Effects of Modified Montmorillonite Nanocomposite on Growing/Finishing Pigs during Aflatoxicosis

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ABSTRACT : Experiments were conducted to determine the efficacy of modified montmorillonite nanocomposite (MMN) to reduce the toxic effects of aflatoxin (AF) in growing/finishing pigs. 96 weaned pigs were assigned to four dietary treatment groups (0 g of MMN and 0 mg of AF/kg feed, 3 g of MMN/kg feed, 0.1 mg of AF/kg feed, and 3 g of MMN plus 0.1 mg of AF/kg feed). Body weight gain (BW gain), feed/gain ratio, serum biochemical values and enzyme activities were evaluated. Compared with the control, AF alone markedly reduced BW gain and resulted in a significantly higher feed/gain ratio. There were no differences in BW gain and feed/gain ratio between 0.3% MMN or 0.3% MMN plus AF and the control. These results suggested that the deleterious effects of AF were ameliorated by MMN addition. AF intake markedly increased relative organ weights of liver, kidney, spleen and pancreas, and resulted in significant alterations of serum parameters. However, these parameters for pigs fed diets containing MMN and AF returned to normal values, indicating that MMN had the ability to recover the AF-decreased performance, organ damage and to correct aberrations in serum parameters. These findings in our study suggested that MMN can effectively modulate the toxicity of AF in growing/finishing pigs and may offer a novel approach to the preventive management of aflatoxicosis in animals. *(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 9 : 1305-1309)*

Key Words : Aflatoxin, Modified Montmorillonite Nanocomposite (MMN), Detoxification, Pigs

INTRODUCTION

The mycotoxin, aflatoxin (AF) B_1, G_1, B_2 and G_2, are highly toxic, mutagenic, teratogenic and carcinogenic compounds produced by species of *A. flavus* and *A. parasiticus* (Smela et al., 2001; Mishra et al., 2003). Liver damage, gastrointestinal dysfunction, lower reproductivity, reduced feed utilization and efficiency, anemia, and jaundice are toxic manifestations linked to aflatoxicosis in various species of animals (Huff et al., 1986; Harvey et al., 1988; Robens et al., 1992). Pigs are highly sensitive to the effects of AF, and exposure to AF-contaminated feed seriously affects the economy of the swine industry. Therefore, practical and cost-effective methods to detoxify AF-containing feedstuffs are in great demand. One of the more encouraging approaches is the addition of non-nutritive sorptive materials to feedstuffs with the consequent reduction of the gastrointestinal absorption of these fungal metabolites. Studies showed that dietary addition of montmorillonite, bentonite and hydrated sodium calcium aluminosilicate (HSCAS) can reduce detrimental effects on farm animals of several mycotoxins like AF and T-2 toxin (Bonna et al., 1991; Harvey et al., 1991, 1994; Kubena et al., 1990, 1991, 1993; Abdel-Wahhab et al., 1999). But natural montmorillonite is always congregated with some impurities, which make their adsorptive effect difficult to exert and make their addition level high.

Modified montmorillonite nanocomposite (MMN) is a new sorptive additive developed by our institute.

We made preparation to natural montmorillonite via separation, purification, depolymerization and dispersion and got nanoparticles which size was 10-60 nm and purity was above 98%, and then made modification to these nanoparticles by pillar composition, intercalation composition and spot polymerization (Vaia et al., 1997a, b; Ishida et al., 2000), finally constructed a new inorganic nanocomposite (MMN).

Because MMN possess sizable surface areas, high porosity, and variable cation exchange activities along with active sites, it can interact with and immobilize AF molecules via macro quantum tunnel effect or weak electrostatic forces or through the formation of strong bonds (Dale et al., 1991), then reduce gastrointestinal absorption of toxins and ameliorate adverse effects of AF on animals.

The objective of this experiment was to evaluate the efficacy of MMN to reduce bioavailability and to diminish the toxic effects of AF in pigs.

MATERIALS AND METHODS

Materials

AF was produced via fermentation of milled corn by *A. parasiticus* NRRL 2999. The sterile substrate was inoculated with 100 ml of the mold aqueous suspension containing 10^6 spores^{-1} ml, cultures were allowed to grow for 15 d at 28°C in darkness. On the fifteenth day, culture materials were autoclaved and dried. The AF content was measured by HPLC according to AOAC (1995). The AF

Table 1. The composition of normal and AF-contaminated corn/soybean diets

Item	Growing phase[1]		Finishing phase[2]	
	Normal diet	AF diet	Normal diet	AF diet
Uncontaminated corn	50.00	40.00	50.60	40.60
Contaminated corn[3]	0.00	10.00	0.00	10.00
Soybean meal	19.00	19.00	17.40	17.40
Rapeseed meal	4.00	4.00	5.00	5.00
Whaet meddling	18.00	18.00	17.00	17.00
Wheat bran	5.10	5.10	6.00	6.00
Limestone	1.30	1.30	1.30	1.30
Dicalcium phosphate	1.20	1.20	1.20	1.20
Salt	0.40	0.40	0.50	0.50
Premix[4]	1.00	1.00	1.00	1.00

[1] Calculated to supply 17.24% CP, 0.83% lysine, 0.86% Ca and 0.35% available P.

[2] Calculated to supply 16.92% CP, 0.81% lysine, 0.86% Ca, and 0.35% available P.

[3] Contaminated corn contained 1.000 μg/kg of aflatoxin B_1.

[4] Supplied per kg of diet: 180 mg of Zn; 150 mg of Cu; 50 mg of Mn; 0.3 mg of I; 0.3 mg of Se; 0.3 mg of Co; 6.500 IU of vitamin A; 750 IU of vitamin D3; 20 IU of vitamin E; 3.5 mg of vitamin K3; 2.8 mg of vitamin B1; 6.2 mg of vitamin B2; 33 mg of niacin; 18 mg of d-pantothenic acid; 3.5 mg of vitamin B6; 0.85 mg of folic acid; 60.0 μg of biotin; 35 μg of vitamin B12; and 600 mg choline chloride. Premix also provided 80 mg of chlortetracycline and 1.000 mg L-lysine HCL (purity, 98%) per kg of diet.

content in the milled corn was 84% AFB_1, 8% AFG_1, 6% AFB_2 and 2% AFG_2. The milled corn was incorporated into the basal diet to provide the desired level of 0.1 mg AFB_1/kg of diet. MMN was obtained from Feed Science Institute, Zhejiang University, Hangzhou, China.

Animals and diets

A total of 96 crossbred (Yorkshire×Landrace× Hampshire) weaned barrows and gilts (mean body weight, 29.8±1.32 kg) were identified by individual ear tags and tattoos, and randomly assigned to four treatment groups by sex (three replicates per treatment with eight pigs per replicate), housed in concrete-floored indoor pens, and provided with corn/soybean diets and water *ad libitum*. The diets either met or exceeded critical nutrient concentrations, as recommended by the National Research Council (1998). Four dietary treatments of 0 g of MMN and 0 mg of AF/kg feed (control), 3 g of MMN/kg feed (0.3% MMN), 0.1 mg of AF/kg feed, and 3 g of MMN plus 0.1 mg of AF/kg feed were fed to treatment groups for 90 days (Table 1).

Pigs were weighed and group feed consumption were recorded weekly. Blood samples were taken via jugular venipuncture for serum biochemical and enzymatic measurements at the end of the trial. Serum analysis for glutamic-pyruvic transaminase (GPT), glutamic-oxalacetic transaminase (GOT), alkaline phosphatase (ALP), lactic dehydrogenase (LDH) activities and for total protein, albumin (ALB), globulin (GLOB), urea nitrogen, cholesterol, triglycerides were accomplished by described methods using an biochemical autoanalyzer (Beckman Instruments, Inc.).

Histopathology

At the end of the trial, pigs were euthanatized and necropsied. Liver, kidney, spleen and pancreas were weighed, and organ weight was calculated as a percentage of body weight (g/100 g). Liver and kidney Specimens were fixed in 10% neutral buffered formalin. Fixed tissues were trimmed, embedded in paraffin, and stained with hematoxylin and eosin for histopathological examination.

Statistical analysis

All data were subjected to statistical analysis using the General Linear Models Procedure of the Statistical Analysis System (SAS Institute, 1985). The significance of the differences among treatment groups with variable means was determined by Duncan's new multiple range test. All statements of significance were based on probability $p \leq 0.05$.

RESULTS

Pigs of the control group, 0.3% MMN, and 0.3% MMN plus AF treatment groups did not have clinical signs of disease or abnormal behavior, whereas AF-treated pigs were listless, had rough coat, and were noticeably smaller than pigs of the other groups. The effects of MMN and AF diets on growth performance were presented in Table 2. The results showed that 0.3% MMN in the diet significantly diminished the inhibitory effects of feeding 0.1 mg AF/kg diet. Mortality did not occur. Compared with the control group, a significant decrease in BW gain was observed in pigs fed AF alone (p<0.05), but no differences in BW gain were noted in other diets. The feed/gain ratio was significantly increased by AF intake (p<0.05), the other treatments did not show significant differences.

The effects of dietary treatments on serum parameters

Table 2. Effects of MMN and AF on growth performance in pigs[1]

Item	Treatment 1	Treatment 2	Treatment 3	Treatment 4
BW gain (kg)	0.62±0.01[a]	0.64±0.03[a]	0.54±0.03[b]	0.61±0.02[a]
Feed/gain ratio	2.52±0.07[b]	2.51±0.05[b]	2.71±0.05[a]	2.58±0.06[b]
Mortality (%)	0	0	1.39	0

[1] Values within a row with the different superscript letters differ significantly (p<0.05).

Table 3. Effects of MMN and AF on serum biochemical values of pigs[1]

Item	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Total protein (g/dl)	9.40±0.37[a]	9.35±0.56[a]	8.79±0.45[b]	9.30±0.44[a]
Albumin (g/dl)	1.54±0.17[a]	1.52±0.14[a]	1.27±0.19[b]	1.51±0.19[a]
Globulin (g/dl)	7.85+0.22[a]	7.83+0.24[a]	7.54+0.22[b]	7.80+0.18[a]
Urea nitrogen (mg/dl)	18.73±2.17[a]	18.45±1.82[a]	15.51±2.16[b]	18.12±2.52[a]
Cholesterol (mg/dl)	87.49±3.72[a]	87.32±4.86[a]	73.38±7.62[b]	84.27±7.92[a]
Triglycerides (mg/dl)	84.30±4.07[a]	83.53±3.90[a]	72.44±4.53[b]	83.25±4.79[a]

[1] Values within a row with the different superscript letters differ significantly (p 0.05).

Table 4. Effects of MMN and AF on serum enzyme activities of pigs[1]

Item	Treatment 1	Treatment 2	Treatment 3	Treatment 4
GPT (U/ml)	20.76±2.49[b]	20.75±3.42[b]	24.08±3.00[a]	20.96±2.56[b]
GOT (U/ml)	14.67±2.18[b]	14.18±1.61[b]	18.46±3.27[a]	15.08±2.01[b]
ALP (U/100 ml)	16.42±1.57[b]	16.06±2.49[b]	20.02±2.37[a]	17.20±2.21[b]
LDH (U/ml)	4.36±0.61[b]	4.18±0.42[b]	4.92±0.37[a]	4.37±0.34[b]

[1] Values within a row with the different superscript letters differ significantly (p 0.05).
[2] GPT: glutamic-pyruvic transaminase; GOT: glutamic-oxalacetic transaminase; ALP: alkaline phosphatase; LDH: lactic dehydrogenase.

Table 5. Effects of MMN and AF on relative organ weights of pigs[1]

Item	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Liver (g/100 g)	1.82±0.13[b]	1.81±0.13[b]	1.97±0.12[a]	1.84±0.08[b]
Kidney (g/100 g)	0.44±0.05[b]	0.43±0.05[b]	0.56±0.05[a]	0.46±0.04[b]
Spleen (g/100 g)	0.17±0.02[bc]	0.16±0.02[c]	0.24±0.02[a]	0.18±0.02[b]
Pancreas (g/100 g)	0.14±0.02[b]	0.14±0.02[b]	0.20±0.03[a]	0.15±0.02[b]

[1] Values within a row with the different superscript letters differ significantly (p 0.05).
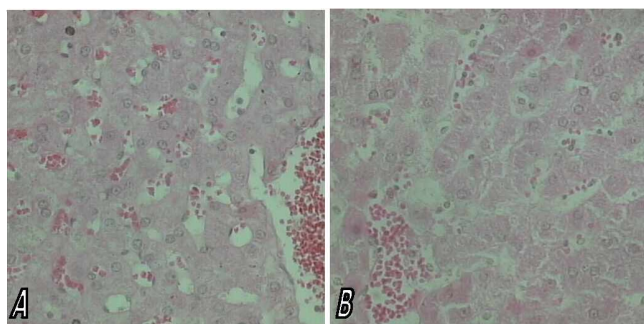

Figure 1. Photomicrographs of sections of porcine liver specimens. (A) AF group showing diffuse vacuolization and hepatic steatosis. (B) MMN plus AF group showing the lack of lesions. Hematoxylin and eosin stain, ×200.

were shown in Table 3 and 4. Toxicity of AF expressed through significant changes in serum biochemical values and enzymatic activities. When compared to the control. Levels of serum total protein, ALB, GLOB, urea nitrogen, cholesterol and triglycerides were markedly decreased in pigs fed AF alone (p<0.05), whereas GPT, GOT, ALP and LDH activities were significantly increased in pigs fed AF alone (p<0.05). But there were no significant differences in these parameters between 0.3% MMN or 0.3% MMN plus AF and the control. This demonstrated that addition of 0.3% MMN made the levels of these parameters return to the normal.

Table 5 showed the effects of MMN and AF on relative

organ weights. Compared with the control, the diet containing AF alone significantly increased relative liver, kidney, spleen and pancreas weights (p<0.05). However, addition of 0.3% MMN to the AF-contaminated diet prevented an increase in weight of these organs. The significant increases in relative organ weights were prevent by the MMN addition, that is, there was no significant difference between 0.3% MMN plus AF and the control.

Microscopically, hepatic lesions in pigs of the AF-alone treatment group were characterized as severe diffuse cytoplasmatic vacuolization accompanied by centrilobular and peripheral-lobular lipidosis and bile duct hyperplasia (Figure 1A). When AF fed to pigs, renal damage including localized degeneration and vacuolization in cytoplasm of convoluted tubular epithelia were observed (Figure 2A). Microscopic lesions of the liver and kidney were not observed in pigs of the control, MMN, or MMN plus AF treatment groups (Figure 1B, 2B).

DISCUSSION

AF is very harmful to the swine industry because of its toxicity and occurrence in feedstuffs. In our study, aflatoxicosis was induced by a diet of 0.1 mg of AF/kg feed. The toxic effects of AF were expressed as reduced BW gain, higher feed/gain ratio, increased relative organ weights and serum biochemical alterations. These results agreed with
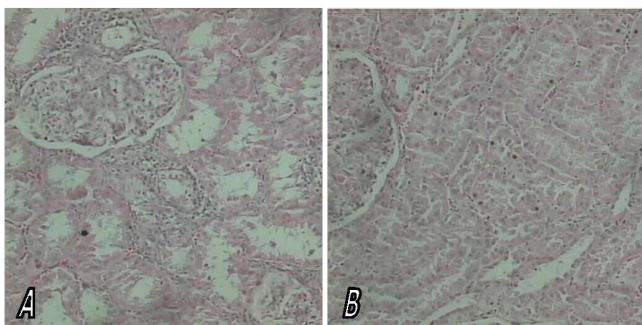
Figure 2. Photomicrographs of sections of porcine kidney specimens. (A) AF group showing many vacuoles of varying sizes in cytoplasm of convoluted tubules. (B) MMN plus AF group showing normal kidney. Hematoxylin and eosin stain, ×200.

previous reports by Harvey et al. (1989a, 1990). No significant difference was found between pigs fed the control diet and fed the diet containing MMN alone, indicating that the adsorbent was inert and nontoxic. The better growth performance of pigs fed the diet of 3 g of MMN plus 0.1 mg of AF/kg feed demonstrated that the ability of MMN to ameliorate the inhibitory effects produced by AF.

AF has been reported to cause inhibition of protein synthesis (Tung et al., 1975; Yu, 1977). As a result, hypoproteinemia is a common effect of aflatoxicosis. Reduced levels of serum total protein, ALB, and GLOB are indictors of aflatoxicosis. Serum biochemical values and enzymatic activities of GPT, GOT, ALP and LDH are sensitive serological indicators of liver and kidney toxicity. These parameters significantly altered in our study, suggesting that AF caused critical injury to these organs. Serum profiles in the trial were similar to those obtained by Harvey et al. (1989b), who reported that AF reduced indicators of protein synthesis such as serum total protein, ALB, GLOB and urea nitrogen. The high levels of serum enzymes found in the study were consistent with the results of Harvey et al. (1990) and Lindemann et al. (1993), who reported high levels of AST, GGT and ALP in the serum of pigs fed AF-contaminated diets. However, these parameters for pigs fed diets containing MMN and AF returned to normal values, showing beneficial effects of MMN on aflatoxicosis. The results were in agreement with the study by Kececi et al. (1998) who reported that some serum biochemical changes could be ameliorated by bentonite administration to the diet at doses of 5 mg AF/kg diet. Schell et al. (1993a, b) also reported that sodium bentonite or HSCA were effective in reducing the high levels of some serum enzymes.

The relative weights of liver, kidney, spleen and pancreas were significantly increased in our study. AF has been known to irritate gastrointestinal tract and gut and make them dysfunction, thus causing an increase in the relative weights of these organs, the target organs were liver and kidney (Huff et al., 1981). However, there was no

significant difference between 0.3% MMN plus AF and the control, which showed that MMN addition diminished the adverse effects of AF and organ damage were prevented.

The histological changes observed in the maternal liver and kidney induced by AF have been documented previously (Heathcote et al., 1978; Ikegwuonu et al., 1980). We also observed bile duct proliferation and periportal hepatocellular vacuolar degeneration in the livers of pigs treated with AF alone. Arora et al. (1978) and Grosman et al. (1983) reported that rats were acutely sensitive to the nephrotoxic effects of AF. In our study, the kidney from pigs fed AF alone showed localized degeneration and vacuolization in convoluted tubules at the corticomedullary junction. However, the liver and kidney of pigs fed MMN plus AF diet were normal. These findings indicated that organ lesions induced by AF were eliminated by the addition of MMN in the feed, and apparently, MMN was effective in preventing the maternal and developmental toxicity of AF in pigs. The results obtained were in agreement with previous reported by Mayura et al. (1998) and Harvey et al. (1989b).

In our study, on the basis of performance data, serum biochemical values and enzyme activities, as well as histopathologic findings, it was apparent that MMN had the ability to recover the AF-decreased performance and to correct aberrations in serum parameters, and 0.3% MMN in the diet protected pigs from the deleterious effects of AF. The serum parameters reflected the growth performance data. It should be noted that because montmorillonite has been demonstrated to bind AF in aqueous solution (Ramos et al., 1996; Grant et al., 1998), the presumed mechanism of action of MMN is to bind with the toxin in the gut and form stable sorption complex, thereby to prevent absorption of the toxin across the gastrointestinal wall, then the recovery, or restoration of performance were obtained.

IMPLICATIONS

When diets containing AF are fed to growing/finish pigs, there are reductions in growth rate and metabolic alterations reflected in an altered serum profile. The addition of MMN can ameliorate the toxic effects of AF (mainly via sequestration and reduced bioavailability *in vivo*). Our work suggests that MMN is a high affinity adsorbent for AF in pigs, and that the use of MMN may offer a novel approach to the preventive management of aflatoxicosis in animals.

REFERENCES

Abdel-Wahhab, M. A., S. A. Nada and H. A. Amra. 1999. Effect of aluminosilicates and bentonite on aflatoxin-induced developmental toxicity in rat. J. Appl. Toxicol. 19:199-204.

AOAC. 1995. Aflatoxins in corn and peanut butter. In: (Ed. P. Cunniff), Official Methods of Analysis of AOAC International.

Vol. 2. Method 990. 33, AOAC International, Gaithersburg, MD, p. 18.

Arora, R. G., L. E. Appelgren and A. Bergman. 1978. Distribution of ^{14}C-labeled aflatoxin B$_1$ in mice. Acta Pharmacol. Toxicol. 43:273-279.

Beckman Instruments, Inc. Scientific Instruments Division, Fullerton, CA.

Bonna, R. J., R. J. Aulerich and S. J. Bursian. 1991. Efficacy of hydrated sodium calcium aluminosilicate and activated charcoal in reducing the toxicity of dietary aflatoxin to mink. Arch. Environ. Contam. Toxicol. 21:441-447.

Dale, J., M. Kowalska and D. L. Cocke. 1991. Interactions of montmorillonite with organic compounds-adsorptive and catalytic properties. Chemosphere 22:769-798.

Grant, P. G. and T. D. Phillips. 1998. Isothermal adsorption of aflatoxin B$_1$ on HSCAS clay. J. Agric. Food Chem. 46:599-605.

Grosman, M. E., M. M. Elias and E. J. Comin. 1983. Alterations in renal function induced by aflatoxin B$_1$ in the rat. Toxicol. Appl. Pharmacol. 69:319-325.

Harvey, R. B., W. E. Huff and L. F. Kubena. 1988. Progression of aflatoxicosis in growing barrows. Am. J. Vet. Res. 49:482-487.

Harvey, R. B., L. F. Kubena and W. E. Huff. 1989a. Effects of aflatoxin, deoxynivalenol, and their combinations in the diets of growing pigs. Am. J. Vet. Res. 50:602-608.

Harvey, R. B., L. F. Kubena and T. D. Phillips. 1989b. Prevention of aflatoxicosis by addition of hydrate sodium calcium aluminosilicate to the diets of growing barrows. Am. J. Vet. Res. 50:416-420.

Harvey, R. B., L. F. Kubena and W. E. Huff. 1990. Effects of treatment of growing swine with aflatoxin and T-2 toxin. Am. J. Vet. Res. 51:1688-1693.

Harvey, R. B., L. F. Kubena and T. D. Phillips. 1991. Diminution of aflatoxin toxicity to growing lambs by dietary supplementation with hydrate sodium calcium aluminosilicate. Am. J. Vet. Res. 52:152-156.

Harvey, R. B., L. F. Kubena and M. H. Elissalde. 1994. Comparison of two hydrate sodium calcium aluminosilicate compounds to experimentally protect growing barrows from aflatoxicosis. J. Vet. Diagn. Invest. 6:88-92.

Heathcote, J. G. and J. R. Hibbert. 1978. Pathological effects. In aflatoxins: Chemical and Biological Aspects, pp. 83-111. Elsevier Scientific, Amsterdam/Oxford/New York.

Huff, W. E. and J. A. Doeer. 1981. Synergism between aflatoxin and ochratoxin A in broiler chickens. Poult. Sci. 60:550-555.

Huff, W. E., L. F. Kubena and R. B. Harvey. 1986. Progression of aflatoxicosis in broiler chichens. Poult. Sci. 65:1891-1899.

Ikegwuonu, F. L., G. N. Egbunike and G. O. Emerole. 1980. The effects of aflatoxin B$_1$ on some testicular and kidney enzyme activity in rat. Toxicol. 17:9-16.

Ishida, H. and S. Campbell. 2000. General approach to nanocomposite preparation. Chem. Mater. 12(5):1260-1267.

Kececi, T. H., V. Kurtoglu and O. Demet. 1998. Effects of polyvinylpolypyrolidone, synthetic zeolite and bentonite on serum biochemical and haematological characters of broiler chickens during aflatoxicosis. Br. Poult. Sci. 39:452-458.

Kubena, L. F., T. D. Phillips and W. E. Huff. 1990. Diminution of aflatoxicosis in growing chickens by addition of hydrate sodium calcium aluminosilicate. Poult. Sci. 69:727-735.

Kubena, L. F., W. E. Huff and R. B. Harvey. 1991. Effects of a hydrate sodium calcium aluminosilicate on growing turkey poults during aflatoxicosis. Poult. Sci. 70:1823-1830.

Kubena, L. F., R. B. Harvey and T. D. Phillips. 1993. Effects of hydrate sodium calcium aluminosilicate on aflatoxicosis in broiler chicks. Poult. Sci. 72:651-657.

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

Mayura, K., M. A. Abdel-Wahhab and K. S. Mckenzie. 1998. Prevention maternal and developmental toxicity in rats via dietary inclusion of common aflatoxin sorbents: potential for hidden risks. Toxicol. Sci. 41:175-182.

Mishra, H. N. and Chitrangada Das. 2003. A review on biological control and metabolism of aflatoxin. Crit. Rev. Food Sci. Nutr. 43(3): 245-264.

National Research Council. 1994. Nutrient Requirements of Swine. 4th rev. ed. National Academy Press, Washington, DC.

Ramos, A. J. and E. Hernandez. 1996. In vitro aflatoxin adsorption by means of a montmorillonite silicate. A study of adsorption isotherms. Anim. Feed Sci. Technol. 62:263-269.

Robens, J. F. and J. L. Richard. 1992. Aflatoxins in animal and human health. Rev. Environ. Contam. Toxicol. 127:69-94.

SAS Institute. 1985. SAS User's Guide: Statistics. Version 6 Edition. SAS Institute Inc., Cary, NC.

Schell, T. C., M. D. Lindemann and E. T. Kornegay. 1993a. Effectiveness of different types of clay for reducing the detrimental effects of aflatoxin-contaminated diets on performance and serum profiles of weaning pigs. J. Anim. Sci. 71:1223-1231.

Schell, T. C., M. D. Lindemann and E. T. Kornegay. 1993b. Effects of feeding aflatoxin-contaminated diets with and without clay to weaning and growing pigs on performance, liver function, and mineral metabolism. J. Anim. Sci. 71:1209-1218.

Smela, M. E., S. S. Currier and E. A. Bailey. 2001. The chemistry and biology of aflatoxin B1: from mutational spectrometry to carcinogenesis. Carcinogenesis. 22(4):535-545.

Tung, H. T., R. D. Wyatt and P. Thaxton. 1975. Concentrations of serum proteins during aflatoxicosis. Toxicol. Appl. Pharm. 34:320-326.

Vaia, R. A. and E. P. Giannelis. 1997a. Lattice model of polymer melt intercalation in organically-modified layered silicates. Macromolecules. 30:7990-7999.

Vaia, R. A., B. B. Sauer and O. K. Tse. 1997b. Relaxations of confined chains in polymer nanocomposites glass transition properties of poly intercalated in montmorillonite. Polym. Sci. PartB. Polym. Phys. 35:59-67.

Yu, S. 1977. Mechanism of aflatoxin B$_1$ inhibition of rat hepatic nuclear RNA-synthesis. J. Biol. Chem. 252:3245-3251.