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Innovative methods for the amelioration of aflatoxin (afb_1) effect in broiler chicks

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ABSTRACT: The aim of this study was to evaluate the ability of Myco-Detox (Invention) in counteracting the deleterious effects of aflatoxin B1 (AFB_1) in broiler chicks. A total of 120 Ross 308 one-day-old male broiler chicks were assigned to 8 treatments for 42 d. The experiment had a 2 × 4 factorial arrangement of treatments involving 0 and 1 mg of AFB_1/kg feed and 0, 1, 2, and 5 g of Myco-Detox (*Spirulina platensis* powder + 0.5 gm humic acid) / kg feed. Chicks were fed on Built-up litter the first 7 d and in cages (3 chicks/cage; 5 cages/treatment) from7 to 42 d. Growth performance was measured from d 7 to 42. Histopathological examination of liver, and AFB_1 residues in liver was determined on d 42. Aflatoxin B_1 significantly decreased the BW gain, feed intake, and impaired feed conversion rate ($P < 0.05$). The addition of Myco-Detox in the contaminated diets significantly diminished the inhibitory effects of dietary AFB_1 ($P < 0.05$) on the growth performance with no differences compared to the control diet. Liver tissue of broilers receiving AFB_1 alone had perilobular inflammation and vacuolar degeneration of hepatocytes as compared with the tissue from the control group ($P < 0.05$). Residues of AFB_1 were detected in the liver tissues of broilers fed on the AFB_1 diet (0.166 µg/kg). Supplementation of Myco-Detox reduced the incidence and severity of the hepatic histopathology changes associated with aflatoxicosis and the amount of AFB_1 residue in liver.
Keywords: *Spirulina platensis*; Aflatoxin; Amelioration; Growth performance

Introduction

Mycotoxin contamination is very costly for the animal industry and is a food safety concern because of potential mycotoxin residue in meat, dairy, and eggs (Pandey and Chauhan, 2007; Denli et al., 2009). The most significant economic cost of mycotoxin in poultry is reduced growth rate and spiked mortality.

Aflatoxins are secondary toxic metabolites produced by certain strains of fungi (e.g., *Aspergillus flavus* and *Aspergillus parasiticus* species). Among the main aflatoxins [aflatoxin B_1 (AFB_1), aflatoxin B_2, aflatoxin G_1, and aflatoxin G_2], AFB_1 is known to be the most toxic metabolite, especially on sensitive species such as poultry (Busby and Wogan, 1981; Hussein and Brasel, 2001).

Increased efforts are being undertaken in the areas of developing cost effective and safe procedures and products to effectively deal with the decontamination and remediation of feedstuffs contaminated with mycotoxins, including AF and T-2 toxin. Approaches used have included the physical, chemical, and biological treatment of contaminated products (Goldblatt, 1971; Goldblatt and Dollear, 1979; Anderson, 1983; Park *et al.*, 1984; McKenzie *et al.*, 1997, 1998). Each of these particular methods involves specialized facilities, equipment, or procedures that do not lend themselves to practical application at the producer level.

Aflatoxins are a cause of concern in the poultry industry due to health problems in flocks and potential economic losses (Bailey et al., 1998). Consequently, poultry producers are in need of aids and methods to assist them in the protection of their flocks against these toxins. It is important to adequately test a potential mycotoxin adsorbent, not only for its in vitro binding capabilities, but also for its in vivo ability, because results in the past have indicated that there is great variability in the efficacy of adsorbents in vivo, even though the compounds may show potential for toxin binding in vitro (Bailey et al., 1998). This

emphasizes the importance for industry scrutiny of new products purported to ameliorate the effects of mycotoxins in poultry, to ensure that sound scientific principles have been applied in the evaluation of these products (Dale, 1998).

Spirulina platensis, blue - green algae, is known to be a rich source of important nutrients including several vitamins, minerals, essential amino acids, essential fatty acids, source of carotenoids and possess profound antioxidant property (Verma *et al.*, 2004)

It is known that dietary inclusion of modified mannanoligosaccharides (MOS), extracted from the cell wall of yeast, has some beneficial effects in preventing adverse effects of mycotoxins.

The aim of this study was to evaluate the ability of MycoDetox (Invention) in counteracting the deleterious effects of aflatoxin B_1 (AFB_1) in broiler chicks.

Materials And Methods

Birds, Diets and housing

A total of 120 one-day-old male Ross 308 broiler chicks from a commercial hatchery were used in this study. Chicks were individually weighed (47± 1 g) and divided into 8 treatments. The 8 treatments, arranged according to a 2 × 4 factorial experimental design, consisted of 2 levels of AFB_1 (0 and 1 mg/kg of feed) and 4 levels of Myco-Detox (0, 1, 2, 4 and 5 g *Spirulina platensis* powder + 0.5 gm humic acid / kg of feed). Chicks were placed on the ground during the first week of the experiment. On d 7, all of the chicks were transferred into cages (with 5 replicates per treatment and 3 chicks per replicate). Two diets were formulated according to the NRC (1994) recommendations to meet the nutrient requirements of broilers from d 1 to 21(grower diet) and from d 22 to 42 (finishing diet). The composition of the basal diets is presented in Table 1. Pure crystalline AFB_1 (Acros Organics, Geel, Belgium) was incorporated into the diets by dissolving AFB_1 in chloroform (1 mg/10 mL) followed by mixing the solution with appropriate quantities of ground feed. The contaminated premix feed was left overnight at room temperature for the solvent to evaporate and was then mixed into the basal diet to provide the desired level of AFB_1/kg of diet (1 mg of AFB_1/kg of feed).

After preparing the diet, 2 samples of feed from the control group and AFB_1-contaminated diet were analyzed by HPLC and fluorescence detector (Sharma and Marquez, 2001) to ensure the AFB_1 concentrations in the experimental diets. Aflatoxin B_1 content was 1.03 and1.05 mg/kg in the growing and finishing contaminated diets and <0.001 mg/kg in control diet. Feed and water were provided ad libitum throughout the experiment. The chicks were reared under a conventional temperature regimen (i.e., starting at 30°C and reduced by 3°C/ wk to 21°C). The birds were exposed to 23L: 1D. The experiment lasted 42 d, including 21 d on the grower diet and from d 22 to 42 on the finishing diet.

Growth Performance

Body weight gain, feed intake, and feed conversion rate per cage were recorded weekly from d 7 to 42. On d 42, feed intake was recorded.

Analysis of AFB_1 Residues in Liver and Breast Muscles

Five liver samples from each treatment were kept at −20°C for analyzing the residue of AFB_1. Analysis of AFB_1 residues were performed according to Tavcar-Kalcher et al. (2007). Briefly, 1 ground sample was mixed thoroughly with an aqueous solution of citric acid and diatomaceous earth. The mixture was extracted with dichloromethane. The filtered extract was dried, filtered again, and an aliquot was evaporated to dryness. The residue was dissolved in methanol and mixed with buffer and applied into an immunoaffinity column. Aflatoxin B_1 was eluated from the column and the concentration of AFB_1 in the final solution was determined by an HPLC method with fluorescence detection after derivatization with bromine in the Kobra cell(R-Biopharm Rhone Ltd., Glasgow, UK).

Histopathological Examination

Liver samples from birds fed on the control and contaminated diets were obtained to evaluate lesions and other abnormalities. Samples were obtained from the birds with intermediate weight in each cage (5 chickens from each group) and were fixed in 10% neutral buffered formalin solution, dehydrated in graded alcohol, and embedded in paraffin. Sections of 3 to 5 µm were obtained and stained with hematoxylin-eosin. Two sections of liver tissue from each chick were examined by light microscopy for previously described lesions: vacuolar degeneration of hepatocytes; inter- and perilobular inflammations; bile duct hyperplasia or

hypertrophy, or both; and necrosis (Pandey and Chauhan, 2007). Sections with no, slight, moderate, or intense presence of lesions was given a score of 0, 1, 2, and 3, respectively.

Statistical Analysis

Data were analyzed by using the GLIMMIX procedure of SAS software (SAS Institute, 1996). Tukey's test was used for multiple comparisons when a significant interaction was detected. All statements of significance were based on probability ($P < 0.05$).

Results

Growth Performance

The effects of dietary Myco-Detox and AFB_1 on growth performance are shown in Table 3. A significant interaction was observed between AFB_1 and Myco-Detox on feed intake, BW gain, and feed conversion rate. Consumption of the AFB1 diet (AFB_1: 1 mg/kg; Myco-Detox: 0 g/kg) reduced feed intake, BW gain, and caused poor feed conversion rate compared with the control diet (AFB_1: 0 mg/kg; Myco-Detox: 0 g/ kg). The addition of Myco-Detox to the contaminated diets significantly offset these effects, reaching values not significantly different from the control diet and showing no effects when supplemented to the uncontaminated diets.

Organ Weights and AFB_1 Residues

The relative weights of liver and spleen and AFB_1 residues in the liver are given in Table 2. A significant interaction between Myco-Detox and AFB_1 was observed in the relative weights of liver. Feeding AFB_1-contaminated diet without adsorbent caused significant increases in the relative weight of liver. Addition of Myco-Detox (1, 2, 4 and 5 g *Spirulina platensis* powder + 0.5 gm humic acid / kg of feed) to the diet containing AFB_1 reduced the toxic effects of AFB_1 on the relative weights of liver, showing no effect on the control diet.

There were no detectable residues of AFB_1 in the liver of diets consuming the uncontaminated diets (control and 4 levels of Myco-Detox: (1, 2, 4 and 5 g *Spirulina platensis* powder + 0.5 gm humic acid / kg of feed). A detectable amount of AFB_1 (0.166 µg/ kg) was found in the liver of the chickens fed the AFB_1 alone in diet. The results showed that the feed: liver AFB_1 transmission ratio was approximately 6,000:1.

The supplementation of 4 levels of Myco-Detox to AFB_1 diets (1 mg/kg) resulted in an overall reduction of AFB_1 residue in liver, although without reaching significant differences.

Histopathological Examination

Results of histological analysis showed that there was significant damage in the liver tissues of broilers receiving AFB_1 alone, Table 2. Liver tissue from this treatment had vacuolar degeneration of hepatocytes, perilobular inflammation (mainly mononuclear cells), bile duct hyperplasia, and hypertrophy compared with the tissue of birds fed on the uncontaminated diet. Myco-Detox supplementation to AFB_1 diets significantly avoided these lesions to values not significantly different from the control.

Discussion

AFB_1 dramatically depressed feed intake, weight gain, and resulted in high mortality. Birds fed AFB_1-contaminated feed had enlarged livers and impaired liver function, experiment, both SO and MTB effectively alleviated aflatoxicosis at a low level (1 mg/kg), but the hydrated sodium calcium aluminosilicate product (SO) was more effective than the yeast cell wall product (MTB) in diminishing aflatoxicosis in birds fed diets containing 2 mg/kg of AFB_1. (Zhao et al., 2010).

The most economically significant effect of aflatoxicosis in poultry is reduced growth rate and thet dietary AFB_1 ,1 mg/ kg severely affected the productive performance (Denli et al.,2009; Fernandez et al. ,1994; Denli et al.,2004).

The adverse effects of AFB_1 on growth performance have been related with a decrease in the protein and energy utilization (Dalvi and Ademoyero, 1984; Verma et al., 2002), probably as a consequence of a deterioration of the digestive and metabolic efficiency of the birds.

Liver is considered the target organ for AFB_1 because it is the organ where most aflatoxins are bioactivated to the reactive 8,9-epoxide form, which is known to bind DNA and proteins, damaging the liver structures and increasing liver weight (Miazzo et al., 2005; Bailey et al., 2006; Pasha et al., 2007).

In this study, a pronounced increase in the liver weight and hepatic lesions was observed in chickens treated with AFB_1. Histopathological changes in the livers of chickens exposed to AFB_1 are comparable to those reported in the literature on avian aflatoxicosis (Denli et al., 2005; Miazzo et al., 2005). Significant increases in the relative spleen weight of broilers exposed to aflatoxin-contaminated diets have also been reported by Bailey et al. (2006) and Shi et al. (2006). In current study, the relative weight of spleen also increased from 0.17 to 0.21 g/100 g of BW with the AFB_1 contamination. However, these differences did not reach a significant level.

The most critical aspect of aflatoxins in animal production is the presence of aflatoxins in animal products. The residues of AFB_1 and its metabolites have been found in eggs and poultry tissues after the consumption of diets contaminated with aflatoxins (Pandey and Chauhan, 2007).

In our study, we observed high levels (0.166 µg/kg) of AFB_1 in the liver of birds fed on the contaminated diets. Chen et al. (1984) reported that after feeding the aflatoxin-contaminated diet (containing 2.06 mg of AFB_1/kg) for 35 d, mean values for the combined aflatoxins were less than 3 µg of AFB_1/kg of gizzard, liver, and kidney tissues of broiler chickens. Residues of AFB_1 were also detected in the livers of laying hens given 2.5 mg of AFB_1/kg of feed, at levels that ranged from 1.92 to 4.13 µg/kg (Zaghini et al., 2005).

Adsorbents have been proven to be the most promising and economical approach of preventing aflatoxicosis in poultry. Various products are available on the market, including single ingredients of clays, bentonites, zeolites, phyllosilicates, synthetic aluminosilicates, or a blend of adsorbents with enzyme or yeast cell wall, or both.

Zhao *et al.*,(2010) found that, HSCAS (the hydrated sodium calcium aluminosilicate) effectively ameliorated the negative effects of AFB_1 and recovered the depressed feed intake to 90% of that of the positive control group, whereas MTB (yeast cell wall) partially recovered feed intake at 1 mg/kg but had no effect at 2 mg/kg of AFB_1. The lack of efficacy of MTB especially at the higher level of AFB_1 is perhaps due to saturation or limited binding capacity of yeast cell walls and the type and concentration of clay that was used to prepare the MTB product. Similar results were reported in dairy cows by Kutz et al. (2009), with MTB at 0.5 and 0.56% of diets containing 170 and 112 µg of AFB_1/kg of feed, respectively, not effective in reducing milk AFM_1 concentrations, AFB_1 excretion, or AFB_1 transfer from feed to milk.

Van Rensburg et al., (2006) indicated that oxihumate, but not BDY (brewers dried yeast), could alleviate some of the toxic effects of aflatoxin in growing broilers. Oxihumate might, therefore, prove to be beneficial in the management of aflatoxin- contaminated feedstuffs for poultry when used in combination with other mycotoxin management practices.

The use of feed adsorbents is considered the most promising and economical approach for reducing mycotoxicosis in animals (Dakovic et al., 2005). Several approaches indicated that several adsorbents (a variety of clays, bentonites, zeolites, phyllosilicates, and synthetic aluminosilicates) are capable of binding aflatoxins and preventing or to reducing their detrimental effects on animals (Abdel-Wahhab et al., 1999). The basic mechanism seems to involve chemisorption of aflatoxins in the gastrointestinal tract resulting in a reduction in its bioavailability (Phillips et al., 1990; Abdel-Wahhab et al., 1999). In spite of the claims about protective effects, the efficacy and safety of these products have not always been demonstrated.

Myco-Detox is a binding agent (dried *Spirulina platensis* + 0.5 gm humic acid / kg feed) that can be fed to animals in a safe way, as demonstrated by the results. In this study, broilers fed on uncontaminated diets supplemented with Myco-Detox showed no differences compared with control.

Moreover, in our study, supplementation of Myco-Detox significantly ameliorated the toxic effects of AFB_1 in the broilers. Thus, the addition of Myco-Detox (4, 5 g/kg *Spirulina platensis* powder + 0.5 gm humic acid / kg of feed) to diets containing AFB_1 significantly improved performance and counteracted histopathological changes and reduced the relative weight of liver.

Our results were in agreement with Shi et al. (2006), who reported that a reduced growth rate and serum biochemical changes associated with AFB_1 contamination (0.1 mg/kg) could be ameliorated by the supplementation of a modified montmorillonite nanocomposite at doses of 3 g/kg. Similarly, Bailey et al. (2006) reported that montmorillonite clay (5 g/kg) in broiler diets provided protection on growth performance, serum biochemistry, and the relative organ weights from over 4 mg of AFB_1/kg diets. On the other hand, other authors have not observed protective effects on the biochemical parameters and histopathological changes in liver sections when sodium bentonite (5 and 3 g/kg of feed) was included in the diet (Santurio et al., 1999; Rosa et al., 2001). Differences among studies could be explained by different levels of adsorbents or the AFB_1

exposure dose tested. Notwithstanding, it is necessary to point out that mycotoxins are complex organic compounds and each of them has different functional groups; thus, the binding capacity of an adsorbent depends on its chemical and physical properties and its relation with the physical structure of the target mycotoxins. Thus, the physicochemical differences among the adsorbents used in the studies mentioned above could explain the higher or lower efficacy among them.

The incorporation of Myco-Detox in the diet during the period of exposure to AFB_1 resulted in reduction of the residue of AFB_1 in the liver. This result confirms that the protective effects of Myco-Detox might be due to its capability of specific chemisorption of AFB_1 in gastrointestinal tract, which reduces AFB_1 bioavability. Similarly, Bintvihok and Kositcharoenkul (2006) reported that the AFB_1 residues in muscle and liver of broiler were decreased by addition of calcium propionate in the diet, which was used as a detoxifying agent.

In conclusion, our study clearly indicated that AFB_1 in the diet at levels of 1 mg/kg resulted in a reduced growth performance and an alteration of the liver weight, AFB_1 liver residue, and histological parameters of the birds. The addition of Myco-Detox prevented all of the toxic effects of AFB_1 and reduced the accumulation of AFB_1 residues in the livers as well as improves the growth performance.

Table 1.Effect of aflatoxin B_1 (AFB_1) and Myco-Detox on the liver tissue score of broilers at 42 days of age.

	Treatments		
AFB1 (mg/kg)	Myco-Detox (g/kg)	Hepatocytic Vacuolation	Perilobular Inflammation
0	0	0.0^{b}	1.0^{b}
1	0	0.5^{a}	2.2^{a}
1	1	0.2^{a}	1.8^{ab}
1	2	0.1^{a}	1.2^{b}
1	4	0.0^{a}	0.6^{b}
	5	0.0^{a}	0.0^{b}
	SEM	0.01	0.05
		P-value	

[ab]Mean within a column without a common superscript differ statistically *(P<0.05).* Results are reported as means for 5 broilers each. Sections with no, slight, moderate, or intense lesions were given a score of 0, 1, 2, and 3, respectively.

Table 2.Effects of aflatoxin B_1 (AFB_1) and Myco-Detox on the relative weight of liver and the residual concentration of aflatoxin B_1 (AFB_1) in broilers at 42 days of age.

	Treatments		
AFB1(mg/kg)	Myco-Detox (g/kg)	Liver weight (g/100 g of BW)	AFB1 residue in liver (µg/kg)
0	0	2.0b	ND
1	5	2.05b	ND
1	0	2.6a	2.38
1	1	2.31b	0.132
1	2	2.22b	0.68
1	4	2.0b	ND
1	5	2.0b	ND
	Probability		
AFB1			
Myco-detox		NS	NS
AFB1 x Myco-detox			NS

[ab]Mean within a column without a common superscript differ statistically *(P<0.05).* Results are reported as means for 5 livers samples each. Detection limits of analyzed method 0.05 µg/kg.

Table 3.Effects of aflatoxin B_1 (AFB_1) and Myco-Detox on FCR and the weight in broilers at 42 days of age.

Treatments			
AFB1(mg/kg)	Myco-Detox (g/kg)	FCR	Weight at d 42 (kg)
0	0	1.88B	2.15B
1	0	2.10A	1.85A
1	1	1.96A	1.95A
1	2	1.98AB	2.05AB
1	4	1.88B	2.10B
1	5	1.78B	2.19B
	SEM	0.01	0.05
	P-value		

[a,b] Means with different superscripts within the same column differ significantly *(P<0.05).*

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