ORIGINAL RESEARCH PEER REVIEWED

Assessment of a hydrated sodium calcium aluminosilicate agent and antioxidant blend for mitigation of aflatoxin-induced physiological alterations in pigs

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Summary

Objective: To assess hydrated sodium calcium aluminosilicates (HSCAS) and an antioxidant supplying ethoxyquin and tertiary butyl hydroquinone (TBHQ) as dietary additives to mitigate physiological effects of aflatoxin in feed for pigs.

Materials and methods: Ninety pigs (9.42 ± 0.05 kg) were used in a study with five dietary treatments: an uncontaminated control diet with no additives and four similar diets that were contaminated with 500 ng per g aflatoxin B1 (AB1) and supplemented with no additive, with 0.5% HSCAS, with an antioxidant preparation providing 125 mg per kg of ethoxyquin and 10 mg per kg of TBHQ, or with both HSCAS and the antioxidant preparation.

Results: Feed consumption and growth were poorer ($P < .05$) in pigs consuming AB1-contaminated feed without additives than in pigs fed the uncontaminated control diet. Serum chemistry constituents were altered ($P < .05$) in a manner consistent with ingestion of AB1. Growth performance and serum chemistry constituents did not differ between pigs fed an AB1-contaminated diet supplemented with HSCAS and pigs fed uncontaminated feed. In pigs fed the AB1 diet with antioxidant, growth was poorer than in controls ($P < .05$), but serum gamma glutamyltransferase levels ($P < .05$) were lower than in pigs fed AB1-contaminated feed without supplementation.

Implications: Supplementation of HSCAS is effective in preventing the negative effects of dietary aflatoxin in young pigs as measured by growth and serum chemistry parameters. Supplementing with antioxidant does not mitigate most negative physiological effects associated with aflatoxin consumption.

Keywords: swine, aflatoxin, feed additives, serum chemistry

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Resumen - Valoración de la mezcla de un agente de aluminosilicato de calcio de sodio hidratado y de un antioxidante para mitigar las alteraciones fisiológicas inducidas por una aflatoxina en cerdos

Objetivo: Valorar los aluminosilicatos de calcio de sodio hidratados (HSCAS por sus siglas en inglés) y un antioxidante que suministra etoxiquina y butil-hidroquinona terciaria (TBHQ por sus siglas en inglés) como aditivos dietéticos para mitigar los efectos fisiológicos de la aflatoxina en el alimento para cerdos.

Materiales y métodos: Se utilizaron noventa cerdos (9.42 ± 0.05 kg) en un estudio con cinco tratamientos dietéticos: una dieta control no contaminada y sin aditivos y cuatro dietas similares contaminadas con 500 ng por g de aflatoxina B1 (AB1 por sus siglas en inglés) y se suplementaron sin aditivo, con 0.5% HSCAS, con una preparación antioxidante que provee 125 mg por kg de etoxiquina y 10 mg por kg de TBHQ, ó con ambos HSCAS y la preparación antioxidante.

Resultados: El consumo de alimento y el crecimiento fueron menores ($P < .05$) en los cerdos que consumieron alimento contaminado con AB1 sin aditivo que en los cerdos alimentados con la dieta control no contaminada. La composición química del suero se alteró ($P < .05$) de manera consistente con la ingestión de AB1. El desempeño del crecimiento y la composición química del suero no difirieron entre los cerdos alimentados con la dieta contaminada con AB1 y suplementada con HSCAS y los cerdos alimentados con alimento no contaminado. En cerdos alimentados con la dieta AB1 con antioxidante, el crecimiento fue menor que en los controles ($P < .05$), pero los niveles de gamma glutamiltransferasa del suero ($P < .05$) fueron menores que en los cerdos alimentados con el alimento contaminado con AB1 y sin suplemento.

Implicaciones: Suplementar con HSCAS es efectivo para prevenir los efectos negativos de la aflatoxina dietética en cerdos jóvenes cuando se midieron los parámetros de crecimiento y química del suero. Suplementar con antioxidante no mitiga la mayoría de los efectos fisiológicos negativos asociados con el consumo de aflatoxina.

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Résumé - Évaluation d'un mélange d'aluminosilicate de sodium calcique hydraté et d'antioxydants pour l'atténuation des changements physiologiques induits par l'aflatoxine chez des porcs

Objectif: Évaluer un mélange d'aluminosilicate de sodium calcique

hydraté (HSCAS) et un antioxydant fournissant de l'éthoxyquin et de l'hydroquinone de butyl tertiaire (TBHQ) comme additif alimentaire afin d'atténuer les effets physiologiques de l'aflatoxine dans l'alimentation des porcs.

Matériels et méthodes: Quatre-vingt douze porcs (9.42 ± 0.05 kg) ont été utilisés dans une étude avec cinq traitements alimentaires: une ration témoin non-contaminée sans additif et quatre rations similaires qui ont été contaminées avec 500 ng par g d'aflatoxine B1 (AB1) et supplémentées avec 0.5% de HSCAS, avec une préparation d'antioxydant fournissant 125 mg par kg d'éthoxyquin et 10 mg par kg de TBHQ, avec du HSCAS et la préparation d'antioxydant, et une ration sans additif.

Résultats: La consommation alimentaire et la croissance étaient plus faibles ($P < .05$) chez les porcs consommant la ration contaminée par AB1 ne contenant aucun additif que chez les porcs nourris avec la ration témoin non-contaminée. Les constituants chimiques sériques étaient altérés ($P < .05$) d'une manière compatible avec l'ingestion d'AB1. Les performances de croissance et les constituants chimiques sériques ne différaient pas entre les porcs nourris avec une ration contaminée avec AB1 et supplémentée avec HSCAS et les porcs nourris avec la ration non-contaminée. Chez les porcs nourris avec la ration contaminée avec AB1 contenant l'antioxydant, la croissance était plus faible comparativement aux animaux témoins ($P < .05$), mais les niveaux sériques de gamma glutamyl-transférase étaient inférieurs ($P < .05$) à ceux des porcs nourris avec la ration contaminée par AB1 sans aucun supplément.

Implications: L'ajout de HSCAS est efficace pour prévenir les effets négatifs de l'aflatoxine alimentaire chez les jeunes porcs tel que mesuré par la croissance et les paramètres chimiques sanguins. L'ajout d'antioxydant ne peut mitiger la plupart des effets physiologiques négatifs associés à la consommation d'aflatoxine.

Aflatoxins, produced primarily by *Aspergillus flavus* and *Aspergillus parasiticus,* act as potent hepatotoxins and carcinogens and may occur naturally in feed grains, peanuts, and cottonseed. Corn, the predominant feed grain in North America, is most commonly infected. Principle metabolites that occur in grains have been characterized and identified as aflatoxin B1 (AB1), G1, B2, and G2, but the AB1 metabolite occurs most frequently and is considered the most toxic.[1] Infection can occur while corn grain is in the field, at and soon after harvest, and during storage before or after the grain is processed into feed. Above-normal temperatures during the growing season, drought stress, and heavy insect damage promote fungal production of aflatoxin in the field, so incidence and severity of aflatoxin problems vary from year to year and across regions. When aflatoxin-infected corn is used in the production of ethanol, the toxin may be concentrated in the resulting distiller's dried grains with solubles (DDGS) feed by-product.[2]

Among food-producing animals, swine are particularly susceptible to negative effects associated with consumption of aflatoxin-contaminated feeds. In young growing pigs, reduced feed consumption and impaired growth have been reported at feed contamination levels of 125 to 140 ng per g, with more pronounced effects at higher concentrations.[3,4] Other clinical effects include liver damage, greater susceptibility to hemorrhage and bruising, and impaired immune function.[5,6] The US Food and Drug Administration has published action levels for aflatoxin concentration in corn and other feedstuffs. For immature pigs and other immature livestock, the maximum level is 20 ng per g, which is equivalent to the maximum level for human consumption in all products. Action levels for breeding and finishing swine are 100 and 200 ng per g, respectively.[6] However, these levels are not always in accordance with levels associated with field outbreaks, since they do not take into consideration interactions with other mycotoxins and other stressors animals may be exposed to in production settings.[6]

Avoiding purchase and use of aflatoxin-contaminated grain for feed production is based on sampling and assaying suspect grain. Inexpensive rapid-screening test kits (enzyme-linked immunosorbent assay; ELISA) are routinely used. Samples testing positive for a threshold level using rapid-screening tests may subsequently be assayed more precisely for contamination level by high performance liquid chromatography (HPLC). However, a practical discovery for prevention of toxic effects when feed contains aflatoxins was that certain non-nutritive feed additives labeled to improve feed flow and feed pellet quality can prevent clinical signs of aflatoxicosis. Harvey et al[7] were the first to report this in pigs, demonstrating that 0.5% dietary inclusion of hydrated sodium calcium aluminosilicates (HSCAS) to an aflatoxin-contaminated diet prevented growth depression and abnormal prothrombin times and improved blood profiles of specific liver enzymes indicative of toxicity. Sodium and calcium bentonites and other clay-based feed additives produce similar amelioration in pigs consuming aflatoxin-contaminated diets.[8,9] Research evidence indicates that the principle mechanism of action for these products is binding of aflatoxin by HSCAS or other clay-based additives in the gastrointestinal tract and, as a result, prevention of absorption of the toxin.[10] However, structural and chemical characteristics of clay-based products may differ, resulting in altered capacity to sequester aflatoxins. For example, Diaz et al[11] demonstrated that different sources of bentonite clays had variable capacities to prevent expression of aflatoxin M1 in the milk of lactating cows consuming feed-borne AB1.

Antioxidants, another class of non-nutritive feed additive that might affect clinical signs of aflatoxicosis, may be added to compounded swine feeds to reduce oxidative destruction of fats and fat-soluble vitamins. These synthetic additives include ethoxyquin, butylated hydroxyanisole, butylated hydroxytoluene, and tertiary butyl hydroquinone (TBHQ).[12] Some research evidence indicates that synthetic antioxidant compounds can act systemically to metabolize aflatoxin and reduce its toxicity. For example, studies with rats have shown that supplementing diets with ethoxyquin induces hepatic resistance to aflatoxin, as measured by increased glutathione conjugation with activated AB1.[13,14]

The objective of this study was to assess dietary addition of a commercial source of HSCAS and an antioxidant mixture containing ethoxyquin and TBHQ as practical methods to prevent negative physiological effects of AB1 in the feed of young growing pigs.

Materials and methods

The study protocol, including all aspects of animal housing and care, were reviewed and approved by the Virginia Tech Institutional Animal Care and Use Committee.

Study animals, feeding, and housing

Eighteen litters of crossbred pigs (Yorkshire × Landrace) farrowed at the Virginia Tech Tidewater Agricultural Research and Extension Center swine unit in Suffolk, Virginia, were managed according to standard practice. This included administration of vaccine against atrophic rhinitis, *Pasteurella multocida* types A and D, and erysipelas (Rhinogen BPE; Intervet Schering-Plough Animal Health, Millsboro, Delaware) at 7 days of age and at weaning, and administration of 200 mg of iron dextran and castration of male piglets at 7 days of age. All pigs were weaned as a group at 22 ± 2 days of age and penned as intact litters during the pre-test period in an environmentally controlled nursery unit. Pen floors were galvanized steel bar slats designed specifically for swine nursery pens. Pigs were allowed ad libitum access to feed and water via a stainless steel feeder and nipple drinker in each pen. The pre-test postweaning diet was a complex formulation designed to meet all nutritional requirements and promote rapid adaptation to solid feed (Table 1).

Study design

Pigs were fed the pre-test diet for 9 days, at which time all pigs were individually weighed. Weights were recorded for use in allotting pigs to study groups at 31 ± 2 days of age (Day 0). From the original 18 litters weaned, 90 pigs were selected and blocked by body weight, litter of origin, and sex to balance these factors across five experimental dietary treatments as a randomized complete block design. Three pigs were housed in each pen (0.91 × 1.22 m), with six replicate pens for each dietary treatment. The dietary treatments (Table 1) included an uncontaminated corn-soy-based control diet with no experimental additives and four similar diets that were artificially contaminated with 500 ng per g AB1, and supplemented either with no experimental additives, with 0.5% HSCAS, with an antioxidant preparation providing 125 mg per kg of ethoxyquin and 10 mg per kg of TBHQ, or with both HSCAS and the antioxidant preparation (combination treatment). The HSCAS additive, Solis, was a commercially available anticaking feed additive provided by Novus International Inc, St Charles, Missouri. The antioxidant additive, Agrado Plus, was also produced and supplied by Novus International Inc.

Pigs had ad libitum access to water from a nipple drinker and to the diets via a stainless steel feeder with four feeding spaces in each pen. Feeders were equipped with adjustable baffles to control feed flow. Feeder troughs were inspected daily and baffle adjustments made when necessary to minimize potential for feed waste. On Day 4, all test pigs were vaccinated with *Mycoplasma hyopneumoniae* bacterin (Respisure; Pfizer Animal Health, Exton, Pennsylvania) and porcine circovirus vaccine type 1-type 2 chimera (Suvaxyn PCV2 One Dose; Fort Dodge Animal Health, Fort Dodge, Iowa). After a study period of 21 days, all test pigs were transferred to a separate finisher barn and fed a standard corn-soy-based diet in accordance with the institutional animal care and use plan.

Preparation of aflatoxin (AB1) contaminant

The AB1 preparation used for artificial contamination of the experimental diets was prepared at and graciously provided by the laboratory of Dr George Rottinghaus, College of Veterinary Medicine, University of Missouri, Columbia. Briefly, whole grain rice was inoculated with *Aspergillus paraciticus* in 1-L flasks, which were placed on a rotator shaker at 250 rpm for 7 days at room temperature. After 1 week, chloroform was added to each flask and allowed to sit overnight to kill the fungus. The culture material was dried, ground, blended, and stored at -20°C. Multiple subsamples of the culture material were analyzed for aflatoxins by HPLC with fluorescence, and contained 850 mg per kg AB1, 34 mg per kg aflatoxin B2, 345 mg per kg aflatoxin G1, and 12 mg per kg of aflatoxin G2. The culture material was blended for 15 minutes with a cornmeal carrier using a Hobart mixer (Hobart Corporation, Troy, Ohio) to a final AB1 concentration of 120 mg per kg, which was confirmed by HPLC analysis. The AB1 metabolite was selected and used as the basis for preparing the experimental premix because it is recognized as the most toxic and most predominant form in feed grains,[1] with the understanding that the premix preparation also contained 4.80, 48.71, and 1.41 mg per kg of aflatoxins B2, G1, and G2, respectively. A final test diet concentration of 500 ng of AB1 per g of feed was selected, on the basis of previous research,[3,4] as a level likely to cause impaired growth and physiological responses in young pigs.

Preparation of experimental diets

A basal diet was first prepared, containing most of the corn and all of the common ingredients for each diet except soy oil, test ingredients, and AB1 preparation. To prepare each experimental diet, the appropriate quantities of ground corn, soy oil, AB1, and test feed additives were then added as required and mixed with the basal diet. An 80-kg horizontal ribbon mixer was used to premix lesser ingredients with the basal diet before transferring to a larger vertical screw mixer for final mixing of each experimental diet. Prior to the experiment, corn used to prepare the diets was screened and determined to be free of aflatoxins (< 20 ng per g) and deoxynivalenol using commercial ELISA kits certified by the Association of Official Analytical Chemists (Veratox AST and Veratox 5/5; Neogen Corporation, Lansing, Michigan). Concentration of AB1 in experimental diets was verified by HPLC analysis at an independent laboratory (Trilogy Analytical Laboratory, Washington, Missouri). Analysis indicated 14, 505, 488, 480, and 506 ng per g of AB1 for the control, unsupplemented, HSCAS, antioxidant, and combination HSCAS-antioxidant diets, respectively.

Data collection, calculations, and blood assays

Pen feed consumption (based on disappearance) and individual pig weights were determined on Day 11 and again at the conclusion of the growth assay on Day 21. Average daily gain (ADG) for each pen was calculated as the cumulative pig weight gain over the period divided by the number of pig-days (three pigs per pen × period days). Feed efficiency or feed-to-gain ratio (F:G) for each pen was calculated as pen feed consumption divided by cumulative pig weight gain during the period. Also on Day 21, two blood samples were collected from each pig by jugular venapuncture: one into a plain vacuum tube for serum collection and one into a tube containing EDTA for plasma collection. Serum and plasma were harvested following centrifugation and stored frozen at -20°C until subsequent analyses. Serum samples were subjected to complete clinical chemistry profile analysis using an Olympus AU400 clinical chemistry analyzer (Olympus America Inc, Center Valley, Pennsylvania) in the pathology laboratory of the Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, Virginia. Specific indicators of hepatic function assessed included aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), and direct, indirect, and total bilirubin, along with specific measures of nitrogen and protein metabolism. Plasma samples were assayed for concentration of vitamins

Table 1: Composition of pre-test and test diets for assessment of feed additives to mitigate physiological effects of aflatoxin B1 in young growing pigs*

Feed component	Diet					
	Pre-test diet	Control	Aflatoxin†	HSCAS+ aflatoxin‡	AOX+ aflatoxin§	HSCAS+AOX +aflatoxin
Ground corn (%)	42.69	65.84	65.42	64.92	65.28	64.78
Dried whey (%)	21.00	0.00	0.00	0.00	0.00	0.00
Lactose (%)	4.00	0.00	0.00	0.00	0.00	0.00
Plasma protein (%)¶	5.00	0.00	0.00	0.00	0.00	0.00
Fishmeal (%)	4.00	0.00	0.00	0.00	0.00	0.00
Soybean meal (dehulled) (%)	15.00	22.50	22.50	22.50	22.50	22.50
Soy protein concentrate (%)	4.00	6.60	6.60	6.60	6.60	6.60
Dicalcium phosphate (%)	0.49	1.26	1.26	1.26	1.26	1.26
Limestone (%)	0.76	0.94	0.94	0.94	0.94	0.94
Salt (%)	0.25	0.35	0.35	0.35	0.35	0.35
Lysine (%)	0.03	0.20	0.20	0.20	0.20	0.20
D-L methionine (%)	0.03	0.02	0.02	0.02	0.02	0.02
Vitamin premix (%)	0.35	0.30	0.30	0.30	0.30	0.30
Trace mineral premix (%)	0.15	0.15	0.15	0.15	0.15	0.15
Carbadox premix (%)**	0.25	0.25	0.25	0.25	0.25	0.25
Fat (prilled blend) (%)	2.00	0.00	0.00	0.00	0.00	0.00
Soybean oil (%)	0.00	1.59	1.59	1.59	0.00	0.00
AOX (%)§	0.00	0.00	0.00	0.00	1.73	1.73
HSCAS (%)‡	0.00	0.00	0.00	0.50	0.00	0.50
Aflatoxin preparation (%)	0.00	0.00	0.42	0.42	0.42	0.42
Totals	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis						
Crude protein (%)	22.15	20.38	20.34	20.30	20.34	20.29
Lysine (%)	1.48	1.28	1.28	1.28	1.28	1.28
Calcium (%)	0.85	0.76	0.76	0.76	0.76	0.76
Phosphorus (%)	0.70	0.63	0.62	0.62	0.62	0.62
ME (kcal/kg)	3411	3376	3362	3345	3369	3352

* Experimental diets were composed of the test ingredient(s) mixed with a basal diet (Control) consisting of the major portion of the ground corn and all other common ingredients. Pigs weaned at 22 ± 2 days of age were fed the common pre-test diet for 9 days, then treatment diets for 21 days.

† Experimental diets artificially contaminated with aflatoxin B1, prepared in a corn-based carrier containing 120 μg/g of aflatoxin B1 (graciously provided from the laboratory of Dr George Rottinghaus, College of Veterinary Medicine, University of Missouri, Columbia, Missouri). Intended final concentration 500 ng aflatoxin B1/g feed. Concentrations assayed by high performance liquid chromatography (Trilogy Analytical Laboratory, Washington, Missouri) were 14, 505, 488, 480, and 506 ng aflatoxin B1/g feed for the control, unsupplemented, and HSCAS-, AOX-, and HSCAS + AOX-supplemented diets, respectively,

‡ HSCAS: Solis; Novus International, Inc, St Charles, Missouri.

§ AOX in oil mixture providing 125 mg/kg of ethoxyquin and 10 mg/kg of tertiary butylhydroquinone in the final diet (Agrado Plus, Novus International, Inc).

¶ Appetein; American Proteins Corporation, Ankeny, Iowa.

** Mecadox; Phibro Animal Health, Ridgefield Park, New Jersey; 55 mg/kg carbadox in the final diet.

HSCAS = hydrated sodium calcium aluminosilicate; AOX = antioxidant; ME = metabolizable energy.

A and E and malondialdehyde. Plasma retinol and α-tocopherol concentrations were determined by HPLC using previously described methods.[15,16] A commercial kit (Cayman Chemical, Ann Arbor, Michigan) was used to determine malondialdehyde according to the instructions supplied by the manufacturer.[17]

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using the general linear models procedure of the SAS program (SAS Institute, Inc, Cary, North Carolina). The pen mean was the experimental unit for growth and serum and plasma chemistry variables. The statistical model included effects of block and dietary treatment. When a significant F-statistic for dietary treatment was observed ($P < .05$), paired *t*-tests were performed using the P-DIFF option of SAS for comparison of individual treatment means.

Results

General appearance and growth

Throughout the 21-day test period, pigs appeared healthy. All 90 pigs completed the experiment with no individual cases of lethargy, rough hair coat, or other overt symptoms of poor health.

Growth performance results are summarized in Table 2. When the pigs were assessed on Day 11, it was apparent that those fed the diet contaminated with 500 ng per g AB1 and provided no dietary additive were growing at a slower rate ($P < .05$) than control pigs fed an uncontaminated diet. This pattern continued through to the end of the growth assay at Day 21. The slower growth rate for the pigs fed the contaminated diet with no feed additive was associated with lower daily feed consumption ($P < .05$), but there was no difference in feed efficiency for these two groups. For the overall 3-week growth assay, pigs fed the diet contaminated with AB1 and no dietary additives grew 27% slower and consumed 29% less feed than pigs fed the uncontaminated control diet. This observation confirms that the experimental model to test responses of pigs consuming aflatoxin was effective.

During the initial 11 days, addition of 0.5% HSCAS to the AB1-contaminated diet resulted in growth rates that were intermediate to and significantly different from growth rates of pigs fed the contaminated diet and the uncontaminated control diet ($P < .05$) (Table 2). During the period

Table 2: Growth performance responses of growing pigs to dietary addition of a source of hydrated sodium calcium aluminosilicates (HSCAS) or an antioxidant (AOX) product or both in a diet contaminated with 500 ng/g of aflatoxin B1*

Parameter‡	Treatment					SEM
	Control	Aflatoxin	HSCAS+ aflatoxin†	AOX+ aflatoxin‡	HSCAS+AOX +aflatoxin†‡	
Pens	6	6	6	6	6	NA
Body weight (kg)						
Day 0	9.45	9.40	9.39	9.36	9.48	0.05
Day 11	15.56[a]	13.76[b]	14.99[c]	14.05[b]	14.62[c]	0.19
Day 21	22.58[a]	18.91[b]	22.35[a]	19.66[b]	21.77[a]	0.32
ADG (g)						
Days 0-11	555[a]	397[b]	509[c]	426[b]	467[c]	15
Days 12-21	702[a]	515[b]	736[a]	560[b]	715[a]	24
Overall	625[a]	453[b]	617[a]	490[b]	585[a]	15
ADFI (g)						
Days 0-11	871[a]	650[b]	823[a]	660[b]	784[a]	37
Days 12-21	1186[a]	803[b]	1207[a]	893[b]	1140[a]	35
Overall	1021[a]	723[b]	1006[a]	771[b]	953[a]	31
Feed:gain						
Days 0-11	1.56	1.64	1.64	1.55	1.67	0.06
Days 12-21	1.69	1.56	1.66	1.60	1.60	0.05
Overall	1.63	1.60	1.64	1.57	1.63	0.04

* Pigs and experimental diets described in Table 1. A randomized complete block design was used, with six pens per treatment and three pigs per pen. Pen mean was the experimental unit.

† HSCAS product supplemented at 0.5% of diet (Solis; Novus International, Inc, St Charles, Missouri).

‡ AOX product provided in the final diet, 125 mg/kg of ethoxyquin and 10 mg/kg of tertiary butylhydroquinone (Agrado Plus; Novus International, Inc, St Charles, Missouri).

[abc] Means in the same row with no common superscript differ ($P < .05$; post-ANOVA paired *t*-tests).

NA = not applicable; ADG = average daily gain; ADFI = average daily feed intake.

from Day 12 to 21 and for the overall trial, growth rates of pigs fed the contaminated diet supplemented with HSCAS and those fed the uncontaminated control diet did not differ. Furthermore, throughout the trial, feed consumption of pigs fed the contaminated diets supplemented with HSCAS and the pigs fed the uncontaminated control diet did not differ.

Throughout the growth assay, growth rate and feed consumption were significantly poorer in pigs fed the AB1-contaminated diet supplemented with antioxidant alone (no HSCAS) than in pigs fed the uncontaminated control diet ($P < .05$). Growth performance at Day 11 and overall did not differ between pigs fed the combination treatment of HSCAS and AOX and pigs fed the diet supplemented with HSCAS alone, and growth performance of the pigs fed the diet supplemented with HSCAS alone did not differ from that of pigs fed the control diet for the overall trial (Table 2).

Serum biochemistry

There were no effects of AB1 contamination or of diet supplements on serum AST concentration. However, serum GGT levels were markedly higher in pigs fed the diet with AB1 and no supplements than in pigs fed the uncontaminated control diet (Table 3; $P < .05$). Serum GGT concentration did not differ between pigs fed the AB1-contaminated diet with supplemental HSCAS, either with or without antioxidant, and pigs fed the uncontaminated control diet. Serum GGT concentration in pigs fed the AB1-contaminated feed supplemented with antioxidant alone was higher than in pigs fed the uncontaminated control diet, but lower than in pigs fed the contaminated unsupplemented diet ($P < .05$).

There were no significant effects of dietary treatment on indirect or total bilirubin concentrations. However, direct or conjugated bilirubin was higher in pigs fed the unsupplemented AB1-contaminated diet than in pigs in all other dietary treatments ($P < .05$).

Serum urea nitrogen, total protein, and albumin were each lower in pigs fed the AB1-contaminated diet containing no additives than in pigs fed the uncontaminated control diet (Table 3; $P < .05$). Serum urea nitrogen concentration in pigs fed the contaminated diet supplemented with combined HSCAS and antioxidant did not differ from that of pigs fed the uncontaminated control diet. Furthermore, total serum protein and albumin concentrations in pigs fed the diet supplemented with HSCAS alone or with combined HSCAS and antioxidant did not differ from those of pigs fed the uncontaminated control diet. Serum globulin, as calculated by the difference between total protein and albumin concentrations, was higher in pigs fed the AB1-contaminated diet than in pigs fed the uncontaminated control diet ($P < .05$). However, serum globulin concentration in pigs fed the HSCAS-supplemented diet did not differ from that of pigs fed the uncontaminated control diet.

Serum chloride concentration was higher in pigs fed the AB1-contaminated diet than in pigs fed either the uncontaminated control diet or the contaminated diets supplemented with HSCAS (Table 3; $P < .05$).

Other serum metabolites and minerals (Table 3) were not significantly affected by diet (data not shown; $P > .05$).

Plasma vitamins A and E and malondialdehyde

Plasma vitamin A and E concentrations were 58% and 50% lower, respectively, in pigs fed the unsupplemented AB1-contaminated diet than in pigs fed the uncontaminated control diet (Table 4; $P < .05$). Vitamin A and E were intermediate in pigs fed

Table 3: Serum chemistry responses of growing pigs to dietary addition of a source of hydrated sodium calcium aluminosilicate (HSCAS) or an antioxidant (AOX) product or both when the diet is contaminated with aflatoxin B1 at 500 ng/g feed*

Parameter†	Treatment					SEM
	Control	Aflatoxin	HSCAS+ aflatoxin‡	AOX+ aflatoxin§	HSCAS +AOX + aflatoxin‡§	
Pens	6	6	6	6	6	NA
Urea N (mg/dL)	13.06[a]	10.06[b]	11.58[ab]	10.72[b]	12.94[a]	0.65
Total protein (g/dL)	5.44[ac]	5.11[b]	5.61[a]	5.32[bc]	5.53[ac]	0.09
Albumin (g/dL)	3.72[a]	3.11[b]	3.71[a]	3.25[b]	3.67[a]	0.06
Globulin (g/dL)	1.73[a]	2.00[b]	1.90[a]	2.07[b]	1.87[ab]	0.07
GGT (U/L)	38.61[a]	64.50[b]	37.92[a]	50.78[c]	39.78[ac]	3.75
Direct bilirubin (mg/dL)	0.02[a]	0.09[b]	0.00[a]	0.02[a]	0.00[a]	0.02
Chloride (mEq/L)	102.61[a]	103.72[b]	101.92[a]	102.67[ab]	102.56[a]	0.36

* Pigs and diets described in Table 1; study design described in Table 2.

† Data in which a significant F-statistic was observed ($P < .05$) are shown. No treatment effects were observed in the clinical chemistry profile for serum glucose, creatinine, phosphorus, calcium, magnesium, aspartate aminotransferase, total bilirubin, indirect bilirubin, creatine kinase, sodium, carbon dioxide, or anion gap.

‡ HSCAS product supplemented at 0.5% of diet (Solis; Novus International, Inc, St Charles, Missouri).

§ AOX product provided in the final diet, 125 mg/kg of ethoxyquin and 10 mg/kg of tertiary butylhydroquinone (Agrado Plus; Novus International, Inc).

[abc] Means in the same row with no common superscript differ ($P < .05$; post-ANOVA paired *t*-tests)

NA = not applicable; N = nitrogen; GGT = gamma-glutamyltransferase.

Table 4: Plasma concentrations of vitamin A, vitamin E, and malondialdehyde (MDA) in growing pigs in response to dietary addition of a hydrated sodium calcium aluminosilicate (HSCAS) or an antioxidant (AOX) product or both when the diet is contaminated with aflatoxin B1 at 500 ng/g feed*

Parameter	Treatment					SEM
	Control	Aflatoxin	HSCAS+ Aflatoxin†	AOX+ aflatoxin‡	HSCAS+AOX + aflatoxin†‡	
Pens	6	6	6	6	6	
Vitamin A (μg/dL)	8.44[a]	3.54[b]	6.22[c]	5.23[bc]	7.93[ac]	0.73
Vitamin E (μg/dL)	75.01[a]	37.37[b]	58.36[c]	50.98[bc]	83.88[a]	5.87
Plasma MDA (μM)	7.48	7.54	7.23	7.41	7.36	0.42

* Pigs and diets described in Table 1. Experimental design described in Table 2.

† HSCAS product supplemented at 0.5% of diet (Solis; Novus International, Inc, St Charles, Missouri).

‡ AOX product provided in the final diet, 125 mg/kg of ethoxyquin and 10 mg/kg of tertiary butylhydroquinone (Agrado Plus; Novus International, Inc).

[abc] Means in the same row with no common superscript differ ($P < .05$; post-ANOVA paired *t*-tests).

the AB1-contaminated diet supplemented with HSCAS; that is, levels were significantly higher than in pigs fed the unsupplemented contaminated diet ($P < .05$), but significantly lower than in pigs fed the uncontaminated control diet ($P < .05$). However, vitamin A and E levels in pigs fed the contaminated diet supplemented with antioxidant alone did not differ from those in pigs fed the unsupplemented AB1-contaminated diet ($P > .05$), and were lower than those in the uncontaminated controls ($P < .05$). Plasma vitamin A and E levels in pigs fed the contaminated diet supplemented with combined HSCAS and antioxidant did not differ from those in pigs fed the uncontaminated control diet.

Plasma malondialdehyde levels did not differ among any of the five dietary treatment groups.

Discussion

That pigs fed the diet containing 500 ng per g of AB1 with no feed additive grew 27% slower and consumed 29% less feed over the 3-week trial is consistent with prior studies.[3,8,9] Blood chemistry profiles were also consistent with previous reports,[8,9] in that indicators of protein synthetic capacity, including total protein, urea nitrogen, and albumin, were lower, while indicators of hepatic stress, including GGT and direct bilirubin concentrations, were higher than those in pigs fed the uncontaminated diet. Within the scope of this study, we cannot distinguish precisely the specific cause of these physiological responses as resulting from aflatoxicosis per se or inadequate feed consumption or a combination of these factors. Nevertheless, these responses are consistent with field and research reports[3,6,8,9] of growing pigs fed similar levels of dietary AB1.

Supplementing with HSCAS in this study resulted in acceptable growth performance when growing pigs were fed a diet contaminated with 500 ng per g of AB1. This observation was apparent during the latter period (Days 12 to 21) and for the overall 3-week trial, in that growth and feed consumption did not differ among pigs fed the AB1-diet supplemented with HSCAS and pigs fed the uncontaminated control diet, while growth and feed consumption for pigs fed the unsupplemented AB1-contaminated diet were markedly lower. Blood chemistry profiles were generally in agreement with the performance data, in that serum chemistry values in pigs fed the AB1-contaminated diet supplemented with HSCAS generally did not differ from those of pigs fed the uncontaminated control diet, but several indicators of protein synthesis and hepatic stress in pigs fed the unsupplemented AB1-contaminated diet differed from those in pigs fed the uncontaminated control diet. An exception to this was that plasma vitamin A and E concentrations for pigs fed an AB1-contaminated diet supplemented with HSCAS were intermediate between the low levels in pigs fed the unsupplemented AB1-contaminated diet and the higher levels in pigs fed the uncontaminated control diet.

That supplemental HSCAS can prevent negative production and health effects of dietary AB1 contamination in pigs was first reported by Harvey et al.[7] These researchers demonstrated that supplementing with either 0.5% or 2.0% of a feed anti-caking agent providing HSCAS to diets artificially contaminated with AB1 maintained growth performance and serum chemistry profiles at normal levels. In subsequent studies with diets prepared using corn naturally contaminated with aflatoxin, Lindemann et al[8] and Schell et al[9] confirmed the effectiveness of HSCAS in mitigating effects of aflatoxin-tainted feed for growing pigs. These studies further demonstrated that sodium and calcium bentonites, and several other clay-based additives labeled as feed anti-caking or pelleting aids, were similarly effective. The proposed mode of action for the preventive effect of these additives is that they have a strong affinity for physical and chemical adsorption to AB1, sequestering the toxin and preventing systemic absorption from the gastrointestinal tract. This is supported by a series of in-vitro and in-vivo studies by Phillips et al[10] using Leghorn and broiler chicks and radiolabeled AB1. Indirectly, results of the current study agree with such a mechanism, in that pigs fed a diet supplemented with HSCAS consumed, over the 3-week trial, a calculated mean daily dose of 0.50 mg of AB1 and grew normally, while those fed the contaminated diet with no feed additive consumed 0.36 mg of AB1 per day and displayed poorer performance. A logical concern with this mode of action would be that adsorption products may also bind minerals or other nutrients, rendering them unavailable to the pig. However, a mineral balance study indicated little or no negative effect on essential mineral absorption and retention,[18] and growth-performance studies do not support this as a potential problem.

Our hypothesis for the mechanism by which antioxidant supplementation may prevent or reduce negative effects of aflatoxin was a systemic mode of action in which AB1 may be metabolized in the liver to less toxic forms. Using laboratory rats as a model for cancer research, Mandel et al[13] demonstrated that 0.5% dietary ethoxyquin promoted metabolic conversion of AB1 in the liver to less toxic metabolites, designated aflatoxins Q1 and M1, through induction of cytochrome P450 and P448 pathways. The study also reported ethoxyquin-induced reduction in binding of AB1-epoxide to liver DNA associated with increased glutathione S-transferase activity and conjugation of AB1-epoxide to glutathione. Subsequent rat studies confirm the ability of ethoxyquin to induce glutathione S-transferase with high specific activity to catalyze conjugation of AB1-epoxide to glutathione[14] and also to induce aldehyde reductase enzyme pathways involved in transforming AB1-epoxide to less toxic AB1 dialcohol forms.[19] Further evidence of hepatic protection against AB1 toxicity in nonhuman primates and a descriptive overview of the associated mechanisms induced by antioxidants such as ethoxyquin have been presented by Bammler et al.[20]

The methods in the present study did not involve intricate assessment of hepatic metabolism of AB1, as did the studies[13,14] with laboratory animals using ethoxyquin as a chemo-protectant against AB1, but rather focused on performance and serum indicators typically affected when pigs consume AB1. Therefore, we cannot determine if our antioxidant treatment induced similar hepatic responses as seen in the laboratory-animal studies. However, it is clear that antioxidant supplementation did not produce the level of preventive response to AB1 as was observed with HSCAS. This was apparent from the growth and feed consumption data, as well as the serum and plasma chemistry profiles. Nevertheless, there was some indication that the antioxidant may have altered some aspects of the pigs' response to AB1 in the diet. Serum GGT levels were lower than in pigs fed the unsupplemented AB1-contaminated diet, and direct or conjugated bilirubin was maintained at a level not different from that of the pigs fed the uncontaminated control diet. Both of these observations are consistent with some degree of altered liver function in pigs fed AB1-contaminated feed. In addition, plasma vitamin A and E levels did not differ among pigs fed the uncontaminated control diet and those fed the AB1-contaminated diet supplemented with both HSCAS and antioxidant. However, malondialdehyde as a measure of cellular oxidative stress was not influenced by any of the dietary treatments.

Implications

- A feed-additive product providing HSCAS at 0.5% of the diet is effective in preventing the negative effects of moderate dietary aflatoxin contamination on growth performance and serum chemistry alterations in growing pigs.
- Supplementing the feed with an antioxidant product providing 125 mg per kg of ethoxyquin and 10 mg per kg of tertiary butylhydroquinone is not effective in correcting the negative effects of dietary aflatoxin contamination on feed intake and growth, but appears to have some positive influence on certain physiological indicators, including serum GGT, conjugated bilirubin, and plasma vitamins A and E.
- In the presence of moderate dietary aflatoxin concentrations, the antioxidant product tested does not enhance the beneficial effect of HSCAS on growing pig performance.

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