or decreased absorption of aflatoxin, there is an increase in performance as seen here and elsewhere (Lindemman and Blodgett, 1991, Colvin et al., 1989). Thus, when aflatoxins are fed with clays there is a partial recovery of normal mineral metabolism.

Liver Function. Similar to results reported by Harvey et al. (1989, 1990), serum ALP and GGT were increased by the aflatoxin treatment in the nursery phase, indicating apparent liver damage. After a 5 wk withdrawal of aflatoxins, these serum enzymes associated with liver function had returned to normal levels. However, the pigs that continued on the aflatoxin treatment in the metabolism phase showed sustained liver damage by having higher GGT levels than pigs not fed aflatoxin. The return of serum ALP levels to normal in the metabolism phase is in contrast to results obtained by Harvey et al. (1989b, 1990), who found serum ALP levels increased in growing pigs fed aflatoxin compared with pigs fed control corn. However, the pigs in their study were fed aflatoxin for 3 wk, whereas pigs in the present study were fed aflatoxin for 10 wk. The results in this study, may indicate a reduction in the production of these enzymes after prolonged exposure to aflatoxins, or the standardization of feed intake may neutralize the increase in the level of these enzymes in the serum.

The clay treatment in the nursery phase was effective in reducing the high levels of these enzymes which is in agreement with results reported by Lindemann et al. (1990). Even after removal of aflatoxin-contaminated corn, the clay reduced ALP levels in pigs previously fed aflatoxin. However, in contrast to results in the nursery phase and the results of Beaver et al. (1990) and Lindemann et al. (1989, 1990), feeding the clay had no effect on serum enzymes of pigs fed aflatoxin diets in the metabolism phase. The lack of a response may be due to the lack of a significant increase in AST and ALP serum levels, possibly because the feed intake was restricted. In the nursery phase, clay was more effective in reducing enzyme levels when levels were elevated by aflatoxins.

The aflatoxin-induced lower levels of serum urea N in the nursery pigs, indicate a lower level of protein catabolism with a concurrent lowering of protein synthesis as evident by lower serum albumin (Kaneko, 1989). These results are consistent with previous reports of decreased protein synthesis in pigs during aflatoxicosis (CAST, 1989). The lack of a response for these protein indicators in the pigs fed aflatoxin in the metabolism phase suggests a lowered sensitivity toward aflatoxin. Armbrecht et al. (1971) found graded responses to different levels of aflatoxin when fed to grower pigs, indicating different

levels of sensitivity. In the grower phase, the lack of difference in serum total protein, albumin, and urea N levels suggest the recovery of normal protein synthesis after withdrawal from aflatoxin.

The clay was effective in raising serum albumin levels in both the nursery phase and the metabolism phase, with a greater effect on pigs fed aflatoxin. This along with lowered serum bilirubin levels in clay-fed pigs indicates a prevention of liver damage due to the addition of clay to the diets (Kaneko, 1989). The lack of a difference between treatments in the serum levels of albumin, urea N, total protein, and bilirubin in the grower phase pigs is indicative of restored protein synthesis and liver function after withdrawal from aflatoxin (Kaneko, 1989).

Serum glucose levels appear to be correlated more to growth performance than to aflatoxin treatment. In the nursery phase, the slow growing aflatoxin-fed pigs had glucose levels that were not different from those of control corn-fed pigs. When aflatoxin-fed pigs from the nursery phase were fed control corn in the grower phase, however, their glucose demands for growth may have been greatly increased, which may have caused the lowering of their serum glucose levels. The aflatoxin-fed pigs in the metabolism phase continued to grow at a slow rate, which may be the

reason that their serum glucose levels were similar to those of the pigs fed the control diets.

Feeding clay increased the serum glucose levels of pigs fed aflatoxin in the nursery phase and in the metabolism phase. This is again probably related to the ability of clay to bind aflatoxin in the gastrointestinal tract.

As reported by Armbrecht et al. (1971), Murthy et al. (1975) and Harvey et al. (1990), livers of pigs fed aflatoxins were enlarged in all the phases. Although the magnitude of relative increased liver sizes of pigs in the grower phase was smaller compared to pigs in the metabolism phase, the lack of recovery of liver size in pigs removed from aflatoxin in the grower phase suggests sustained liver damage even after withdrawal.

The feeding of clay was effective in reducing the relative liver sizes in all phases, especially when aflatoxin was fed. This is in agreement with Harvey et al. (1989) and Kubena et al. (1990) who also saw a prevention of increased liver size when clays were fed with aflatoxins. Kidney weights were affected in the metabolism phase which suggests a long term feeding effect. This is in agreement with Armbrecht et al. (1975), who reported increased kidney weights in pigs fed 400 ppb aflatoxin for 7 wk.

Implications

These experiments show that clay can effectively reduce some of the toxic effects of feeding aflatoxins to pigs. Performance and liver function were enhanced by the addition of clay to aflatoxin-contaminated diets, and mineral metabolism was only slightly affected by the clay. The lack of the ability of the clay to restore all functions indicates a varying degree of sensitivity to aflatoxins of the functions examined. The binding of only part of the available aflatoxins in the gastrointestinal tract, leaving some aflatoxin available for absorption, would allow for the more aflatoxin sensitive parameters to still be adversely affected.

TABLE 1. PERCENTAGE COMPOSITION OF DIETS

Item	Nursery Diets[a]				Grower Diets[b]			
	I	II	III	IV	I	II	III	IV
Non-contaminated Corn	68.95	28.95	67.95	27.95	76.90	36.90	75.90	35.90
Contaminated Corn[c]	-----	40.00	-----	40.00	-----	40.00	-----	40.00
Soybean meal (48% CP)	26.90	26.90	26.90	26.90	19.00	19.00	19.00	19.00
Soybean oil	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.20	1.20	1.20	1.20	1.40	1.40	1.40	1.40
Limestone	1.00	1.00	1.00	1.00	0.90	0.90	0.90	0.90
VPI vitamin premix[d]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[e]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
ASP-250[f]	0.25	0.25	0.25	0.25	----	----	----	----
Aureo-50[g]	----	----	----	----	0.10	0.10	0.10	0.10
Volclay-90[h]	----	----	1.00	1.00	----	----	1.00	1.00

[a]Calculated to supply 18.9% crude protein, .96% Ca, .74% P and 1.01% lysine.
[b]Calculated to supply 15.9% crude protein, .74% Ca, .59% P and .78% lysine.
[c]Corn contained 2305 ppb aflatoxin B_1, diets calculated to contain 992 ppb aflatoxin.
[d]Supplied per kilogram of diet: 4,400 IU vitamin A, 440 IU vitamin D, 11 IU vitamin E, 4.4 mg riboflavin, 22 mg d-pantothenic acid, 22 mg niacin, 489.5 mg choline, .022 mg vitamin B_{12}, .5 mg menadione, .44 mg d-biotin and .3 mg Se.
[e]Supplied per kilogram of diet: 150 mg Zn, 176 mg Fe, 60 mg Mn, 17 mg Cu, 2 mg I.
[f]Supplied 110 mg chlortetracycline, 110 mg sulfamethazine and 55 mg penicillin per kilogram diet.
[g]Supplied 5.1 mg chlortetracycline per kilogram diet.
[h]Volclay-90, American Colloid Co., Arlington Heights, IL.

TABLE 2. PERFORMANCE OF PIGS FED NON-CONTAMINATED (NC) AND AFLATOXIN-CONTAMINATED (AC) CORN WITH AND WITHOUT CLAY. NURSERY PHASE AND GROWER PHASE.

	Treatments				
	NC		AC[a]		
Items	No clay	Clay[b]	No clay	Clay[b]	SEM
Nursery phase (n=24/trt)[c]					
Body weights, kg					
Initial	8.78	8.78	8.80	8.72	.04
Final[dfh]	29.99	31.19	25.27	28.49	.47
ADG, kg[dfh]	.505	.534	.392	.471	.011
Average daily feed intake, kg[dfh]	1.10	1.15	.88	1.07	.03
Feed/gain[e]	2.17	2.16	2.25	2.27	.04
Similar final wt[e]	1.98	1.92	2.25	2.18	.08
Grower study (n=12/trt)[i]					
Body weights, kg					
Initial[e]	29.82	30.38	26.06	28.58	1.01
Final	59.42	58.50	61.85	58.73	1.42
ADG, kg	.903	.876	.975	.883	.042
Average daily feed intake, kg	2.05	1.97	2.38	2.20	.07
Feed/gain	2.27	2.26	2.44	2.49	.05

[a]Contaminated with 922 ppb aflatoxin B_1 in the diet.
[b]Volclay-90, American Colloid Co., Arlington Heights, IL.
[c]Nursery trial lasted 6 wk.
[de]Aflatoxin effect (contaminated diets vs non-contaminated diets; $P < .01$, .05 respectively).
[fg]Clay effect (clay diets vs non-clay diets; $P < .10$, .01 respectively).
[h]Aflatoxin*clay interaction effect ($P < .10$).
[i]All pigs given non-contaminated corn in grower phase, 5 wk trial. Performance values were adjusted using initial weight as a covariant.

TABLE 3. SERUM CHEMISTRY OF PIGS FED NON-CONTAMINATED (NC) AND AFLATOXIN-CONTAMINATED (AC) CORN WITH AND WITHOUT CLAY. NURSERY PHASE.

	Treatments				
	NC		AC[a]		
Items	No clay	Clay[b]	No clay	Clay[b]	SEM
Nursery phase (n=24/trt)					
Enzymes					
GGT, U/L[cg]	46.7	51.0	67.0	57.5	4.1
AST, U/L[eh]	66.1	68.0	81.6	53.6	5.9
ALP, U/L[df]	129.1	118.3	135.0	127.6	3.7
Minerals					
Fe, mg/L[d]	1.75	2.06	2.31	2.12	.16
Cu, mg/L[c]	1.58	1.67	1.41	1.44	.05
Other					
Urea N, mg/dl[d]	13.4	12.1	10.4	11.8	1.0
Glucose, mg/dl[i]	116.6	108.5	100.1	117.0	3.6
Albumin, g/dl[fh]	3.5	3.4	3.0	3.7	.2
Total protein, g/dl[g]	6.1	5.8	5.6	6.2	.2
Bilirubin, mg/dl[f]	.13	.12	.17	.12	.02

[a]Contaminated with 922 ppb aflatoxin B_1 in the diet.
[b]Volclay-90, American Colloid Co. Arlington Heights, IL.
[cd]Aflatoxin effect (contaminated diets vs non-contaminated diets; $P < .01$, .10 respectively).
[ef]Clay effect (clay diets vs non-clay diets; $P < .05$, .10 respectively).
[ghi]Aflatoxin*clay interaction effect ($P < .10$, .05, .01 respectively).

TABLE 4. WEIGHTS OF LIVERS AND KIDNEYS OF PIGS FED NON-CONTAMINATED (NC) AND AFLATOXIN-CONTAMINATED (AC) CORN WITH AND WITHOUT CLAY, EXPRESSED AS A PERCENTAGE OF LIVE BODY WEIGHT.

	Treatments				
	NC		AC[a]		
Items	No clay	Clay[b]	No clay	Clay[b]	SEM
Nursery phase (n=6/trt)					
Liver[ce]	3.16	2.99	3.44	3.05	.24
Kidneys	.55	.57	.54	.54	.02
Metabolism phase (n=6/trt)					
Liver[df]	2.13	2.33	2.40	2.43	.12
Kidneys	.41	.44	.34	.44	.03
Grower phase (n=6/trt)[g]					
Liver[ce]	1.78	1.67	1.90	1.78	.05
Kidneys[c]	.35	.36	.39	.38	.01

[a]Contaminated with 922 ppb aflatoxin B_1 in the diet.
[b]Volclay-90, American Colloid Co., Arlington Heights, IL.
[cd]Aflatoxin effect (contaminated diets vs non-contaminated diets; $P < .05$, .01 respectively).
[e]Clay effect (clay diets vs non-clay diets; $P < .05$).
[f]Aflatoxin*clay interaction effect ($P < .05$).
[g]All pigs given non-contaminated corn in grower phase.

TABLE 5. SERUM CHEMISTRY OF PIGS PREVIOUSLY FED NON-CONTAMINATED (NC) AND AFLATOXIN-CONTAMINATED (AC) CORN WITH AND WITHOUT CLAY. GROWER PHASE.

	Grower phase treatments				
	NC		NC[a]		
Items[b]	No clay	Clay[c]	No clay	Clay[c]	SEM
Enzymes					
GGT, U/L	57.0	50.6	45.5	51.0	4.8
AST, U/L	57.3	37.5	46.2	41.5	7.8
ALP, U/L[de]	169.3	136.4	137.5	142.9	7.8
Minerals					
Ca, mg/dl[eh]	10.7	9.7	8.3	9.5	.4
Fe, mg/L[h]	3.21	2.75	2.68	3.18	.14
Cu, mg/L[d]	1.60	1.67	1.57	1.75	.06
K, mmol/L[ei]	5.6	5.2	4.1	5.2	.3
Na, mmol/L[f]	150.6	140.1	118.8	136.5	6.3
Cl, mmol/L[g]	105.4	101.6	80.0	94.7	5.0
Other					
Glucose, mg/dl[e]	105.3	98.1	78.9	90.8	4.8

[a]Fed contaminated diets with 922 ppb aflatoxin B_1 in the nursery phase.
[b]Eight pigs per mean.
[c]Volclay-90, American Colloid Co., Arlington Heights, IL.
[d]Clay effect (clay diets vs. non-clay diets; $P < .10$).
[efg]Aflatoxin*clay interaction effect ($P < .05$, .10, .01 respectively.
[hi]Previous diet, aflatoxin effect (contaminated diets vs. non-contaminated diets; $P < .05$, .01 respectively).

TABLE 6. SERUM CHEMISTRY OF PIGS FED NON-CONTAMINATED (NC) AND AFLATOXIN-CONTAMINATED (AC) CORN WITH AND WITHOUT CLAY. METABOLISM PHASE.

	Treatments				
	NC		AC[a]		
Items[b]	No clay	Clay[c]	No clay	Clay[c]	SEM
Enzymes					
GGT, U/L[d]	41.2	52.3	72.2	60.2	8.6
ALP, U/L	151.3	127.5	144.5	155.3	9.9
Minerals					
Ca, mg/dl[e]	10.0	10.6	9.9	11.2	.5
P, mg/dl[f]	8.1	9.3	8.3	9.3	.5
Na, mmol/L[e]	145.5	155.2	143.5	159.8	6.3
Cl, mmol/L[e]	106.7	111.8	102.0	115.0	4.7
Zn, ug/L[e]	.93	.98	.76	1.02	.07
Cu, ug/L[gh]	1.90	1.95	1.67	2.03	.07
Other					
Glucose, mg/dl[e]	110.5	122.5	105.3	128.8	9.0
Albumin, g/dl[g]	3.8	4.1	3.6	4.1	.1

[a]Contaminated with 922 ppb aflatoxin B_1 in the diet.
[b]Six pigs per mean.
[c]Volclay-90, American Colloid Co. Arlington Heights, IL.
[d]Aflatoxin effect (contaminated diets vs. non-contaminated diets; $P < .10$).
[efg]Clay effect (clay diets vs. no clay diets; $P < .10$, .05, .01 respectively.
[h]Aflatoxin * clay interaction ($P < .05$).

TABLE 7. CA, P, MG AND MN ABSORPTION AND RETENTION BY GROWER PIGS FED NON-CONTAMINATED (NC) AND AFLATOXIN-CONTAMINATED (AC) CORN WITH AND WITHOUT CLAY.

	Treatments				
	NC		AC[a]		
Items	No clay	Clay[b]	No clay	Clay[b]	SEM
Calcium[cd]					
Intake	596	604	598	600	6.8
Absorption[e]	64.1	67.0	71.3	63.3	1.9
Retention[e]	62.2	64.8	69.6	61.1	1.9
Phosphorus[cd]					
Intake	461	467	463	464	5.2
Absorption[fh]	59.5	58.6	64.2	59.9	1.4
Retention[gi]	54.8	54.0	60.0	54.5	1.5
Magnesium[cd]					
Intake	111	113	112	112	1.3
Absorption[j]	33.0	22.9	34.1	25.0	1.8
Retention[j]	23.8	12.9	23.8	13.2	1.7
Manganese[cd]					
Intake	3.11	3.15	3.12	3.13	.04
Absorption	17.8	18.2	27.9	20.8	3.6
Retention[j]	17.8	14.2	25.1	17.2	3.8

[a]Contaminated with 922 ppb aflatoxin B_1 in the diet.
[b]Volclay-90, American Colloid Co., Arlington Heights, IL.
[c]Intake mg/d*kg$^{.75}$.
[d]Absorption and retention are a percentage of intake.
[e]Aflatoxin*clay interaction effect ($P < .01$).
[fg]Aflatoxin effect (contaminated diets vs non-contaminated diets; $P < .05$, .10 respectively).
[hij]Clay effect (clay diets vs non-clay diets; $P < .10$, .05, .01 respectively).

TABLE 8. NA, K, FE AND ZN ABSORPTION AND RETENTION BY GROWER PIGS FED NON-CONTAMINATED (NC) AND AFLATOXIN-CONTAMINATED (AC) CORN WITH AND WITHOUT CLAY.

	Treatments				
	NC		AC[a]		
Items	No clay	Clay[b]	No clay	Clay[b]	SEM
Sodium[cd]					
Intake	127	129	128	129	1.5
Absorption[eg]	86.9	80.1	88.9	84.7	1.4
Retention[fj]	31.7	32.0	41.9	27.0	3.7
Potassium[cd]					
Intake	628	637	631	633	7.1
Absorption[g]	83.8	80.4	84.4	81.0	1.1
Retention	21.8	26.4	31.3	24.2	3.8
Iron[cd]					
Intake[g]	23.2	35.9	23.3	35.7	.3
Absorption[h]	24.7	19.7	29.9	16.3	3.8
Retention[h]	23.0	28.5	18.7	15.1	3.7
Zinc[cd]					
Intake	9.54	9.67	9.58	9.61	.11
Absorption[fi]	40.3	39.7	47.2	40.6	2.0
Retention[fh]	37.8	37.0	44.7	37.4	2.0

[a]Contaminated with 922 ppb aflatoxin B_1 in the diet.
[b]Volclay-90, American Colloid Co., Arlington Heights, IL.
[c]Intake $mg/d*kg^{.75}$.
[d]Absorption and retention are a percentage of intake.
[ef]Aflatoxin effect (contaminated diets vs non-contaminated diets; $P < .05, .10$ respectively).
[ghi]Clay effect (clay diets vs non-clay diets; $P < .01, .05, .10$ respectively).
[j]Aflatoxin*clay interaction effect ($P < .05, .10$)).

Chapter V

EFFECTIVENESS OF FEEDING DIFFERENT TYPES OF CLAYS FOR REDUCING THE DETERIMENTAL EFFECTS OF AFLATOXIN CONTAMINATED DIETS ON PERFORMANCE, BLOOD CHEMISTRY AND IMMUNE RESPONSE OF WEANLING PIGS

T.C. Schell, M.D. Lindemann, E.T. Kornegay, D.J. Blodgett and J. Doerr

Virginia Polytechnic Institute and State University
Blacksburg, 24061

Abstract

Two trials were conducted using weanling pigs (n=129) to determine the effects of feeding different types of clays in conjunction with aflatoxin-contaminated diets. In trial I, (n=81) pigs were fed one of nine diets for 5 wk, seven diets were contaminated with aflatoxin (500 ppb) and supplemented with .5% of one of seven types of clay, one diet was contaminated with aflatoxin (500 ppb) with no clay and one diet was a control (non-contaminated). Feeding the aflatoxin-contaminated diet depressed ($P < .05$) overall ADG and daily feed intake, and the addition of all the types of clays caused variable improvements ($P < .05$) in ADG and feed intake. Feed efficiency was not affected by the treatments. Sodium bentonite improved ($P < .05$) serum urea N levels which were depressed by feeding aflatoxin, and intermediate nonsignificant improvements were observed by several other clays. Several clays lowered the aflatoxin-induced

elevation of aspartate aminotransferase (AST) and gamma glutamyltransferase (GGT) in the serum. Serum glucose, Ca, Mg, P, Na, and K were not affected by any treatment. In Trial II, weanling pigs (n=48) were assigned to one of four diets; control; aflatoxin control (171 ppb); aflatoxin plus 1.0% hydrated sodium calcium aluminosilicate or aflatoxin plus 1.0% Ca bentonite for 5 wk. Aflatoxin depressed ADG ($P < .05$) for the first 4 wk, with no effect in wk 5. Both clays increased ($P < .05$) ADG and feed intake compared with the aflatoxin controls. Feeding aflatoxin diets did not influence feed to gain ratios. There were no significant treatment effects on the serum levels of total protein, albumin, urea N, GGT, AST, ALP, Ca, P, Mg, Na, K and glucose and humoral immune response taken at the end of the trial. Feeding clays can effectively prevent some of the negative effects of feeding aflatoxin-contaminated diets to weanling pigs. However, the level of prevention is dependant on the type of clay fed.

Key Words: Pigs, Clays, Aflatoxin, Immunity

Introduction

Weanling swine fed aflatoxin contaminated diets grow slower, consume less feed, and in some cases have higher feed to gain ratios (Lindemann et al., 1988; Panangala et al., 1986). Additionally, liver damage characterized by enlargement and the release of enzymes; aspartate

aminotransferase (AST), gammaglutamyl transferase (GGT), and alkaline phosphatase (ALP) into the blood has been reported in pigs fed 500 ppb aflatoxin (Harvey et al., 1990).

Efforts to minimize the effects of aflatoxins have primarily centered on reducing the toxin levels in feeds. Screening, blending, and ammoniation have been used to reduce aflatoxin levels in swine feeds with only limited success (CAST, 1989). Recently, studies have shown that adding clay (.5%) to contaminated swine diets can successfully reduce a major part of the negative effects of aflatoxins (Lindemann et al., 1988, 1990). Lindemann et al. (1988) reported that feeding weanling swine .5% hydrated sodium calcium aluminosilicate (HSCA) with 840 ppb aflatoxin significantly prevented depressions in daily gains and feed intake. Furthermore, Harvey et al. (1990) reported a recovery of elevated serum levels of GGT, AST, and ALP associated with aflatoxin induced liver damage when HSCA was fed with aflatoxin.

The purpose of this research was to evaluate the effectiveness of several types of clays in reducing the negative effects caused by the feeding of aflatoxin-contaminated diets to weanling swine.

Methods and Materials

Two trials using a total of 129 weanling barrows and gilts were conducted to determine the effectiveness of

several types of clays in preventing some of the negative effects of feeding aflatoxin contaminated diets. In Trial I, after a postweaning adjustment period of 7 to 9 d, 81 crossbred pigs with an average initial wt of 9.65 kg and an average age of 38.7 d were allotted based on gender, weight, and litter to one of nine dietary treatments (Table 1). Diets II-IX were contaminated with 500 ppb aflatoxin B_1 on rice starch using the procedure of Shotwell et al. (1966) and 7 types of clay[2] were added at .5%.

Pigs were housed in .91 m x 1.22 m plastic-coated welded-wire floored pens (three pigs/pen) in an enclosed nursery. The room temperature was set initially at 26°C and was reduced weekly according to the body weight of the pigs. Access to feed and water was ad libitum. Pigs were weighed and feed intake per pen was determined weekly. One replicate (27 pigs, one pen per treatment) was removed from the trial on d 33, and the other two replicates were removed on d 40. Blood was taken from each pig via jugular venipuncture at the termination of each replicate for serum blood chemistry determination.

In Trial II, after a postweaning adjustment period of 5 d, 48 crossbred pigs with an average initial weight of 9.08

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[2]15 RVM, 55 RVM, 80 RVM obtained from Floridin, Tallahassee, Fl, Zeobrite obtained from Zeotech Corp., Albuquerque, NM, Novasil marketed by Englehard Corp., Cleveland, OH. FD-181 obtained from American Colloid Co. Arlington Heights, IL, AB-20 obtained from ECC America, Inc., Gonzales, TX.

kg and an average age of 28 d were assigned to one of four dietary treatments (Table 2) based on weight, gender, and litter. Naturally contaminated corn was used to formulated diets that contained 171 ppb aflatoxin B_1. Pigs were housed in cages (.61 m x .91 m) with plastic-coated welded-wire floors (two pigs/pen). Access to feed and water was ad libitum for the 5 wk trial. Pigs were weighed and feed intake was determined weekly. On d 7 and d 21, all animals were bled via anterior vena cava puncture, and were then injected im with 1 ml of an antigen solution [1 mg ovalbumin (Sigma) in .5 ml Freund's Incomplete Adjuvant (Sigma) and .5 ml physiological saline] for determination of humoral immune response. The immune response to ovalbumin was determined using a modified ELISA procedure (Appendix Table 32). Blood samples were taken again at d 35 for determination of serum chemistry and immune response. Serum chemistry analysis was performed at the Virginia-Maryland Regional College of Veterinary Medicine using a Kodak Ektachem 700 (Eastman Kodak, Rochester, New York). One pig on diet 3 was removed from the study after wk 2 because of an undetermined illness. Data from this pig were eliminated from the analysis.

The data from both studies was analyzed using the General Linear Models procedure of SAS (1988). Models contained diet and replicate effects. Tukey studentized

range test was performed to compare differences between the diets for performance and serum chemistry.

Results

Trial I. Average daily gain and feed intake were depressed ($P < .05$) for pigs fed the aflatoxin contaminated diet (with no clay) compared with pigs fed the control diet (Table 3). The addition of all clays to the aflatoxin diet (attapulgites, sepolite, zeolite, HSCA, Na bentonite and Ca bentonite) improved ($P < .05$) ADG and ADFI over those of pigs fed the aflatoxin control diet. There was no effect of aflatoxin or clay on feed efficiency.

Pigs fed the aflatoxin diet (with no clay) had depressed ($P < .05$) serum urea N levels compared with control pigs (Table 4). The addition of Na bentonite increased ($P < .05$) urea N compared with pigs fed aflatoxin diets with no clay, but some of the other clays had nonsignificant intermediate values.

Although not significant, serum albumin levels were lower in pigs fed aflatoxin diets with no clay, but were returned to normal or near normal for pigs fed aflatoxin with zeolite added ($P < .05$). Serum glucose (117 mg/dl), Ca (10.9 mg/dl), Mg (2.0 mg/dl), P (8.6 mg/dl), Na (148 mmol/L) and K (6.3 mmol/L) were not affected by aflatoxin or clay treatments (Appendix Tables 27 and 28).

Serum AST and GGT levels were higher ($P < .05$) in pigs fed aflatoxin with no clay compared with control fed pigs. Increased AST levels were prevented by the addition of HSCA and AB-20 ($P < .05$) to the aflatoxin diet compared with pigs fed the aflatoxin diet alone. Serum GGT levels were also reduced by all the clays, except both of the attapulgites, compared with pigs fed the aflatoxin diet with no clay.

Trial II. Feeding the diet with contaminated corn depressed ADG through wk 4 ($P < .05$) when compared to pigs fed non-contaminated diets; there was no effect for wk 5 (Table 5). Pigs fed aflatoxin diets with HSCA or Ca bentonite had higher ADG than those of pigs fed the contaminated diet with no clay ($P < .05$). Feed intake was higher ($P < .05$) for pigs consuming both clay diets compared with pigs fed the aflatoxin diet with no clay. However, the HSCA fed pigs had a higher feed to gain ratio than control pigs ($P < .05$). There were no effects of aflatoxin or clay on serum total protein (5.04 g/dl), albumin (2.78 g/dl), urea N (9.6 mg/dl), GGT (40.3 u/L), AST (46.7 u/L), ALP (166 u/L), Ca (8.9 mg/dl), Mg (2.1 mg/dl), P (8.1 mg/dl), Na (129.6 mmol/L), K (4.7 mmol/L) and glucose (87.7 mg/dl); all were within the normal range (Appendix table 31). Humoral immune response was not affected by aflatoxin or clay (data not shown).

Discussion

Performance. The results of these studies are in agreement with other reports in which marked improvement in performance were observed when clays were added to aflatoxin contaminated diets (Harvey et al., 1990; Lindemann et al., 1990). In Trial I, the inclusion of 500 ppb aflatoxin to the diets produced a 28% depression in growth when clay was not added; the addition of .5% of several clays recovered from 31 to 76% of the depression. Similarly, ADFI was depressed 31% by the aflatoxin when compared with controls; the addition of clays recovered 40 to 112% of this depression. As reported by Lindemann et al. (1988), feed efficiency was unaffected by aflatoxin or clay treatment.

The magnitude of the aflatoxin effect on performance was much less in Trial II. There was only a depression in ADG for the first 4 wk of the 5 wk trial. Lindemann et al. (1990) reported reduced feed intake and daily gains for weanling pigs fed similar levels of aflatoxin for 5 wk. The discrepency in results may be attributed to the level and/or the source of aflatoxin used in this trial. Coffey et al. (1989) reported a linear depression in performance due to increases in aflatoxin levels in the diets of weanling pigs. The level of aflatoxin in this trial may have only been sufficient to illicite a response for the first 4 wk. Also, previous work has shown that younger pigs are more affected by aflatoxin (Heathcote and Hibbert, 1978), and the age of

the pigs at the end of this trial may have made them unresponsive to the low level of aflatoxin. The high feed to gain ratio for the pigs fed HSCA has not been previously reported and contradicts Trial I and previous work by Lindemann et al. (1990).

Serum chemistry. Serum chemistry profiles in Trial I were similar to those obtained by Harvey et al., (1989b). Aflatoxin reduced indicators of protein synthesis such as serum urea N, and serum levels of AST and GGT were elevated by aflatoxin as seen by Lindemann et al. (1988, 1990) and Harvey et al. (1990). The lack of response to aflatoxin for protein synthesis indicators and serum enzymes related to liver damage in Trial II is consistent with the performance data for that study immediately prior to sampling. The recovery of performance during the last week of the study along with the the normal serum chemistry profiles obtained during this period suggests a recover of normal metabolism. Again, this recovery may be attributed to the low level of aflatoxin in this trial.

In Trial I, the various clays had differing abilities to recover abnormal blood chemistry values caused by the aflatoxins. The Na bentonite, zeolite and sepiolite all significantly increased the serum levels of protein synthesis indicators when aflatoxin was fed. These results are similar to those obtained by Harvey et al. (1989a) who

found the addition of .5% HSCA increased serum albumin, total protein and urea N when aflatoxin was fed. The reduction of aflatoxin-induced high serum enzyme levels was achieved by all of the clays to varying degrees, except the attapulgites. Lindemann et al. (1990) reported a similar serum enzyme reduction with the addition of .5% sodium bentonites and HSCA. The lack of a response of serum glucose, Ca, Mg, P, Na and K to aflatoxin treatment also agrees with the results of Lindemann et al. (1990) in which no effects were found on these parameters.

Immunity. Contrary to work by Richard et al. (1975), which suggests a humoral immune response to aflatoxins in guinea pigs, there was no effect on humoral immune response in this study. This study supports the work of Panangala et al. (1986), which found the primary influence of aflatoxin on the immune system to be on cell-mediated immunity. However, the level of aflatoxin in this trial may not have been sufficient to illicite a change in humoral immune response.

The varying abilities of the clays to prevent depressions in performance and abnormal serum chemistry profiles reflects the varying ablities of these clays to bind aflatoxins (Blodgett et al, unpublished data). The binding of only some of the aflatoxins in the

gastrointestinal tract may account for limited recovery of the responses examined in this study.

Implications

Overall, when aflatoxin contaminated diets are fed to swine, the addition of clays can prevent many of the negative toxin-induced effects. The degree to which the clays can accomplish this is dependant upon the specific clay. Pending FDA approval, the addition of clays to aflatoxin contaminated diets may offer producers another alternative way of dealing with their contaminated grains in the future.

TABLE 1. PERCENTAGE COMPOSITION OF NURSERY DIETS IN TRIAL I[a]

Item	Diets								
	I	II	III	IV	V	VI	VII	VIII	IX
Corn	70.50	70.00	70.00	70.00	70.00	70.00	70.00	70.00	70.00
Soybean meal (48% CP)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Soybean oil	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Limestone	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
VPI vitamin premix[b]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral premix[c]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salt	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
ASP-250[d]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
15 RVM (attapulgite 1)[e]	--	--	0.50	--	--	--	--	--	--
55 RVM (attapulgite 2)[e]	--	--	--	0.50	--	--	--	--	--
80 RVM (sepiolite)[e]	--	--	--	--	0.50	--	--	--	--
Zeobrite (zeolite)[f]	--	--	--	--	--	0.50	--	--	--
Novasil (hydrated sodium calcium aluminosilicate)[g]	--	--	--	--	--	--	0.50	--	--
FD-181 (Na bentonite)[h]	--	--	--	--	--	--	--	0.50	--
AB-20 (treated Ca bentonite)[i]	--	--	--	--	--	--	--	--	0.50
Aflatoxin[j]	--	+	+	+	+	+	+	+	+

[a]Calculated to supply 18.2% crude protein, .96% lysine, .77% Ca, and .59% P.
[b]Supplied per kilogram of diet: 4,400 IU vitamin A, 440 IU vitamin D, 11 IU vitamin E, 4.4 mg riboflavin, 22 mg d-pantothenic acid, 22 mg niacin, 489.5 mg choline, .022 mg vitamin B_{12}, .5 mg menadione, .44 mg d-biotin and .3 mg Se.
[c]Supplied per kilogram of diet: 150 mg Zn, 176 mg Fe, 60 mg Mn, 17 mg Cu, 34 mg I.
[d]American Cyanamid Co., Princeton, NJ, supplied 220 mg chlortetracycline, 220 mg sulfamethazine, and 110 mg penicillin per kilogram of diet.
[e]Obtained from Floridin, Tallahassee, FL.
[f]Obtained from Zeotech Corp., Albuquerque, NM.
[g]Marketed by Englehard Corp., Cleveland, OH.
[h]Obtained from American Colloid Co. Arlington Heights, IL.
[i]Obtained from ECC America, Inc., Gonzales, TX.
[j]Diets II-IX contained 500 ppb mixed aflatoxin (88% aflatoxin B_1) obtained from a culture of A. Flavus on rice starch.

Table 2. PERCENTAGE COMPOSITION OF DIETS IN TRIAL II[ab]

Item	Diets			
	I	II	III	IV
Normal corn	64.70	28.70	27.70	27.70
Contaminated corn	-----	40.00	40.00	40.00
Soybean meal (44% CP)	26.90	26.90	26.90	26.90
Soybean oil	1.00	1.00	1.00	1.00
Dicalcium phosphate	1.20	1.20	1.20	1.20
Limestone	1.00	1.00	1.00	1.00
VPI vitamin premix[c]	0.25	0.25	.25	.25
Trace mineral premix[d]	0.05	0.05	.05	.05
Salt	0.40	0.40	.40	.40
CSP-125[e]	0.50	0.50	.50	.50
Hydrated Sodium Calcium Aluminosilicate[f]	----	----	1.00	----
Ca Bentonite[g]	----	----	----	1.00

[a]Calculated to supply 17.7% crude protein, .95% lysine, .77% Ca, and .59% P.
[b]Diets II-IV were calculated to contain 171 ppb aflatoxin B_1.
[c]Supplied per kilogram of diet: 4,400 IU vitamin A, 440 IU vitamin D_3, 11 IU vitamin E, 4.4 mg riboflavin, 22 mg d-pantothenic acid, 22 mg niacin, 489.5 mg choline, .022 mg vitamin B_{12}, .5 mg menadione, .44 mg d-biotin and .3 mg Se.
[d]Supplied per kilogram of diet: 150 mg Zn, 176 mg Fe, 60 mg Mn, 17 mg Cu, 34 mg I.
[e]Supplied per kilogram of diet; 110 mg chlortetracyclene, 110 mg sulfathiozole and 55 mg penicillin.
[f]Tradename NovaSil, Englehardt Corp. Cleveland, Ohio.
[g]Tradename Red Crown Bentonite, Prince Agri Products Inc. Quincy, Illinois.

TABLE 3. PERFORMANCE OF WEANLING PIGS FED AFLATOXIN-CONTAMINATED DIETS AND SEVERAL TYPES OF CLAYS. TRIAL I.

	Diets[a]									
Items	I Normal Corn	II Aflatoxin Corn(AC)[b]	III AC+.5% 15 RVM	IV AC+.5% 55 RVM	V AC+.5% 80 RVM	VI AC+.5% Zeobrite	VII AC+.5% Novasil	VIII AC+.5% FD-181	IX AC+.5% AB-20	SEM
Body weights, kg										
Initial	9.63	9.61	9.66	9.67	9.67	9.68	9.65	9.68	9.67	.09
Final	31.88^{c}	24.93^{e}	27.55^{cd}	28.40^{cd}	29.07^{cd}	29.02^{cde}	30.35^{cd}	30.10^{cd}	28.94^{cde}	.81
ADG, kg										
Wk 1-5	.648^{c}	.466^{d}	.522^{cd}	.546^{cd}	.567^{cd}	.565^{cd}	.605^{c}	.597^{c}	.562^{cd}	.02
Average daily feed intake, kg										
Wk 1-5	1.35^{cd}	.93^{f}	1.10^{df}	1.14^{def}	1.25^{cde}	1.20^{cde}	1.40^{c}	1.30^{cde}	1.25^{cde}	.05
Feed to gain ratio										
Wk 1-5	2.08	2.10	2.11	2.07	2.22	2.12	2.31	2.11	2.24	.09

[a] n=9 for all treatments.
[b] Diets contaminated with 500 ppb aflatoxin.
[c,d,e,f] Means with different superscripts differ ($P < .05$).

TABLE 4. SERUM CHEMISTRY OF WEANLING PIGS FED AFLATOXIN-CONTAMINATED DIETS AND SEVERAL TYPES OF CLAYS. TRIAL I.

	Diets[a]									
Items	I Normal Corn	II Aflatoxin Corn(AC)[b]	III AC+.5% 15 RVM	IV AC+.5% 55 RVM	V AC+.5% 80 RVM	VI AC+.5% Zeobrite	VII AC+.5% Novasil	VIII AC+.5% FD-181	IX AC+.5% AB-20	SEM
Urea N, mg/dL	13.6^{c}	8.0^{d}	9.8^{cd}	8.8^{d}	11.3^{cd}	10.3^{cd}	12.0^{cd}	13.7^{c}	11.2^{cd}	.78
Albumin, g/dL	4.02^{cd}	3.13^{d}	3.67^{cd}	3.88^{cd}	4.22^{c}	3.82^{cd}	3.92^{cd}	3.92^{cd}	4.00^{cd}	.17
AST, U/L	63^{d}	82^{c}	69^{cd}	71^{cd}	77^{cd}	73^{cd}	57^{d}	81^{c}	66^{d}	4.81
GGT, U/L	52^{cd}	92^{c}	63^{cd}	77^{cd}	44^{d}	50^{d}	40^{d}	51^{d}	42^{d}	7.20

[a] n=9 for all treatments.
[b] Diets contaminated with 500 ppb aflatoxin.
[c,d] Means with different superscripts differ ($P < .05$).

TABLE 5. PERFORMANCE OF WEANLING PIGS FED NONCONTAMINATED AND AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAYS. TRIAL II.

Item[a]	Treatments				
	Normal Corn(NC)	Aflatoxin Corn(AC)[b]	AC+1% HSCA[c]	AC+1% Ca Ben.[d]	SEM
	(n=12)	(n=12)	(n=11)	(n=12)	
Body weights, kg					
Initial	9.05	9.05	9.09	9.11	.092
Final	23.64^{ef}	22.71^{f}	24.83^{e}	24.82^{e}	.462
ADG, kg					
Wk 1-4	.399^{e}	.350^{f}	.420^{e}	.403^{e}	.013
Wk 4-5	.488	.526	.533	.571	.023
Wk 1-5	.417^{ef}	.390^{f}	.450^{e}	.449^{e}	.014
Average daily feed intake, kg					
Wk 1-4	.734f^{g}	.668^{g}	.860^{e}	.765^{f}	.023
Wk 4-5	1.095	1.061	1.268	1.152	.043
Wk 1-5	.819^{fg}	.769^{g}	.962^{e}	.867^{ef}	.023
F/G					
Wk 1-4	1.84^{f}	1.91^{ef}	2.07^{e}	1.90^{ef}	.050
Wk 4-5	2.24	2.02	2.39	2.04	.062
Wk 1-5	1.96^{f}	1.97^{ef}	2.15^{e}	1.93^{f}	.046

[a]Means with different superscripts differ ($P < .05$).
[b]Contaminated with aflatoxin 171 ppb in the diet.
[c]Hydrated Sodium Calcium Aluminosilicate, tradename Novasil, Englehard Corp., Cleveland, Ohio.
[d]Tradename Red Crown Bentonite, Prince Agri Products Inc. Quincy, Illinois.

Chapter VI

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APPENDIX TABLE 1. INTAKE AND FECES DATA FOR THE METABOLISM PHASE.

Items	Treatments				SEM	P-values[b]		
	Normal Corn(NC)	Aflatoxin Corn(AC)[a]	NC+1% Volclay	AC+1% Volclay		A	C	A*C
Initial wt, kg	38.8	33.4	42.4	38.9	1.2	.01	.01	.22
Feed intake, g	4909	4402	5220	4957	139	.01	.01	.40
Feed refusal, g	105	52	27	43	34	.60	.22	.32
Water intake, L	32	24	31	31	4	.50	.35	.48
Feces wet wt, g	1491	1286	1807	1617	95	.05	.01	.93
Feces DM, %	37.5	36.5	36.4	37.7	2	.89	.97	.44
DM digest. %	87.5	88.6	86.3	86.5	.5	.23	.01	.39

[a]Diet contains 922 ppb aflatoxin.
[b]A- Aflatoxin effect (aflatoxin diets vs. no aflatoxin diets)
C- Clay effect (clay diets vs. no clay diets).
A*C- Aflatoxin * clay interaction.

APPENDIX TABLE 2. BODY WEIGHTS AND AVERAGE DAILY GAINS OF WEANLING PIGS FED NON-CONTAMINATED AND AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT VOLCLAY-90. NURSERY PHASE.

Items	Treatments					P-values			
							Contrasts[b]		
	Normal Corn(NC)	Aflatoxin Corn(AC)[a]	NC+1% Volclay	AC+1% Volclay	SEM	Trt	A	C	A*C
	(n=24)	(n=24)	(n=24)	(n=24)					
Body weights, kg									
Initial	8.78	8.80	8.78	8.72	.041	.53	.66	.32	.34
Week 1	11.28	10.84	11.34	10.99	.219	.39	.12	.64	.83
Week 2	14.83	13.31	14.76	13.90	.393	.09	.02	.54	.44
Week 3	18.59	16.47	18.64	16.96	.501	.04	.01	.61	.67
Week 4	22.78	19.67	23.52	20.84	.469	.01	.01	.09	.67
Week 5	26.77	22.71	27.71	24.99	.060	.01	.01	.03	.28
Week 6	29.99	25.27	31.19	28.49	.466	.01	.01	.01	.07
Average daily gain, g									
Week 1	357	290	366	325	29.0	.33	.11	.49	.67
Week 2	507	354	489	415	39.4	.10	.03	.61	.35
Week 3	537	451	554	438	36.7	.15	.03	.96	.70
Week 4	598	457	697	553	42.8	.04	.02	.06	.97
Week 5	570	433	598	593	59.8	.26	.28	.17	.31
Week 6	461	366	498	500	40.9	.17	.30	.08	.28
Wk 1-2	432	322	427	370	25.8	.11	.02	.44	.35
Wk 1-3	467	365	470	393	22.5	.04	.01	.53	.60
Wk 1-4	500	388	526	433	16.4	.01	.01	.07	.60
Wk 1-5	514	397	541	465	15.7	.01	.01	.02	.24
Wk 1-6	505	392	534	471	10.7	.01	.01	.01	.06

[a]Contaminated with 922 ppb in the diet
[b]Contrasts
A- Aflatoxin effect (Aflatoxin corn diets vs normal corn diets)
C- Clay effect (Volclay diets vs no Volclay diets)
A*C- Interaction effect

APPENDIX TABLE 3. AVERAGE DAILY FEED INTAKE AND FEED TO GAIN RATIO OF WEANLING PIGS FED NON-CONTAMINATED AND AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAY. NURSERY PHASE.

	Treatments					P-values			
							Contrasts[b]		
Items	Normal Corn(NC)	Aflatoxin Corn(AC)[a]	NC+1% Volclay	AC+1% Volclay	SEM	Trt	A	C	A*C
ADFI, g	(n=24)	(n=24)	(n=24)	(n=24)					
Week 1	540	489	486	477	17	.11	.11	.09	.23
Week 2	964	748	926	872	60	.16	.07	.50	.23
Week 3	1204	718	1250	1036	28	.01	.01	.03	.24
Week 4	1171	1034	1379	1258	88	.14	.19	.05	.93
Week 5	1385	1058	1495	1420	39	.01	.01	.01	.02
Week 6	1333	1042	1377	1361	29	.01	.01	.01	.01
Wk 1-2	754	618	706	674	37	.18	.07	.92	.21
Wk 1-3	904	718	887	795	28	.01	.01	.33	.15
Wk 1-4	971	797	1010	911	39	.04	.01	.10	.34
Wk 1-5	1054	849	1108	1013	37	.01	.01	.03	.19
Wk 1-6	1101	883	1152	1071	31	.01	.01	.01	.07
Feed to gain									
Week 1	1.57	1.69	1.34	1.55	.13	.43	.28	.24	.76
Week 2	1.91	2.16	1.91	2.10	.07	.14	.03	.75	.73
Week 3	2.25	2.06	2.28	2.37	.11	.37	.68	.20	.29
Week 4	2.06	2.31	2.01	2.27	.24	.77	.33	.88	.97
Week 5	2.61	2.46	2.62	2.40	.28	.93	.54	.93	.92
Week 6	3.05	2.84	2.80	2.74	.21	.77	.57	.44	.74
Wk 1-2	1.76	1.94	1.65	1.83	.05	.05	.02	.09	.96
Wk 1-3	1.94	1.99	1.89	2.03	.06	.48	.20	.92	.45
Wk 1-4	1.94	2.05	1.92	2.11	.06	.22	.06	.77	.57
Wk 1-5	2.05	2.14	2.05	2.18	.04	.17	.04	.64	.65
Wk 1-6	2.17	2.25	2.16	2.27	.03	.08	.04	.90	.58

[a]Contaminated with 922 ppb in the diet.
[b]Contrasts
A- Aflatoxin effect (Aflatoxin corn diets vs. normal corn diets)
C- Clay effect (Volclay-90 diets vs no Volclay-90 diets)
A*C- Interaction effect.

APPENDIX TABLE 4. BLOOD CHEMISTRY OF WEANLING PIGS FED NON-CONTAMINATED CORN OR AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAY. NURSERY PHASE.

Items	Treatments				SEM	P-values[b]			
	Normal Corn(NC)	Aflatoxin Corn(AC)[a]	NC+1% Volclay	AC+1% Volclay		Trt	A	C	A*C
	(n=24)	(n=24)	(n=24)	(n=24)					
BUN [c]	13.4	10.4	12.1	11.8	.97	.22	.10	.99	.19
T Protein[d]	6.1	5.6	5.8	6.2	.21	.19	.72	.33	.06
Glucose[c]	116.6	100.1	108.5	117.0	3.66	.01	.29	.25	.01
Creatine[c]	1.2	1.2	1.2	1.2	.04	.96	.85	.63	.94
Albumin[d]	3.5	3.0	3.4	3.7	.15	.04	.63	.07	.02
GGT, U/L	46.7	67.0	51.0	57.5	4.1	.01	.01	.53	.11
AST, U/L	66.1	81.6	68.0	53.6	5.91	.03	.93	.04	.02
ALP, U/L	129.1	135.0	118.3	127.6	3.72	.04	.06	.03	.66
Ca[c]	9.6	9.2	9.5	9.9	.22	.27	.85	.26	.10
Mg[c]	2.0	1.9	1.9	1.9	.06	.68	.49	.61	.39
P[c]	7.3	7.4	7.6	7.8	.20	.33	.32	.13	.73
Na[e]	145.3	137.4	140.5	143.1	2.76	.24	.34	.87	.07
K[e]	6.6	6.6	6.5	6.6	.18	.99	.85	.90	.83
Cl[e]	101.9	95.9	97.0	100.3	2.23	.23	.56	.90	.05
CO_2[e]	27.5	27.0	28.5	26.7	.88	.54	.23	.70	.47
Anion gap[e]	22.6	21.2	21.6	22.7	.64	.30	.77	.69	.07
Total bili[c]	.13	.17	.12	.12	.02	.17	.39	.09	.22

[a]Contaminated with 922 ppb in the diet
[b]Contrasts
A- Aflatoxin effect (Aflatoxin corn diets vs normal corn diets)
C- Clay effect (Volclay diets vs no Volclay diets)
A*C- Interaction effect
[c]mg/dL
[d]g/dL
[e]mmol/L

APPENDIX TABLE 5. MINERAL CONTENT OF LIVERS OF WEANLING PIGS FED NON-CONTAMINATED CORN OR AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAY. NURSERY PHASE.

Items[c]	Treatments					P-values		
						Contrasts[b]		
	Normal Corn(NC)	Aflatoxin Corn(AC)[a]	NC+1% Volclay	AC+1% Volclay	SEM	A	C	A*C
	(n=24)	(n=24)	(n=24)	(n=24)				
Ca	.41	.34	.38	.42	.03	.65	.47	.13
P	7.84	7.49	8.01	8.44	.44	.93	.25	.41
Mg	.97	.97	.99	1.04	.10	.83	.70	.83
Na	7.93	7.20	7.31	6.96	.36	.19	.29	.59
K	16.4	16.7	15.3	17.2	1.77	.54	.87	.67
Cu	3.36	4.69	4.39	4.05	.79	.55	.81	.32
Fe	63.7	66.8	67.5	79.2	8.7	.43	.39	.64
Zn	18.8	16.9	20.2	23.1	2.6	.85	.20	.40

[a]Contaminated with 922 ppb in the diet.
[b]Contrasts
A- Aflatoxin effect (Aflatoxin corn diets vs. normal corn diets)
C- Clay effect (Volclay-90 diets vs. no Volclay-90 diets)
A*C- Interaction effect
[c]ppm, dry matter basis.

APPENDIX TABLE 6. BODY WEIGHTS AND AVERAGE DAILY GAINS OF GROWER PIGS FED NON-CONTAMINATED CORN WITH AND WITHOUT VOLCLAY-90. GROWER PHASE.

	Treatments							
Nursery Diets	Normal Corn(NC)	Aflatoxin Corn(AC)[a]	NC+1% Volclay	AC+1% Volclay		P-Values[b]		
Grower Diets	NC	NC	NC+1% Volclay	NC+1% Volclay	SEM	A	C	A*C
	(n=12)	(n=12)	(n=12)	(n=12)				
Body weight, kg								
Initial	29.82	26.06	30.38	28.58	1.00	.03	.18	.37
Week 1	38.05	34.63	38.20	36.53	1.22	.08	.43	.50
Week 2	43.73	40.20	43.07	41.59	1.34	.11	.79	.47
Week 3	49.04	46.06	48.95	47.25	1.53	.18	.73	.69
Week 4	55.45	52.18	54.96	53.79	1.69	.24	.75	.56
Week 5	60.93	58.23	60.78	58.55	1.63	.18	.96	.89
Average daily gain, g								
Week 1	1176	1224	1118	1136	47.6	.51	.18	.76
Week 2	813	795	696	724	47.1	.92	.09	.65
Week 3	759	837	840	807	61.4	.72	.69	.40
Week 4	915	875	859	934	51.0	.74	.98	.30
Week 5	913	1008	970	794	57.6	.50	.22	.06
Wk 1-2	994	1010	907	930	28.6	.53	.03	.90
Wk 1-3	916	952	885	889	31.3	.54	.18	.63
Wk 1-4	916	932	878	900	31.0	.55	.30	.94
Wk 1-5	915	946	894	882	28.3	.76	.18	.47

[a]Contaminated with 922 ppb in the diet.
[b]Contrasts
A- Aflatoxin effect (Aflatoxin corn diets vs. normal corn diets)
C- Clay effect (Volclay-90 diets vs. no Volclay-90 diets)
A*C- Interaction effect.

APPENDIX TABLE 7. AVERAGE DAILY FEED INTAKE AND FEED TO GAIN RATIO OF GROWER PIGS FED NON-CONTAMINATED CORN AND CLAY. GROWER PHASE.

	Treatments							
Nursery Diets	Normal Corn(NC)	Aflatoxin Corn(AC)[a]	NC+1% Volclay	AC+1% Volclay		P-Values[b]		
Grower Diets	NC	NC	NC+1% Volclay	NC+1% Volclay	SEM	A	C	A*C
ADFI, g	(n=12)	(n=12)	(n=12)	(n=12)				
Week 1	1717	1777	1674	1642	108	.90	.44	.69
Week 2	2187	1971	1971	2349	125	.54	.54	.06
Week 3	1674	2187	2106	1971	182	.34	.58	.13
Week 4	2754	2511	2349	2403	181	.62	.21	.44
Week 5	2299	2614	2362	2646	187	.16	.81	.94
Wk 1-2	1952	1874	1822	1995	86	.61	.96	.20
Wk 1-3	1859	1978	1917	1987	86	.32	.71	.79
Wk 1-4	2083	2111	2025	2091	80	.58	.64	.82
Wk 1-5	2121	2200	2085	2189	81	.31	.78	.88
Feed to gain								
Week 1	1.47	1.46	1.50	1.44	.08	.68	.91	.80
Week 2	2.73	2.55	2.86	3.25	.23	.67	.13	.27
Week 3	2.32	2.62	2.53	2.44	.33	.77	.95	.58
Week 4	3.02	2.86	2.76	2.58	.18	.40	.19	.96
Week 5	2.53	2.62	2.43	3.35	.22	.07	.22	.12
Wk 1-2	1.96	1.86	2.01	2.14	.05	.80	.02	.07
Wk 1-3	2.03	2.08	2.17	2.23	.08	.58	.15	.93
Wk 1-4	2.28	2.26	2.31	2.32	.04	.97	.34	.81
Wk 1-5	2.32	2.32	2.33	2.48	.05	.22	.17	.25

[a]Contaminated with 922 ppb in the diet.
[b]Contrasts
A- Aflatoxin effect (Aflatoxin corn diets vs. normal corn diets)
C- Clay effect (Volcay-90 diets vs. no Volclay-90 diets)
A*C- Interaction effect.

APPENDIX TABLE 8. BLOOD CHEMISTRY OF GROWER PIGS FED NON-CONTAMINATED CORN AND CLAY. GROWER PHASE.

Items	Treatments[a] Normal Corn(NC)	Aflatoxin Corn(AC)[b]	NC+1% Volclay	AC+1% Volclay	SEM	P-values[c] A	C	A*C
	(n=8)	(n=8)	(n=8)	(n=8)				
BUN[d]	10.4	8.3	11.8	8.9	1.31	.47	.61	.82
T Protein[e]	7.1	5.2	6.3	6.3	.38	.17	.43	.33
Glucose[d]	105.3	78.9	98.1	90.8	4.82	.03	.87	.06
Creatine[d]	1.2	.8	1.0	1.1	.09	.22	.91	.14
Albumin[e]	4.1	3.0	3.7	3.6	.21	.14	.68	.35
GGT, U/L	57.0	45.5	50.6	51.0	4.76	.57	.64	.87
AST, U/L	57.3	46.2	37.5	41.5	7.78	.39	.15	.68
ALP, U/L	169.3	137.5	136.4	142.9	7.76	.15	.10	.04
Ca[d]	10.7	8.3	9.7	9.5	.40	.05	.43	.09
Mg[d]	2.1	1.6	1.8	1.8	.10	.18	.52	.29
P[d]	7.8	6.0	7.1	7.5	.38	.28	.22	.13
Na[f]	150.6	118.8	140.1	136.5	6.31	.08	.52	.20
K[f]	5.6	4.1	5.2	5.2	.28	.03	.21	.02
Cl[f]	105.4	80.0	101.6	94.7	5.04	.04	.37	.17
CO_2[f]	31.3	23.2	22.6	26.2	2.69	.80	.97	.46
Anion gap[f]	19.8	20.0	21.3	20.6	.87	.54	.90	.99
Total bili[d]	.21	.1	.19	.18	.06	.19	.86	.39

[a]Nursery diets
Grower diets: I and II - normal corn with no Volclay
III and IV - normal corn with 1% Volclay
[b]Contaminated with 922 ppb in the diet.
[c]A=aflatoxin effect, C=clay effect, A*C=interaction (2 reps).
[d]mg/dL
[e]g/dL
[f]mmol/L

APPENDIX TABLE 9. MINERAL CONTENT OF LIVERS OF GROWER PIGS FED NON-CONTAMINATED CORN WITH AND WITHOUT CLAY. GROWER PHASE.

Items[d]	Treatments[a]				SEM	P-Values[c]
	Normal Corn(NC)	Aflatoxin Corn(AC)[b]	NC+1% Volclay	AC+1% Volclay		
	(n=6)	(n=6)	(n=6)	(n=6)		
Ca	.40	.41	.43	.41	.03	.90
P	10.12	10.09	10.56	10.70	.40	.63
Mg	1.37	1.41	1.43	1.49	.07	.75
Na	6.32	6.33	6.76	6.86	.29	.44
K	12.5	11.1	13.0	14.2	1.1	.31
Cu	4.02	3.83	4.40	3.67	.31	.40
Fe	105.5	96.7	110.6	105.1	9.7	.79
Zn	37.4	36.4	35.3	43.4	4.9	.61

[a]Nursery diets
Grower diets: I and II - normal corn with no Volclay
III and IV - normal corn with 1% Volclay
[b]Contaminated with 922 ppb in the diet.
[c]Diet effect.
[d]ppm DM basis.

APPENDIX TABLE 10. MICROMINERAL CONTENT OF THE SERUM OF PIGS FED NON-CONTAMINATED OR AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAY.

	Treatments							
	Normal Corn(NC)	Aflatoxin Corn(AC)[a]	NC+1% Volclay	AC+1% Volclay	SEM	Contrasts[b] A	C	A*C
Initial[c]								
Fe[d]	4.35	4.61	4.46	4.94	.45	.77	.54	.39
Cu	1.55	1.66	1.64	1.59	.11	.81	.93	.49
Zn	.64	.77	.74	.63	.06	.86	.72	.08
Nursery phase[c]								
Fe	1.75	2.31	2.06	2.12	.16	.07	.72	.15
Cu	1.58	1.41	1.67	1.44	.05	.01	.23	.48
Zn	.63	.65	.57	.70	.05	.17	.92	.28
Grower phase[e]								
Fe	3.21	2.68	2.75	3.18	.14	.92	.93	.01
Cu	1.60	1.57	1.67	1.75	.06	.84	.07	.39
Zn	1.04	.97	1.03	1.03	.04	.11	.41	.36
Metabolism phase[f]								
Fe	2.54	2.36	2.84	2.48	.25	.30	.42	.73
Cu	1.90	1.67	1.95	2.03	.07	.34	.01	.05
Zn	.93	.76	.98	1.02	.07	.36	.06	.18

[a]Contaminated with 922 ppb in the diet.
[b]A- Aflatoxin effect (aflatoxin diets vs. normal corn diets)
C- Volclay effect (Volclay-90 diets vs. no Volclay-90 diets)
A*C- Interaction effect.
[c]n=24 for all treatments.
[d]All values ug/L.
[e]Diets were changed to only I or III in grower phase, diet II and IV animals were given I or III respectively. (16%CP)
Diet I n=12, Diet II n=8, Diet III n=12, Diet IV n=8.
[f]n=6 for all treatments.

APPENDIX TABLE 11. WEIGHTS OF LIVERS AND KIDNEYS, EXPRESSED AS A PERCENTAGE OF LIVE BODY WEIGHT, OF PIGS FED NON-CONTAMINATED OR AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAY.

	Treatments							
	Normal Corn(NC)	Aflatoxin Corn(AC)[a]	NC+1% Volclay	AC+1% Volclay	SEM	Contrasts[b] A	C	A*C
Initial (n=6)								
Liver g	2.54							
Kidneys g	.51							
End of nursery phase (n=6/trt)								
Liver g	3.16	3.44	2.99	3.05	.24	.56	.24	.63
Kidneys g	.55	.54	.57	.54	.02	.45	.76	.65
End of metabolism phase (n=6/trt)								
Liver g	2.13	2.40	2.33	2.43	.07	.02	.11	.23
Kidneys g	.41	.34	.44	.42	.02	.01	.01	.34
End of grower phase (n=6/trt)								
Liver g	1.78	1.90	1.67	1.78	.05	.02	.04	.97
Kidneys g	.35	.39	.36	.38	.01	.05	.91	.42

[a]Contaminated with 922 ppb in the diet.
[b]Contrasts
A- Aflatoxin effect (aflatoxin diets vs. no aflatoxin diets)
C- Volclay effect (Volclay-90 diets vs. no Volclay-90 diets)
A*C- Interaction effect.

APPENDIX TABLE 12. BLOOD CHEMISTRY OF GROWER PIGS FED NON-CONTAMINATED AND AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAY. METABOLISM PHASE.

Items	Treatments					P-values[b]		
	Normal Corn(NC)	Aflatoxin Corn(AC)[a]	NC+1% Volclay	AC+1% Volclay	SEM	Trt	A	C	A*C
	(n=24)	(n=24)	(n=24)	(n=24)					
BUN[c]	9.5	11.0	9.3	9.7	.83	.52	.31	.40	.51
T Protein[d]	6.8	6.4	7.2	5.5	.63	.34	.14	.70	.34
Glucose[c]	110.5	105.3	122.5	128.8	9.0	.32	.95	.10	.55
Creatine[c]	1.3	1.4	1.5	1.6	.1	.23	.58	.06	.69
Albumin[d]	3.8	3.6	4.1	4.1	.13	.06	.53	.01	.53
GGT,U/L	41.2	72.2	52.3	60.2	8.64	.18	.07	.96	.23
ALP,U/L	151.3	144.5	127.5	155.3	9.85	.29	.33	.53	.13
Ca[c]	10.0	9.9	10.6	11.2	.47	.31	.66	.10	.51
Mg[c]	2.0	1.9	2.1	2.3	.17	.4	.82	.16	.39
p[c]	8.1	8.3	9.3	9.3	.47	.21	.78	.05	.84
Na[e]	145.5	143.5	155.2	159.8	6.25	.3	.84	.08	.61
K[e]	7.7	7.7	7.8	8.7	.6	.64	.55	.41	.48
Cl[e]	106.7	102.0	111.8	115.0	4.74	.32	.88	.10	.44
CO_2[e]	25.5	24.6	27.1	31.5	4.31	.69	.69	.37	.55
Anion gap[e]	21.0	24.8	24.3	22.2	2.49	.68	.75	.90	.27
Total bili[d]	.12	.10	.22	.12	.04	.28	.21	.21	.36

[a]Diet contains 922 ppb aflatoxin.
[b]A- Aflatoxin effect (aflatoxin diets vs. no aflatoxin diets)
C- Clay effect (clay diets vs. no clay diets).
A*C- Aflatoxin * clay interaction.
[c]mg/dL
[d]g/dL
[e]mmol/L

APPENDIX TABLE 13. MINERAL CONTENT OF LIVERS OF GROWER PIGS FED NON-CONTAMINATED OR AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAY. METABOLISM PHASE.

Items[a]	Treatments				SEM	P-values			
	Normal Corn(NC)	Aflatoxin Corn(AC)[b]	NC+1% Volclay	AC+1% Volclay		Trt	Contrasts[c] A	C	A*C
	(n=6)	(n=6)	(n=6)	(n=6)					
Ca	.38	.36	.38	.40	.02	.51	.84	.32	.27
P	8.97	8.34	8.69	8.92	.15	.04	.21	.36	.01
Mg	1.13	1.07	1.20	1.11	.06	.49	.23	.37	.77
Na	6.82	6.55	6.88	6.62	.18	.50	.15	.72	.97
K	15.0	12.7	13.8	13.6	.98	.28	.13	.89	.22
Cu	3.90	3.96	4.07	4.70	.36	.42	.37	.23	.47
Fe	68.0	60.8	64.7	71.7	5.4	.55	.98	.49	.21
Zn	32.7	28.0	32.4	31.7	2.3	.45	.25	.45	.38

[a]ppm, dry matter basis.
[b]Contaminated with 922 ppb in the diet.
[c]Contrasts
A- Aflatoxin effect (Aflatoxin corn diets vs. normal corn diets)
C- Clay effect (Volclay-90 diets vs. no Volclay-90 diets)
A*C- Interaction effect

APPENDIX TABLE 14. CALCIUM ABSORPTION AND RETENTION IN GROWING PIGS FED NON-CONTAMINATED OR AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAY.

Items[a]	Treatments					P-values			
							Contrasts[c]		
	Normal Corn(NC)	Aflatoxin Corn(AC)[b]	NC+1% Volclay	AC+1% Volclay	SEM	Trt	A	C	A*C
Intake[d]	596	598	604	600	6.8	.85	.92	.45	.64
Feces[d]	214	171	199	220	11.8	.03	.36	.16	.01
Urine[d]	11.2	10.3	13.0	12.8	1.5	.52	.70	.16	.84
Abs 1[e]	5926	5779	6575	5898	199	.04	.05	.07	.20
Abs 2[d]	382	427	405	380	12.5	.04	.41	.34	.01
Abs 3[f]	64.1	71.3	67.0	63.3	1.91	.03	.36	.19	.01
RTN 1[e]	5750	5640	6358	5692	198	.06	.06	.11	.17
RTN 2[d]	370	417	392	367	12.7	.04	.39	.27	.01
RTN 3[g]	97.0	97.6	96.7	96.6	.41	.38	.58	.17	.37
RTN 4[f]	62.2	69.6	64.8	61.1	1.94	.02	.35	.14	.01

[a] n=12/treatment.
[b] Contaminated with 922 ppb in the diet.
[c] Contrasts
A- Aflatoxin effect
C- Clay effect
A*C- Aflatoxin-Clay interaction.
[d] expressed in $mg/d*kg^{.75}$.
[e] expressed in mg/d.
[f] expressed as a percent of intake.
[g] expressed as a percent of absorbed.

APPENDIX TABLE 15. PHOSPHORUS ABSORPTION AND RETENTION IN GROWING PIGS FED NON-CONTAMINATED OR AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAY.

Items[a]	Treatments					P-values			
							Contrasts[c]		
	Normal Corn(NC)	Aflatoxin Corn(AC)[b]	NC+1% Volclay	AC+1% Volclay	SEM	Trt	A	C	A*C
Intake[d]	461	463	467	464	5.24	.85	.92	.45	.64
Feces[d]	186.5	165.6	193.2	186.0	6.51	.04	.04	.05	.30
Urine[d]	21.8	19.7	21.5	25.2	2.42	.45	.73	.29	.24
Abs 1[e]	4280	4043	4446	4334	126	.17	.18	.08	.62
Abs 2[d]	274	297	274	278	7.35	.11	.08	.21	.22
Abs 3[f]	59.5	64.2	58.6	59.9	1.36	.04	.04	.07	.22
RTN 1[e]	3937	3766	4089	3926	125	.37	.20	.23	.97
RTN 2[d]	252	277	252	253	7.88	.09	.12	.13	.13
RTN 3[g]	92.0	93.4	92.1	90.9	.90	.32	.89	.19	.19
RTN 4[f]	54.8	60.0	54.0	54.5	1.47	.03	.07	.04	.12

[a] n=12/treatment.
[b] Contaminated with 922 ppb in the diet.
[c] Contrasts
A- Aflatoxin effect
C- Clay effect
A*C- Aflatoxin-Clay interaction.
[d] expressed in $mg/d*kg^{.75}$.
[e] expressed in mg/d.
[f] expressed as a percent of intake.
[g] expressed as a percent of absorbed.

APPENDIX TABLE 16. MAGNESIUM ABSORPTION AND RETENTION IN GROWING PIGS FED NON-CONTAMINATED OR AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAY.

Items[a]	Treatments				P-values			
						Contrasts[c]		
	Normal Corn(NC)	Aflatoxin Corn(AC)[b]	NC+1% Volclay	AC+1% Volclay	SEM	Trt	A	C	A*C
Intake[d]	111	112	113	112	1.27	.84	.92	.45	.64
Feces[d]	74.4	73.7	87.0	84.1	1.96	.01	.37	.01	.59
Urine[d]	10.2	11.6	11.4	13.2	.98	.20	.11	.16	.80
Abs 1[e]	580	520	425	446	36.4	.02	.59	.01	.28
Abs 2[d]	36.9	38.1	25.9	28.1	2.08	.01	.44	.01	.82
Abs 3[f]	33.0	34.1	22.9	25.0	1.77	.01	.37	.01	.80
RTN 1[e]	419	362	241	235	32.6	.01	.33	.01	.44
RTN 2[d]	26.7	26.5	14.6	14.8	1.98	.01	.99	.01	.91
RTN 3[g]	72.0	68.3	55.2	50.3	3.95	.01	.29	.01	.88
RTN 4[f]	23.8	23.8	12.9	13.2	1.72	.01	.94	.01	.92

[a] n=12/treatment.
[b] Contaminated with 922 ppb in the diet.
[c] Contrasts
A- Aflatoxin effect
C- Clay effect
A*C- Aflatoxin-Clay interaction.
[d] expressed in $mg/d*kg^{.75}$.
[e] expressed in mg/d.
[f] expressed as a percent of intake.
[g] expressed as a percent of absorbed.

APPENDIX TABLE 17. SODIUM ABSORPTION AND RETENTION IN GROWING PIGS FED NON-CONTAMINATED OR AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAY.

Items[a]	Treatments					P-values			
							Contrasts[c]		
	Normal Corn(NC)	Aflatoxin Corn(AC)[b]	NC+1% Volclay	AC+1% Volclay	SEM	Trt	A	C	A*C
Intake[d]	127	128	129	129	1.47	.83	.96	.38	.75
Feces[d]	16.6	14.2	25.7	19.7	1.70	.01	.02	.01	.31
Urine[d]	70.2	60.0	67.9	74.3	6.02	.41	.76	.33	.18
Abs 1[e]	1726	1551	1687	1693	42.7	.04	.06	.24	.04
Abs 2[d]	111	114	103	109	2.33	.04	.08	.02	.58
Abs 3[f]	86.9	88.9	80.1	84.7	1.36	.01	.02	.01	.36
RTN 1[e]	626	696	684	540	69.7	.39	.60	.49	.14
RTN 2[d]	40.5	53.2	41.4	34.8	4.86	.08	.54	.08	.06
RTN 3[g]	36.6	47.2	39.5	31.9	4.49	.14	.74	.18	.06
RTN 4[f]	31.7	41.9	32.0	27.0	3.70	.06	.49	.06	.05

[a] n=12/treatment.
[b] Diet contains 922 ppb aflatoxin.
[c] Contrasts
A- Aflatoxin effect (aflatoxin diets vs. no aflatoxin diets)
C- Clay effect (clay diets vs. no clay diets)
A*C- Aflatoxin-Clay interaction.
[d] expressed in $mg/d*kg^{.75}$.
[e] expressed in mg/d.
[f] expressed as a percent of intake.
[g] expressed as a percent of absorbed.

APPENDIX TABLE 18. POTASSIUM ABSORPTION AND RETENTION IN GROWING PIGS FED NON-CONTAMINATED OR AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAY.

Items[a]	Treatments				P-values			
						Contrasts[c]		
	Normal Corn(NC)	Aflatoxin Corn(AC)[b]	NC+1% Volclay	AC+1% Volclay	SEM	Trt	A	C	A*C
Intake[d]	628	631	637	633	7.14	.85	.92	.45	.64
Feces[d]	102	100	125	121	6.91	.03	.59	.01	.88
Urine[d]	388	334	345	359	20.7	.31	.36	.67	.11
Abs 1[e]	8193	7248	8326	7968	191	.01	.01	.04	.14
Abs 2[d]	527	532	512	512	8.45	.27	.78	.06	.75
Abs 3[f]	83.8	84.4	80.4	81.0	1.07	.03	.62	.01	.97
RTN 1[e]	2153	2661	2710	2302	365	.65	.89	.79	.22
RTN 2[d]	139	197	167	153	24.4	.39	.38	.74	.15
RTN 3[g]	28.6	37.0	32.7	30.0	4.61	.55	.52	.74	.21
RTN 4[f]	21.8	31.3	26.4	24.2	3.78	.35	.34	.72	.14

[a] n=12/treatment.
[b] Contaminated with 922 ppb in the diet.
[c] Contrasts
A- Aflatoxin effect
C- Clay effect
A*C- Aflatoxin-Clay interaction.
[d] expressed in $mg/d*kg^{.75}$.
[e] expressed in mg/d.
[f] expressed as a percent of intake.
[g] expressed as a percent of absorbed.

APPENDIX TABLE 19. IRON ABSORPTION AND RETENTION IN GROWING PIGS FED NON-CONTAMINATED OR AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAY.

Items[a]	Treatments					P-values			
							Contrasts[c]		
	Normal Corn(NC)	Aflatoxin Corn(AC)[b]	NC+1% Volclay	AC+1% Volclay	SEM	Trt	A	C	A*C
Intake[d]	23.2	23.3	35.9	35.7	.29	.01	.82	.01	.58
Feces[d]	18.6	16.4	28.9	31.3	2.88	.01	.94	.01	.08
Urine[d]	.41	.34	.36	.39	.03	.46	.57	.95	.14
Abs 1[e]	92.0	92.8	111.2	93.9	18.1	.83	.65	.58	.61
Abs 2[d]	5.71	6.94	7.11	5.83	1.10	.71	.99	.90	.26
Abs 3[f]	24.7	29.9	19.7	16.3	3.75	.08	.81	.02	.25
RTN 1[e]	85.9	88.3	105.3	87.5	17.9	.83	.67	.60	.57
RTN 2[d]	5.32	6.60	6.74	5.42	1.09	.68	.99	.91	.24
RTN 3[g]	91.5	94.7	91.4	83.9	3.29	.17	.51	.11	.11
RTN 4[f]	23.0	28.5	18.7	15.1	3.69	.09	.80	.03	.22

[a] n=12/treatment.
[b] Contaminated with 922 ppb in the diet.
[c] Contrasts
A- Aflatoxin effect
C- Clay effect
A*C- Aflatoxin-Clay interaction.
[d] expressed in $mg/d*kg^{.75}$.
[e] expressed in mg/d.
[f] expressed as a percent of intake.
[g] expressed as a percent of absorbed.

APPENDIX TABLE 20. ZINC ABSORPTION AND RETENTION IN GROWING PIGS FED NON-CONTAMINATED OR AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAY.

Items[a]	Treatments					P-values			
							Contrasts[c]		
	Normal Corn(NC)	Aflatoxin Corn(AC)[b]	NC+1% Volclay	AC+1% Volclay	SEM	Trt	A	C	A*C
Intake[d]	9.54	9.58	9.67	9.61	.11	.84	.93	.45	.64
Feces[d]	5.69	5.07	5.83	5.71	.20	.06	.08	.06	.22
Urine[d]	.24	.24	.27	.31	.03	.36	.48	.14	.48
Abs 1[e]	60.4	60.9	62.4	60.8	3.4	.98	.88	.78	.76
Abs 2[d]	3.85	4.51	3.84	3.90	.19	.05	.07	.12	.12
Abs 3[f]	40.3	47.2	39.7	40.6	2.0	.04	.06	.08	.13
RTN 1[e]	56.7	57.7	58.0	56.1	3.4	.98	.90	.97	.68
RTN 2[d]	3.60	4.27	3.58	3.59	.19	.04	.09	.08	.10
RTN 3[g]	93.5	94.6	92.8	92.1	.84	.20	.81	.06	.29
RTN 4[f]	37.8	44.7	37.0	37.4	2.0	.03	.08	.05	.11

[a] n=12/treatment.
[b] Contaminated with 922 ppb in the diet.
[c] Contrasts
A- Aflatoxin effect
C- Clay effect
A*C- Aflatoxin-Clay interaction.
[d] expressed in $mg/d*kg^{.75}$.
[e] expressed in mg/d.
[f] expressed as a percent of intake.
[g] expressed as a percent of absorbed.

APPENDIX TABLE 21. COPPER ABSORPTION AND RETENTION IN GROWING PIGS FED NON-CONTAMINATED OR AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAY.

Items[a]	Treatments				P-values			
						Contrasts[c]		
	Normal Corn(NC)	Aflatoxin Corn(AC)[b]	NC+1% Volclay	AC+1% Volclay	SEM	Trt	A	C	A*C
Intake[d]	1.74	1.75	1.77	1.76	.02	.83	.98	.44	.60
Feces[d]	1.57	1.39	1.46	1.45	.08	.36	.56	.33	.18
Urine[d]	.04	.03	.03	.04	.01	.27	.23	.84	.12
Abs 1[e]	3.34	5.21	7.26	5.87	1.37	.23	.86	.10	.23
Abs 2[d]	.22	.39	.42	.36	.08	.36	.56	.33	.18
Abs 3[f]	12.9	22.6	23.8	20.3	5.1	.40	.54	.39	.19
RTN 1[e]	2.62	5.37	6.64	5.43	1.41	.19	.58	.16	.17
RTN 2[d]	.18	.40	.38	.33	.09	.24	.34	.44	.12
RTN 3[g]	80.5	88.6	82.1	83.6	6.1	.84	.43	.78	.59
RTN 4[f]	10.1	23.2	21.6	18.6	5.1	.27	.33	.50	.13

[a] n=12/treatment.
[b] Contaminated with 922 ppb in the diet.
[c] Contrasts
A- Aflatoxin effect
C- Clay effect
A*C- Aflatoxin-Clay interaction.
[d] expressed in $mg/d*kg^{.75}$.
[e] expressed in mg/d.
[f] expressed as a percent of intake.
[g] expressed as a percent of absorbed.

APPENDIX TABLE 22. MANGANESE ABSORPTION AND RETENTION IN GROWING PIGS FED NON-CONTAMINATED OR AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAY.

Items[a]	Treatments					P-values			
							Contrasts[c]		
	Normal Corn(NC)	Aflatoxin Corn(AC)[b]	NC+1% Volclay	AC+1% Volclay	SEM	Trt	A	C	A*C
Intake[d]	3.11	3.12	3.15	3.13	.04	.85	.90	.47	.64
Feces[d]	2.67	2.26	2.67	2.48	.13	.09	.03	.39	.38
Urine[d]	.11	.09	.11	.11	.02	.55	.41	.38	.44
Abs 1[e]	8.58	11.37	9.33	10.07	1.84	.73	.34	.88	.58
Abs 2[d]	.55	.87	.57	.65	.13	.25	.12	.43	.35
Abs 3[f]	17.8	27.9	18.2	20.8	3.6	.23	.11	.39	.33
RTN 1[e]	8.4	10.2	7.3	8.3	1.8	.61	.39	.36	.82
RTN 2[d]	.56	.78	.44	.54	.12	.15	.14	.12	.58
RTN 3[g]	72.6	88.5	76.3	76.6	5.2	.10	.09	.39	.11
RTN 4[f]	17.8	25.1	14.2	17.2	3.8	.13	.14	.10	.54

[a] n=12/treatment.
[b] Contaminated with 922 ppb in the diet.
[c] Contrasts
A- Aflatoxin effect
C- Clay effect
A*C- Aflatoxin-Clay interaction.
[d] expressed in $mg/d*kg^{.75}$.
[e] expressed in mg/d.
[f] expressed as a percent of intake.
[g] expressed as a percent of absorbed.

APPENDIX TABLE 23. BODY WEIGHTS OF WEANLING PIGS FED A NON-CONTAMINATED DIET OR AN AFLATOXIN-CONTAMINATED DIET WITH SEVERAL TYPES OF CLAYS.

	Diets									
Items[a]	I Normal Corn	II Aflatoxin Corn(AC)[b]	III AC+.5% 15 RVM	IV AC+.5% 55 RVM	V AC+.5% 80 RVM	VI AC+.5% Zeobrite	VII AC+.5% Novasil	VIII AC+.5% FD-181	IX AC+.5% AB-20	SEM
Body weights, kg										
Initial	9.63	9.61	9.66	9.67	9.67	9.68	9.65	9.68	9.67	.09
Week 1	12.47	11.63	11.80	12.17	11.55	12.21	12.06	12.21	11.59	.23
Week 2	16.75^{c}	14.77^{d}	14.93^{c}	15.71^{cd}	15.20^{d}	16.12^{cd}	15.95^{cd}	15.98^{cd}	15.14^{c}	.30
Week 3	21.62^{c}	17.67^{e}	18.65^{de}	19.84^{cd}	19.85^{cd}	20.00^{cd}	20.71^{cd}	20.54^{cd}	19.89^{cd}	.43
Week 4	26.79^{c}	21.12^{e}	23.40^{de}	24.53^{de}	24.58^{cd}	24.98^{cd}	26.19^{cd}	25.69^{cd}	25.05^{cd}	.58
Week 5	31.88^{c}	24.93^{e}	27.55^{de}	28.40^{cde}	29.07^{cd}	29.02^{cde}	30.35^{cd}	30.10^{cd}	28.94^{cde}	.81

[a] n=9/treatment.
[b] Contaminated with 500 ppb in the diet.
[c,d] Means with different superscripts differ ($P < .05$).

APPENDIX TABLE 24. AVERAGE DAILY GAINS OF WEANLING PIGS FED A NON-CONTAMINATED DIET OR AN AFLATOXIN-CONTAMINATED DIET WITH SEVERAL TYPES OF CLAYS.

	Diets									
Items[a]	I Normal Corn	II Aflatoxin Corn(AC)[b]	III AC+.5% 15 RVM	IV AC+.5% 55 RVM	V AC+.5% 80 RVM	VI AC+.5% Zeobrite	VII AC+.5% Novasil	VIII AC+.5% FD-181	IX AC+.5% AB-20	SEM
Average daily gains, g										
Week 1	407	289	306	357	269	361	345	361	274	36.6
Week 2	611[c]	449[d]	446[d]	506[cd]	521[cd]	559[cd]	555[cd]	539[cd]	507[cd]	27.7
Week 3	696[c]	414[d]	531[cd]	590[cd]	665[c]	554[cd]	680[c]	652[c]	678[c]	40.0
Week 4	739[c]	494[d]	679[cd]	670[cd]	676[cd]	711[cd]	783[c]	735[c]	738[c]	44.2
Week 5	813	596	660	586	722	641	685	714	629	85.6
Weeks 1-5	648[c]	446[d]	522[cd]	546[cd]	567[cd]	565[cd]	605[c]	597[c]	562[cd]	25.3

[a] n=9/treatment.
[b] Contaminated with 500 ppb in the diet.
[c,d] Means with different superscripts differ (P <.05)

APPENDIX TABLE 25. AVERAGE DAILY FEED INTAKE OF WEANLING PIGS FED A NON-CONTAMINATED DIET OR AN AFLATOXIN-CONTAMINATED DIET WITH SEVERAL TYPES OF CLAYS.

	Diets									
Items[a]	I Normal Corn	II Aflatoxin Corn(AC)[b]	III AC+.5% 15 RVM	IV AC+.5% 55 RVM	V AC+.5% 80 RVM	VI AC+.5% Zeobrite	VII AC+.5% Novasil	VIII AC+.5% FD-181	IX AC+.5% AB-20	SEM
Average daily feed intake, g										
Week 1	655	554	662	626	576	670	821	641	576	36.6
Week 2	1188^{cd}	907^{cd}	893^{d}	1022^{cd}	1087^{cd}	1058^{cd}	1231^{c}	1037^{cd}	1080^{cd}	66.8
Week 3	1469^{c}	950^{e}	1130^{de}	1188^{cde}	1418^{cd}	1260^{cd}	1469^{c}	1354^{cd}	1390^{cd}	60.9
Week 4	1691^{cd}	1030^{e}	1354^{de}	1433^{cd}	1570^{cd}	1505^{cd}	1757^{c}	1591^{cd}	1613^{cd}	75.3
Week 5	1812	1228	1555	1421	1665	1536	1755	1744	1655	119.5
Weeks 1-5	1351^{cd}	931^{f}	1104^{ef}	1137^{def}	1251^{cde}	1197^{cde}	1396^{c}	1258^{cde}	1252^{cde}	47.6

[a] n=9/treatment.
[b] Contaminated with 500 ppb in the diet.
[c,d,e,f] Means with different superscripts differ ($P < .05$).

APPENDIX TABLE 26. FEED TO GAIN RATIOS OF WEANLING PIGS FED A NON-CONTAMINATED DIET OR AN AFLATOXIN-CONTAMINATED DIET WITH SEVERAL TYPES OF CLAYS.

	Diets									
Items[a]	I Normal Corn	II Aflatoxin Corn(AC)[b]	III AC+.5% 15 RVM	IV AC+.5% 55 RVM	V AC+.5% 80 RVM	VI AC+.5% Zeobrite	VII AC+.5% Novasil	VIII AC+.5% FD-181	IX AC+.5% AB-20	SEM
Feed to gain ratios										
Week 1	1.63	1.90	2.22	1.74	2.22	1.92	2.40	1.78	2.10	.20
Week 2	1.97	2.04	2.01	2.04	2.11	1.90	2.22	1.92	2.17	.11
Week 3	2.11	2.38	2.12	2.01	2.14	2.29	2.17	2.09	2.05	.17
Week 4	2.33	2.09	2.00	2.13	2.33	2.12	2.24	2.17	2.19	.12
Week 5	2.23	2.09	2.33	2.67	2.30	2.39	2.74	2.44	2.73	.31
Weeks 1-5	2.08	2.10	2.11	2.07	2.22	2.12	2.31	2.11	2.24	.09

[a] n=9/treatment.
[b] Contaminated with 500 ppb in the diet.

APPENDIX TABLE 27. BLOOD CHEMISTRY OF WEANLING PIGS FED A NON-CONTAMINATED DIET OR AN AFLATOXIN-CONTAMINATED DIET WITH SEVERAL TYPES OF CLAYS.

	Diets									
Items[a]	I Normal Corn	II Aflatoxin Corn(AC)[b]	III AC+.5% 15 RVM	IV AC+.5% 55 RVM	V AC+.5% 80 RVM	VI AC+.5% Zeobrite	VII AC+.5% Novasil	VIII AC+.5% FD-181	IX AC+.5% AB-20	SEM
BUN, mg/dl	13.6^{c}	8.0^{d}	9.8^{cd}	8.8^{d}	11.3^{cd}	10.3^{cd}	12.0^{cd}	13.7^{c}	11.2^{cd}	.78
T Protein,g/dl	6.37	5.73	5.81	6.45	6.45	6.05	6.05	6.32	6.27	.16
Glucose, mg/dl	123	118	116	109	121	117	114	116	120	7.1
Albumin, g/dl	4.02^{cd}	3.13^{d}	3.67^{cd}	3.88^{cd}	4.22^{c}	3.82^{cd}	3.92^{cd}	3.92^{cd}	4.00^{cd}	.17
GGT, U/L	52^{cd}	92^{c}	63^{cd}	77^{cd}	44^{d}	50^{d}	40^{d}	51^{d}	42^{d}	7.2
AST, U/L	63^{d}	82^{cd}	69^{cd}	71^{cd}	77^{cd}	73^{c}	57^{c}	81^{cd}	66^{d}	4.8
ALP, U/L	152	431	170	220	197	199	181	158	176	59.2
Ca, mg/dl	10.9	10.9	10.7	11.1	11.0	10.9	10.7	10.9	11.0	.24
Mg, mg/dl	2.0	2.0	2.0	2.1	2.0	2.1	2.0	2.0	2.1	.05
P, mg/dl	8.53	8.30	8.28	8.80	8.80	8.73	8.57	8.62	8.65	.29
Na, mmol/L	152^{c}	148^{cd}	143^{d}	148^{cd}	148^{cd}	151^{c}	148^{cd}	146^{cd}	150^{cd}	1.4
K, mmol/L	6.8	6.3	5.8	6.4	6.5	6.4	6.0	6.1	6.4	.36

[a] n=9/treatment.
[b] Contaminated with 500 ppb in the diet.
[c,d] Means with different superscripts differ ($P < .05$).

APPENDIX TABLE 28. BODY WEIGHTS AND AVERAGE DAILY GAIN OF WEANLING PIGS FED NON-CONTAMINATED CORN OR AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAYS. TRIAL II.

Items	Treatments				SEM	P-values			
							Contrasts[b]		
	Normal Corn(NC)	Aflatoxin Corn(AC)[a]	AC+1% HSCA	AC+1% Ca Ben.		Trt[c]	A	H	C
	(n=12)	(n=12)	(n=11)	(n=12)					
Body weights, kg									
Initial	9.05	9.05	9.09	9.11	.092	.94	.98	.73	.63
Week 1	11.23	11.04	11.37	11.12	.161	.53	.42	.17	.72
Week 2	12.97	12.18	13.49	12.88	.169	.01	.01	.01	.01
Week 3	16.80	15.34	17.37	16.82	.309	.01	.01	.01	.01
Week 4	20.22	18.85	20.84	20.39	.319	.01	.01	.01	.01
Week 5	23.64	22.71	24.83	24.82	.462	.01	.18	.01	.01
ADG, g									
Week 1	311	285	325	287	24.9	.63	.46	.28	.94
Week 2	247	163	304	251	19.3	.01	.01	.01	.01
Week 3	548	451	553	563	34.7	.12	.07	.05	.04
Week 4	489	501	496	509	21.1	.93	.70	.87	.80
Week 5	488	552	570	633	51.4	.29	.39	.80	.28
Wk 1-2	279	224	314	269	14.9	.01	.02	.01	.05
Wk 1-3	369	300	394	367	16.6	.01	.01	.01	.01
Wk 1-4	399	350	420	403	12.8	.01	.02	.01	.01
Wk 4-5	488	526	533	571	23.5	.24	.33	.87	.26
Wk 1-5	417	390	450	449	13.6	.02	.19	.01	.01

[a]Contaminated with 922 ppb in the diet.
[b]Contrasts
A- Aflatoxin effect (Aflatoxin corn diet vs normal corn diet)
H- HSCA effect (HSCA diet vs aflatoxin diet)
C- Ca Bentonite effect (Ca bentonite diet vs aflatoxin diet).

APPENDIX TABLE 29. AVERAGE DAILY FEED INTAKE AND FEED TO GAIN RATIO OF WEANLING PIGS FED NON-CONTAMINATED AND AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAYS. TRIAL II.

Items	Treatments					P-values			
							Contrasts[b]		
	Normal Corn(NC)	Aflatoxin Corn(AC)[a]	AC+1% HSCA	AC+1% Ca Ben	SEM	Trt	A	H	C
ADFI, g	(n=12)	(n=12)	(n=11)	(n=12)					
Week 1	395	394	457	449	29.7	.30	.98	.15	.21
Week 2	573	526	769	597	42.5	.01	.44	.01	.26
Week 3	938	803	1049	988	31.4	.01	.01	.01	.01
Week 4	1030	949	1163	1027	43.1	.02	.20	.01	.22
Week 5	1159	1172	1373	1277	66.1	.12	.89	.05	.28
Wk 1-2	484	460	613	523	32.1	.02	.60	.01	.19
Wk 1-3	635	574	758	678	28.8	.01	.15	.01	.02
Wk 1-4	734	668	860	765	22.6	.01	.06	.01	.01
Wk 1-5	819	769	962	867	23.3	.01	.15	.01	.01
Feed to gain ratio									
Week 1	1.27	1.39	1.44	1.57	.06	.02	.17	.49	.04
Week 2	2.41	3.55	2.66	3.01	.43	.31	.08	.17	.39
Week 3	1.71	1.80	1.92	1.78	.09	.42	.51	.32	.89
Week 4	2.13	1.91	2.35	2.03	.07	.01	.06	.01	.29
Week 5	2.43	2.13	2.56	2.09	.16	.17	.22	.09	.85
Wk 1-2	1.75	2.07	1.98	1.97	.08	.07	.01	.44	.37
Wk 1-3	1.72	1.92	1.96	1.84	.06	.04	.03	.64	.33
Wk 1-4	1.84	1.91	2.07	1.90	.05	.04	.38	.05	.86
Wk 1-5	1.96	1.97	2.15	1.93	.05	.02	.93	.01	.60

[a]Contaminated with 922 ppb in the diet.
[b]Contrasts
A- Aflatoxin effect (Aflatoxin corn diet vs normal corn diet)
H- HSCA effect (HSCA diet vs aflatoxin diet)
C- Ca bentonite effect (Ca bentonite diet vs aflatoxin diet).

APPENDIX TABLE 30. BLOOD CHEMISTRY OF WEANLING PIGS FED NON-CONTAMINATED CORN OR AFLATOXIN-CONTAMINATED CORN WITH AND WITHOUT CLAYS. TRIAL II.

Items	Treatments				P-values			
						Contrasts[b]		
	Normal Corn(NC)	Aflatoxin Corn(AC)[a]	AC+1% HSCA	AC+1% Ca Ben.	SEM	Trt	A	H	C
	(n=12)	(n=12)	(n=11)	(n=12)					
BUN[c]	8.25	10.70	8.19	11.16	.94	.05	.07	.07	.73
T Protein[d]	4.82	4.88	4.95	5.51	.34	.87	.91	.89	.20
Glucose[c]	83.6	88.4	83.8	94.9	5.9	.50	.56	.59	.44
Albumin[d]	2.48	2.76	2.73	3.14	.22	.21	.36	.92	.23
GGT, U/L	42.2	42.0	36.0	41.4	3.9	.66	.97	.29	.91
AST, U/L	45.9	47.2	45.3	48.4	5.2	.98	.86	.80	.87
ALP, U/L	167.7	182.1	151.4	162.7	12.3	.38	.41	.09	.27
CA[c]	8.37	8.86	8.67	9.60	.52	.40	.51	.81	.32
MG[c]	1.97	2.06	1.96	2.27	.16	.50	.71	.69	.34
P[c]	7.66	8.19	8.00	8.60	.57	.70	.51	.82	.61
NA[e]	121.3	129.6	129.4	138.1	7.0	.41	.41	.98	.37
K[e]	4.57	4.64	4.65	4.86	.27	.89	.8	.99	.57

[a]Contaminated with 922 ppb in the diet.
[b]Contrasts
A- Aflatoxin effect (Aflatoxin corn diet vs. normal corn diet)
H- HSCA effect (HSCA diet vs. aflatoxin diet)
C- Ca Bentonite effect (Ca bentonite diet vs aflatoxin diet).
[c]mg/dL.
[d]g/dL.
[e]mmol/L.

APPENDIX TABLE 31. PROCEDURE FOR THE DETERMINATION OF HUMORAL IMMUNE RESPONSE

A. Solutions

1. Coating buffer: sodium carbonate buffer, pH 9.6
   1.59 g $NaCO_3$ granular (anhydrous)
   2.93 g $NaHCO_3$
   Make up to 1000 ml with distilled H_2O

2. PBS/Tween buffer, pH 9.8
   8.0 g NaCl
   0.2 g KH_2PO_4
   1.15 g $Na_2HPO_4 \cdot 12H_2O$
   0.2 g KCl
   0.5 ml Tween 20
   Make up to 1000 ml with distilled H_2O

3. Substrate solution for Peroxidase ABTS
   1 mg ABTS (Sigma #A-1888) per 1 ml sodium citrate buffer, 0.1 mol/l, pH 4.0, add 30 % H_2O_2/ml ABTS (1/5000)

B. Procedure

1. Dilute antigen in coating buffer, 7 ug/ml.

2. Add 100 ul of antigen coating solution to test and control wells of the microtiterplate (Nunc, Maxisorp, 96 well plate, Nunc #4-39454). Incubate overnight at room temp.

3. Wash antigen solution from plate 4 times with PBST and allow to air dry, upside down.

4. Add 100 ul of antibody (or serum with antibody) in PBS/Tween 20 buffer and incubate for 3 hours.

5. Wash the plates 4 times with PBST to remove antibody solutions.

6. Apply Rabbit Anti-Swine IgG (Cappell, 3215-0082, West Chester, PA) diluted in PBST 1:5000, 100 ul per well. Incubate for 2 hours at room temperature.

7. Wash plates 4 times with PBST to remove the Rabbit Anti-Swine solution.

8. Apply Peroxidase substrate, 1 mg/ml ABTS, 100 ul per well. Allow green color to develop (5 min.).

9. Stop reaction with 100 ul per well of 10 mM Sodium Azide.

10. Read absorbance at 414 NM on a spectophotometer (Titertek Multiskan MCC/340).

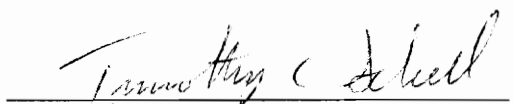
Vita

Timothy Charles Schell, son of Patricia Schell, was born June 27, 1964 in Nampa, Idaho. He graduated from Lafayette High School in Williamsburg, Virginia in June of 1982. He received the Bachelor of Science degree in Animal and Dairy Science at Auburn University in June of 1986.

He spent one year at the University of California, San Francisco in the Neurology department assisting in Alzhiemer's disease related research.

In August 1989, he began study towards a Master of Science degree in Animal Science at Virginia Polytechnic Institute and State University.

He is a member of Omicron Delta Kappa and Delta Tau Delta.



Timothy C. Schell