Efficacy of different adsorbents in reducing the toxic effects of aflatoxin B_1 in broiler diets

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Abstract

The ability of dietary hydrated sodium calcium aluminosilicate (HSCAS), diatomite and activated charcoal (AC) in reducing the detrimental effects of aflatoxin B_1 (AFB_1) in broiler diets was evaluated. Adsorbents were supplemented at 2.5 g/kg to the diets containing 0, 40 or 80 µg AFB_1/kg feed. One hundred and eighty Ross 308, day-old male broilers were assigned to 12 treatments for 42 days. AFB_1 at 80 µg/kg feed resulted in a significantly lower body weight gain and feed efficiency than the control group. Addition of HSCAS in the diets significantly diminished the deleterious effects of dietary AFB_1. Aspartate amino transferase (AST), alanine amino transferase (ALT) activities and total protein concentration in the serum were altered significantly in the birds fed AFB_1 80 µg/kg feed. However, there were no significant differences between treatments in alkaline phosphatase (ALP) activity, and uric acid and creatinine concentrations in the serum. Liver weights of birds fed diets containing 80 µg AFB_1/kg feed were significantly higher than those of the control groups. Histological observations on livers from birds consuming AFB_1 at 80 µg/kg feed showed a yellowish colour, portal leucocytic infiltration, congestion, multifocal fatty degeneration, and dysplasia of parenchymal cells with disorganization of the structure. The addition of HSCAS in the diets prevented an increase in the activity of AST and in the weight of livers and also prevented the histopathological changes induced by AFB_1. However, the addition of diatomite or AC in the diets failed to prevent the harmful effects of AFB_1. It was concluded that HSCAS is the most effective adsorbent to decrease the negative effects of AFB_1 in broiler chickens.

Keywords: Aflatoxin B_1, hydrated sodium calcium aluminosilicate (HSCAS), diatomite, activated charcoal, broiler chickens
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Introduction

Aflatoxins are secondary toxic metabolites produced by certain strains of fungi, e.g. *Aspergillus flavus* and *Aspergillus parasiticus* species. Aflatoxin B_1 (AFB_1), the most toxic of all aflatoxins (AFB_1, AFB_2, AFG_1 and AFG_2), is produced by certain strains of fungi in greater quantities than in others (Sweeney & Dobson, 1998, Akande *et al.*, 2006). The presence or production of aflatoxins in agricultural commodities depends on many factors, including the time and methods of harvesting, and storing and transporting conditions. Their toxicity in animals depends on different factors including the concentration of aflatoxin, the duration of exposure, the species, gender and age and health status of animals (Jewers, 1990).

Aflatoxins affect energy, nucleic acid and protein metabolism (Baptista *et al*., 2004) with described effects in a wide range of animal species such as carcinogenity, hepatotoxicity, mutagenicity, immunusuppression and teratogeny (Busby & Wogan, 1984). Avoidance of contaminated feed is rarely feasible and feeds that contain relatively low concentrations of AFB_1 may still have deleterious effects on sensitive species such as poultry (Doerr *et al*., 1983; Giambrone *et al*., 1985). Poultry are suggested to be the species most sensitive to its toxic effects (Denli *et al*., 2004). Even small amounts of AFB_1 may cause reductions in growth parameters, hatchability and also cause increased susceptibility to disease (Coulombe, 1993; Denli *et al*., 2004). Liver damage, decreased egg production and overall performance, and suppressed immunity have been noted in animals consuming relatively low dietary concentrations of aflatoxin (Robens & Richard, 1992; Okan *et al*., 2004). Liver is the target organ for aflatoxicosis because this is where most aflatoxins are bio-activated to the reactive 8,9-epoxide form, which is capable of binding to both DNA and proteins.

Lately, several approaches to avoid contamination such as decontamination or remediation of feed and feedstuffs have been proposed (Bailey *et al*., 1998; Ledoux *et al*., 1999). A variety of adsorbents such as bentonite (Rosa *et al*., 2001), zeolite (Miazzo *et al*., 2000), hydrated sodium calcium aluminosilicate (HSCAS) (Kubena *et al*., 1993; Scheideler, 1993), *Saccharomyces cerevisiae* (Celik *et al*., 2001) and activated charcoal (AC) (Jindal *et al*., 1994) have been used successfully in detoxifying AFB_1 in contaminated feeds (Ramos & Hernandez, 1997).

Diatomite is a kind of clay that consists of *ca.* 90% silicon dioxide. It is a fine-grained, biogenic siliceous sediment, and is available in large quantities at low cost (Kamikasa & Kato, 2000). Diatomite consists essentially of amorphous silica derived from opalescent frustules of diatoms resulting in an inert, lightweight, highly porous, super-absorbent material, and has a fine porous structure with low density (Wajima *et al*., 2006). Due to these properties diatomite was selected for use in this experiment to compare its aflatoxin adsorption capacity with other adsorbents that have been tested by various researchers.

The objective of this study was to compare the protective role of HSCAS, diatomite and AC against AFB_1 toxicity in growing broiler chicks by observing their effects on growth parameters and serum biochemical variables.

Material and Methods

One hundred and eighty day-old Ross 308 male broiler chickens were used in this experiment. Chicks were weighed and individually caged, numbered and divided into 12 treatment groups of 15 chicks per group. The birds were fed a commercial diet formulated to meet the nutrient requirements of the broilers during days 1 to 14 (starter, 237.5 g CP/kg and 13.22 MJ ME/kg), days 15 to 29 (grower, 237 g CP/kg and 13.91 MJ ME/kg) and days 30 to 42 (finisher, 217.5 g CP/kg and 14.24 MJ ME/kg) of the experimental period. Broilers were provided with water and feed *ad libitum* for the duration of the study. The chicks were reared under a conventional temperature regimen, i.e. starting at 33 ^{o}C, and reduced by 3 °C/week to 21 °C. The relative humidity was maintained at between 60 and 70%. The birds were exposed to continuous lighting.

Birds received the following dietary treatments: 1) 0 (control); 2) 40 µg AFB_1/kg feed; 3) 80 µg AFB_1/kg feed; 4) 2.5 g/kg HSCAS; 5) 40 µg AFB_1/kg feed + 2.5 g/kg HSCAS; 6) 80 µg AFB_1/kg feed + 2.5 g/kg HSCAS; 7) 2.5 g/kg AC; 8) 40 µg AFB_1/kg feed + 2.5 g/kg AC; 9) 80 µg AFB_1/kg feed + 2.5 g/kg AC; 10) 2.5 g/kg diatomite; 11) 40 µg AFB_1/kg feed + 2.5 g/kg diatomite and 12) 80 µg AFB_1/kg feed + 2.5 g/kg diatomite.

Pure crystalline AFB_1 (Sigma Chemical Co.) was incorporated into the diets by dissolving it in chloroform followed by mixing the solution with appropriate quantities of ground feed. The contaminated feed was left overnight at room temperature for the solvent to evaporate and was then mixed into the basal diet to provide the desired levels of AFB_1/kg of diet. The diets were analysed for aflatoxin content using thin layer chromatography (Howel, 1983).

Body weight gain, feed consumption and feed efficiency were calculated weekly. At 42 days of age 10 birds from each treatment were sacrificed by cervical dislocation to perform macroscopic observations of their livers. Livers were immediately dissected and individually weighed. Five livers from each treatment were fixed in 10% buffered formalin for histological analyses. Samples from tissue were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin. Sections of 3-5 µm were obtained and stained with haematoxylin/eosin (H&E). Light microscopy was used to evaluate portal and periportal necrosis, congestion, fatty changes, focal dysplasia and portal lenfocytic infiltration.

At the end of the experiment blood samples were collected from the retroorbital venous plexus from five birds per treatment. Serum was analysed for alkaline phosphatase (ALP, EC 3.1.3.1), alanine aminotransferase (ALT, EC 2.6.1.2) and aspartate aminotransferase (AST, EC 2.6.1.1) activity, using the SNA-12 clinical method (Anonymous, 1974). Serum total protein concentration was measured using the Biuret reaction (Doumas *et al*., 1981) and uric acid concentration by enzymatic-colorimetry (Barham & Trindern, 1972). Data were analysed using the General Linear models (GLM) procedure of SPSS 9.0 (1993). If appropriate, post-hoc analyses were carried out using the Duncan's test for multiple comparisons. Statements of statistical significance are based on $P < 0.05$.

Results and Discussion

Feed contaminated with aflatoxins can cause significant economic losses to the poultry industry due to reduced growth performance and their adverse health effects in the exposed birds. Effects of AFB_1 and of the adsorbents HSCAS, diatomite and AC on body weight gain, feed consumption and feed efficiency in broilers exposed AFB_1 are presented in Table 1.

Table 1 Effect of aflatoxin B_1 (AFB_1), hydrated sodium calcium aluminosilicate (HSCAS), diatomite and activated carbon (AC) on growth performance in broilers fed AFB_1-contaminated diet for 42 days

Treatments	Body weight gain (BWG, g/bird)	Feed intake (FI, g/bird)	Feed efficiency (FI/BWG)
Control	2254.7 ^{ab}	3953.8	1.75 ^{b}
AFB_1 (40 µg AFB_1/kg feed)	2055.2 ^{cd}	3804.1	1.86 ^{ab}
AFB_1 (80 µg AFB_1/kg feed)	2006.9 ^{d}	3775.7	1.89 ^{a}
HSCAS (2.5 g/kg)	2273.7 ^{a}	3966.7	1.75 ^{b}
AFB_1 (40 µg AFB_1/kg feed) + HSCAS (2.5 g/kg)	2200.0 ^{abc}	3937.5	1.79 ^{ab}
AFB_1 (80 µg AFB_1/kg feed) + HSCAS (2.5 g/kg)	2159.0 ^{abc}	3947.0	1.83 ^{ab}
AC (2.5 g/kg)	2185.3 ^{abc}	3933.6	1.80 ^{ab}
AFB_1 (40 µg AFB_1/kg feed) + AC (2.5 g/kg)	2103.1 ^{cd}	3955.1	1.88 ^{ab}
AFB_1 (80 µg AFB_1/kg feed) + AC (2.5 g/kg)	2118.4 ^{bcd}	4007.5	1.89 ^{a}
Diatomite (2.5 g/kg)	2121.0 ^{bcd}	3963.7	1.81 ^{ab}
AFB_1 (40 µg AFB_1/kg feed) + Diatomite (2.5 g/kg)	2140.4 ^{abcd}	3873.9	1.82 ^{ab}
AFB_1 (80 µg AFB_1/kg feed) + Diatomite (2.5 g/kg)	2124.4 ^{bcd}	3950.6	1.86 ^{ab}
s.e.m.	13.87	17.64	0.012
Factors		(P =)	
AFB_1	0.003	0.354	0.011
Adsorbents	0.032	0.071	0.268
AFB_1 * Adsorbents	0.147	0.534	0.940

Pooled s.e.m. - pooled standard error of the mean

^{a,b,c,d} Means in column with different superscripts differ significantly at $P < 0.05$

Results of body weight gain and feed efficiency in this experiment showed that AFB_1 alone significantly decreased body weight gain ($P < 0.05$) as compared to the control group. Growth performance results from our study agreed with those that indicated that AFB_1 severely affected the growth performance of broiler chickens (Fernandez *et al*., 1994; Denli *et al*., 2004). The adverse effects of AFB_1 on growth performance may be due to inhibition of metabolizing capacity (Dalvi & Ademoyero, 1984), and of protein and energy utilisation (Verma *et al*., 2002). Inclusion of HSCAS to the AFB_1 containing diet significantly ($P < 0.05$) alleviated the adverse effects of AFB_1 on body weight gain and improved feed efficiency. Similar effects were reported by Ledoux *et al*. (1999) and Pimpukdee *et al*. (2004). However, the addition of AC

and diatomite to diets containing the AFB_1 at the respective doses used in the present study did not ameliorate these effects. Contrary to our results, Jindal *et al*. (1994) reported that the addition of AC to AFB_1 contaminated diets ameliorated AFB_1 toxicity in broilers. These conflicting results are possibly due to the differences in the cationic compounds or chemical content of the adsorbents tested.

Data presented in Table 2 show the effect of the dietary treatment on liver weight and serum biochemical variables.

Table 2 Effect of aflatoxin B_1 (AFB_1), hydrated sodium calcium aluminosilicate (HSCAS), diatomite and activated carbon (AC) on serum variables and liver weight in broilers fed AFB_1-contaminated diet for 42 days

Treatments	Measurements						
	ALP (U/L)	AST (g/dL)	ALT (U/L)	TP (g/dL)	UA (mg/dL)	CRE (mg/dL)	Liver wt (g)
Control	1886.2 ^{ab}	259.0 ^{d}	2.17 ^{b}	3.42 ^{a}	5.56	0.28	51.3 ^{b}
AFB_1 (40 µg AFB_1/kg feed)	2487.2 ^{a}	355.8 ^{ab}	3.06 ^{ab}	3.01 ^{b}	3.68	0.30	58.4 ^{ab}
AFB_1 (80 µg AFB_1/kg feed)	2072.6 ^{ab}	393.0 ^{a}	3.77 ^{a}	2.98 ^{b}	4.20	0.28	59.5 ^{a}
HSCAS (2.5 g/kg)	1961.2 ^{ab}	265.8 ^{cd}	2.46 ^{ab}	3.29 ^{ab}	5.38	0.28	52.3 ^{ab}
AFB_1 (40 µg AFB_1/kg feed) + HSCAS (2.5 g/kg)	1987.6 ^{ab}	317.8 ^{bcd}	3.15 ^{ab}	3.24 ^{ab}	4.48	0.28	56.2 ^{ab}
AFB_1 (80 µg AFB_1/kg feed) + HSCAS (2.5 g/kg)	1933.4 ^{ab}	291.2 ^{bcd}	2.97 ^{ab}	3.16 ^{ab}	3.76	0.26	55.5 ^{ab}
AC (2.5 g/kg)	1351.8 ^{b}	265.6 ^{cd}	2.70 ^{ab}	3.18 ^{ab}	5.00	0.28	54.0 ^{ab}
AFB_1 (40 µg AFB_1/kg feed) + AC (2.5 g/kg)	1863.6 ^{ab}	304.8 ^{bcd}	3.29 ^{ab}	3.11 ^{ab}	4.94	0.30	55.6 ^{ab}
AFB_1 (80 µg AFB_1/kg feed) + AC (2.5 g/kg)	1395.0 ^{b}	310.2 ^{bcd}	3.46 ^{ab}	3.30 ^{ab}	3.98	0.24	58.8 ^{ab}
Diatomite (2.5 g/kg)	1757.0 ^{ab}	268.4 ^{cd}	2.07 ^{b}	3.31 ^{ab}	4.65	0.26	55.4 ^{ab}
AFB_1 (40 µg AFB_1/kg feed) + Diatomite (2.5 g/kg)	1998.6 ^{ab}	317.4 ^{bcd}	2.77 ^{ab}	3.09 ^{ab}	5.00	0.30	59.3 ^{a}
AFB_1 (80 µg AFB_1/kg feed) + Diatomite (2.5 g/kg)	2581.8 ^{a}	332.8 ^{abc}	3.58 ^{ab}	3.14 ^{ab}	4.90	0.26	59.4 ^{a}
s.e.m.	85.59	7.44	0.14	0.03	0.18	0.01	0.65
Factors	(P =)						
AFB_1	0.205	0.000	0.057	0.048	0.125	0.043	0.008
Adsorbents	0.039	0.047	0.211	0.799	0.904	0.780	0.409
AFB_1 * Adsorbents	0.459	0.247	0.058	0.303	0.551	0.838	0.897

ALP - alkaline phosphatase; AST - aspartate aminotransferase; ALT - alanine aminotransferase; TP – total protein; UA – uric acid; CRE - creatinine

Pooled s.e.m. - pooled standard error of the mean

^{a,b,c,d} Means in column with different superscripts differ significantly at $P < 0.05$

The main effects of aflatoxins are related to liver damage and the classic symptom of aflatoxicosis is an increased liver weight (Miazzo *et al*., 2000). Results from our study showed that the liver weights were significantly higher ($P < 0.05$) in chicks consuming AFB_1 at 80 µg AFB1/kg feed than without any adsorbents, whereas supplementation with HSCAS partly diminished this increase. These results show that HSCAS has the ability to protect birds to some extent against the hepatotoxicity of AFB_1. Kubena *et al*. (1993) and Pimpukdee *et al*. (2004) observed a similar protection by HSCAS against high liver weights caused by AFB_1 in broilers. Histological observations on livers from birds fed AFB_1 at 80 µg AFB1/kg feed alone showed a yellowish colour, portal leucocytic infiltration, congestion, multifocal fatty degeneration, and dysplasia of parenchymal cells with disorganization of the structure.

Macroscopic and histological changes in the livers of chickens exposed to AFB_1 in the present study are comparable to those reported in the literature on avian aflatoxicosis (Miazzo *et al*., 2000; Klein *et al*., 2002; Denli *et al*., 2005; Ortatatli *et al*., 2005). The liver lesions of chickens fed diets containing HSCAS + AFB_1 were less severe and the hepatic structure resembled more that of a healthy liver. The hepatoprotective effects of HSCAS may be attributed to the chemisorptions (i.e. tight binding) of HSCAS to aflatoxins in the gastrointestinal tract, thus reducing toxin bioavailability.

Biochemical changes in serum and alterations in serum hepatic enzyme activities represent a stress on liver function (Abdel-Wahhab *et al*., 1999). In the present study the activity of AST and ALT in the serum increased significantly ($P < 0.05$) when 80 µg AFB_1/kg feed AFB_1 was present in the diet, whereas the concentration of total protein in the serum was decreased by AFB_1 ($P < 0.05$) compared to those of the controls. This is a well-known effect of aflatoxicosis (Huff *et al.*, 1986). The activities of ALT and AST in serum are sensitive indicators of acute hepatic necrosis, and ALP of hepatobiliary disease (Kaplan, 1987). Supplementing HSCAS with the feed prevented the increased concentration of ALT and AST and the decreased content of total protein in serum induced by AFB_1 when compared with birds treated with AFB_1 alone. Similar results were reported by Kubena *et al*. (1993). However, the addition of neither AC nor diatomite to AFB_1 contaminated diet prevented negative effects on these parameters induced by AFB_1. Similarly Kubena *et al*. (1990) reported that the addition of AC to the diet did not show a protective effect against the effect of AFB_1.

Conclusions

The results from this study demonstrate that the growth performances and serum biochemical variables of broilers were negatively affected by AFB_1 at 40 and 80 µg AFB_1/kg feed in the diets. The addition of HSCAS was more protective against the AFB_1 toxicity than AC and diatomite.

Acknowledgements

The authors are grateful to Çukurova University Research Fund for their financial support. Project no: FBE.2003D-11.

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