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Evaluation of the ability of adsorbents to ameliorate the adverse effects of aflatoxin B_1 in broiler chickens

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ABSTRACT

A study was carried out to investigate the efficacy of diatomaceous earth (DE), sodium bentonite (SB) and zeolite (Z), alone or in combinations as aflatoxin adsorbents in the diets of broiler chickens containing 300 ppb aflatoxin B_1 (AFB_1). Day-old broiler chicks (384) were divided into 12 treatment groups (T_1, control; T_2, T_1 + 300 ppb AFB_1; T_3, T_2 + 0.5% DE; T_4, T_2 + 1% DE; T_5, T_2 + 0.5% SB; T_6, T_2 + 1% SB; T_7, T_2 + 0.5% DE +0.5% SB; T_8, T_2 + 0.5% Z; T_9, T_2 + 1% Z; T_{10}, T_2 + 0.5% Z + 0.5% DE; T_{11}, T_2 + 0.5% Z + 0.5% SB; T_{12},T_2 +[Z+DE+SB (0.33% each)]. Each diet was offered from day-old to 42 days of age to 4 replicated groups of 8 birds each. Feeding diet with 300 ppb of AFB_1 in diet caused significant decrease in body weight gain (BWG 1168g) as compared to control (1 429 g). Inclusion of all the binders in aflatoxin contaminated diet, alone or in combinations, improved the overall BWG (1 290 to 1 389 g/bird), however, significantly lower BWG was recorded in DE groups in comparison to other binders during 0–6 weeks growth period. The BWG did not differ between zeolites and sodium bentonite groups. Feed consumption was not affected during the first week of age but reduced thereafter on diet with 300 ppb level of dietary AFB_1. Amongst the binder groups, feed consumption was lowest in DE fed groups. Addition of aflatoxin resulted in poor feed conversion, energy and protein efficiency, which partially improved on inclusions of binders at any levels, alone or in combination. Thus, it may be concluded that addition of 300 ppb AFB_1 in the diet of broiler chickens impaired the performance of broiler chickens during 0–6 weeks of age. All the 3 binders at 0.5 or 1% level, alone or in combination, were partially efficacious in ameliorating the adverse effects of aflatoxin. Among the binders tested, diatomaceous earth appeared to be the least efficacious in ameliorating aflatoxicosis in broiler chickens.

Key words: Adsorbent, Aflatoxin, Broiler chicken, Feed, Energy, Protein

Amongst the most widespread mycotoxins, aflatoxins are of great concern in warm and humid climatic conditions like India (Singh *et al*. 2010). Aflatoxins include B1, B2, G1 and G2, and aflatoxin B1 (AFB1) is the most important toxic secondary metabolites, produced by 3 closely related species of *Aspergillus; A. flavus, A. parasiticus* and *A. nomius* in the feedstuffs. Avoidance of contaminated feed is rarely feasible and feeds that contain relatively low concentrations of AFB1 may have deleterious effects on poultry (Doerr *et al*. 1983, Giambrone *et al*. 1985). Even small amounts of AFB1 may reduce in growth, hatchability and render the birds susceptible to diseases (Coulombe 1993, Denli *et al*. 2004). Liver damage, decreased egg production and overall performance, and suppressed immunity were noted in animals consuming relatively low dietary concentrations of aflatoxin (Robens and Richard 1992, Okan *et al*. 2004). Liver, the major organ involved in nutrient metabolism and detoxifying toxic materials, 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. Use of adsorbents in feed is the most applied user friendly method for protecting animals against mycotoxicosis. They bind the mycotoxins and eliminate through feaces without absorption from the gut (Doll and Danicke 2004). The efficiency of mycotoxin adsorbents, however, differs considerably depending upon the chemical structure of the adsorbent as well as toxin. Therefore, the present study was conducted to evaluate the comparative efficacy of diatomaceous earth, sodium bentonite and zeolites alone or in combination in broiler chickens.

MATERIALS AND METHODS

Production of aflatoxin: Aflatoxin was produced using the fungal strain *Aspergillus flavus* NRRL 6513 that was obtained from U.S. Department of Agriculture, Illinois, USA. To get the fresh spores, the culture was regularly subcultured on potato dextrose agar (PDA) medium slants and stored at

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5°C. Aflatoxin was produced on maize substrate. Fermentations were carried out in batches as per Shotwell *et al.* (1966). The AFB_1 concentration in fermentated maize was 990 mg/kg of maize and the aflatoxin thus produced was very stable under normal conditions.

Aflatoxin analysis: The extraction and estimation of aflatoxin was done as per Pons *et al.* (1966). Aqueous acetone was used for extraction of the toxin. Aflatoxin contents were finally quantified using a spectrophotometer.

Experimental design: Experimental design was completely randomized design (CRD). There were 12 dietary treatments. Each dietary treatment had 4 replicates and each replicate had 8 chicks. The experiment was conducted in broiler chickens from day-old to 6 weeks of age. The various dietary treatments were prepared by mixing the required quantity of adsorbents and mouldy maize to get the desired concentration of 300 ppb AFB1.

Experimental groups and treatment

Treatment no.	Dietary treatment
T_1	Control (basal diet)
T_2	Basal+AFB1 @ 300 ppb
T_3	Basal+AFB1 @ 300 ppb+DE @ 0.5%
T_4	Basal+AFB1 @ 300 ppb+DE @ 1%
T_5	Basal+AFB1 @ 300 ppb+SB @ 0.5%
T_6	Basal+AFB1 @ 300 ppb+SB @ 1%
T_7	Basal+AFB1 @ 300 ppb+DE and SB each @ 0.5%
T_8	Basal+AFB1 @ 300 ppb+Z @ 0.5%
T_9	Basal+AFB1 @ 300 ppb+Z @1%
T_{10}	Basal+AFB1 @ 300 ppb+Z and DE each @ 0.5%
T_{11}	Basal+AFB1 @ 300 ppb+Z and SB each @ 0.5%
T_{12}	Basal+AFB1 @ 300 ppb+Z, DE and SB each @ 0.33%

Biological experiment and analysis: Day-old broiler chicks (384) were obtained from experimental hatchery, CARI, Izatnagar. The chicks were wing banded, weighed individually and distributed randomly into 12 groups. All birds were reared under standard managemental conditions from 0–6 weeks. All birds were fed with broiler starter ration from 1–21 days and broiler finisher ration from 22 to 42 days. The basal diet was prepared as per BIS (2007). Weekly individual body weight and feed consumption of each group were recorded. The compositions of broiler starter and broiler finisher rations are presented in Table 1.

The protein (AOAC 1990) and calcium (Talapatra *et al.*1940) contents were estimated, while the concentrations of lysine, methionine, available P and metabolizable energy values were calculated. Data were analyzed following completely randomized design (CRD) (Snedecor and Cochran 1980). The statistical analysis was done using SPSS 12.0 version.

Table 1. Ingredients and chemical composition of basal feed

Ingredient	Starter (%)	Finisher (%)
Maize	51.7	59.04
Soybean	39.9	32.28
Rapeseed meal	4	4
Oil	1	1.5
Lysine	0.03	0.04
Limestone	0.9	0.9
Di-calcium phosphate	1.7	1.45
DL-methionine	0.11	0.07
Common salt	0.3	0.3
TM premix	0.1	0.1
Vitamin premix	0.15	0.15
B complex	0.015	0.015
Choline chloride	0.05	0.05
Coccidiostat	0.05	0.05
Chemical composition of basal feed		
Crude protein (%)	21.51	19.02
ME (kcal/kg)	2 888	2 991
Calcium (%)	1.02	0.93
Available phosphorus (%)	0.45	0.39
Lysine (%)	1.28	1.09
Methionine (%)	0.52	0.45

RESULTS AND DISCUSSION

Body weight gain (BWG): At first week of age, there was no difference ($P<0.05$) in body weight gains emanated from various dietary treatments. However, thereafter from second week of age, the body weight gains differed ($P<0.01$) due to dietary treatments. On 14 d of age, the average body weight gain of broilers fed AFB_1 (300 ppb) diet reduced significantly ($P<0.01$) than those fed control diet. Inclusion of binders, at different dose rate in AFB_1 contaminated diet, improved body weight gain significantly but could not match the gain observed in control diet except in T_{12}, wherein combination of 3 binders was used. Similar trend existed during subsequent weeks and growth phases (0–21, 22–42 and 0–42d of age). Addition of any binder at 0.5 or 1% improved the body weight gain significantly. During 0–21d of age (Table 2), all the groups fed binders had similar improvement except T_{12} . During 22–42 d of age, BWG observed in diets with DE either at 0.5% or 1% level had lower ($P<0.01$) BWG in comparison to other binders used in this experiment. During overall growth period (0–42d), significantly lower gains were recorded in DE groups in comparison to other binders. There was no significant difference between zeolites and sodium bentonite groups but maximum gain was recorded when all the 3 binders were used together. Our results revealed that inclusion of 300 ppb of AFB_1 caused significant ($P<0.05$) decrease in body weight gain and effect was pronounced on 14d of age. Our observation was in agreement with those of Raju and Devegowda (2000), who also observed reduction in body weight gain of 300 ppb dietary aflatoxin. Results of other studies (Johri *et al.* 1988, Beura *et al.* 1993, Verma 1994 and Rosa *et al.* 2001) also indicated that dietary AFB_1 at 0.5 ppm levels or beyond in commercial broilers diet adversely affected growth in a dose related fashion. In the present study, supplementation of DE at 0.5% and 1% level in the AFB_1 contaminated diet showed improvement (P<0.05) in BWG, which was in line with the observation of Modirsanei *et al*. (2008), wherein addition of DE @ 3% in 1 mg of AFB_1/kg feed significantly (P<0.05) improved BWG. Incorporation of SB in AFB_1 contaminated diet increased the BWG in the present study. Similar results were also obtained by Miazzo *et al.* (2005) on addition of 0.3% SB to the diets containing 2.5 ppm AFB1. Similarly, suppressed body weight of chicks caused by 0.1 mg/kg (0.1ppm) aflatoxin contaminated diet was overcome on addition of 0.5% SB (Pasha *et al.* 2007) in diet. Lopes *et al*. (2006) also reported improved BWG after addition of SB at 0.3% in aflatoxin (300 ppb) contaminated feed. Addition of zeolite at 0.5% and 1% level with the feed with 300 ppb of AFB_1 revealed significant increase in body weight gain in the present study. Miazzo *et al*. (2000) also counteracted partly the effect of 2.5 mg AFB1/kg through addition of 1% zeolite in the diet. Safameher (2008) reported increased weight gain on addition of zeolite at 2% in 0.5 mg of AF/kg feed.

Feed intake (FI): At first week of age, there was difference (P<0.01) in feed consumption among the different dietary groups. However, there was no significant effect on addition of aflatoxin in diet on feed consumption of broilers. The FI decreased in diets containing DE 0.5%+SB 0.5%, 0.5% zeolite and 1% zeolite when compared with control or aflatoxin fed group. On 14^{th} d of age, FI was higher (P<0.01) in control group than AFB_1 or AFB_1 with binder fed groups. The FI differed significantly (P<0.01) due to the dietary treatments in different growth phases also (Table 3). Addition of AFB_1 (300 ppb) resulted in lower feed intake at any growth phase. In 0–21d of age, control group had significantly (P<0.01) higher feed intake than any other group, but FI in AFB_1 fed group did not differ from AFB_1 + binders fed groups. Similar trend was also observed during 22–42 days of age. Feed consumption of the birds kept on different dietary treatments in this study indicated that it remained uninfluenced up to 7d of age but thereafter, reduction in feed consumption was observed in group fed with 300 ppb level of dietary AFB_1. These results are well in agreement with Beura *et al.* (1993), who reported decreased feed consumption in pure bred and commercial broilers at 300 and 800 ppb AFB_1 respectively. Similar results were also recorded by Verma (1994) with 1 ppm of AFB_1. In the present study, inclusion of DE at 0.5% and 1% level in the 300 ppb AFB_1 contaminated diet showed improved FI significantly during overall growth phase. Modirsanei *et al*. (2008) obtained similar result in their study with 1 mg of aflatoxin and DE @ 30g/kg of feed. El-Husseiny *et al*. (2008) reported that addition of diatomaceous earth (DE) at 0.2 and 0.5% significantly increased the feed intake than that of the control, which could not be confirmed in this study as DE was added in diet containing AFB_1. Addition of SB at 0.5 and 1% level with 300 ppb of AFB_1 contaminated diet resulted in decrease in the inhibitory effect on feed intake in experimental aflatoxicosis. This result was in well agreement with Rosa *et al*. (2001), Kermanshahi *et al.* (2009) and Pasha *et al.* (2007), who reported that addition of SB with the aflatoxin feed diminished the effect on feed intake in the level of 0.3% SB into 5 mg aflatoxin/kg feed, 1% SB into 500 ppb aflatoxin and 0.5% SB into 100 ppb aflatoxin feed respectively. Supplementation of 0.5 and 1% zeolite into the 300 ppb of AFB_1 diet increased the feed intake significantly. Safameher (2008) reported that addition of zeolite at 2% with the 0.5 mg of AFB_1/kg (0.5ppm) of feed showed increased feed intake. Oguz *et al*. (2000) observed that use of zeolite at 1.5 and 2.5% concentrations to broiler chickens diet containing 2.5 ppm aflatoxin was effective to avoid decreased feed intake. On critical comparison of different binders for their bio-efficacy in improving feed intake, it revealed sodium bentonite (at 0.5 or 1% level) or zeolites (at 1% level) were better than DE. Similarly, feed intake improved significantly at par with control when the different binders were added together (DE+Z, SB +Z or DE+SB+Z). The DE fed group had lowest feed intake indicating that DE was unable to ameliorate the effect of aflatoxicosis. Feed intake is function of body weight and vice versa. In the present study, growth depression was also more when DE was used as binder in comparison to SB or zeolites.

Feed conversion ratio (FCR): At first week of age, there was no difference (P<0.05) among the dietary treatment in feed conversion ratio. Variable response was observed in different weeks of age thereafter. Overall, aflatoxin fed group had significantly (P<0.01) poorer FCR compared to control group in all the growth phases. During 0–3 weeks of age (Table 4), FCR was similar to control in all the groups barring

Table 2. Body weight gain (g/bird) in different growth phases of broilers fed different dietary treatments

Treatment	0-3 wks	4-6 wks	0-6 wks
1	481.11^{d}	948.28^{e}	1429.39^{e}
2	414.66^{a}	753.59^{a}	1168.26^{a}
3	438.90^{b}	851.16^{b}	1290.05^{b}
4	450.72^{bc}	855.06^{b}	1305.79^{b}
5	455.64^{bc}	889.25^{c}	1344.89^{c}
6	444.22^{bc}	903.88^{cd}	1348.09^{c}
7	447.64^{bc}	899.22^{cd}	1346.86^{c}
8	443.99^{bc}	893.25^{c}	1337.24^{c}
9	449.91^{bc}	897.41^{cd}	1347.31^{c}
10	448.06^{bc}	919.00^{cde}	1367.06^{cd}
11	454.07^{bc}	915.53^{cd}	1369.61^{cd}
12	461.72^{c}	927.53^{de}	1389.25^{d}
SEm	2.565	7.457	9.374
Stat	P<0.01	P<0.01	P<0.01

^{abc}Values in a column with different superscripts differ significantly.

Table 3. Feed intake in different growth phases of broilers fed different dietary treatments

Treatment	0–3	wks 4–6	wks 0–6wks
1	787.8^{c}	1 968.3^{cd}	2 756.1^{e}
2	749.7^{ab}	1 702.2^{a}	2 451.9^{a}
3	743.3^{a}	1 842.3^{b}	2 585.5^{b}
4	756.7^{ab}	1 854.3^{b}	2 611.1^{b}
5	763.4^{ab}	1 932.6^{cd}	2 696.0^{cde}
6	748.9^{ab}	1 947.8^{cd}	2 696.8^{cde}
7	753.5^{ab}	1 936.3^{cd}	2 689. 8^{cd}
8	753.6^{ab}	1 915.1^{c}	2 668.7^{c}
9	762.8^{ab}	1 929.4^{cd}	2 692.2^{cde}
10	754.8^{ab}	1 971.7^{cd}	2 726.6^{cde}
11	766.9^{b}	1 965.2^{cd}	2 732.2^{cde}
12	764.1^{b}	1 986.3^{d}	2 750.4^{de}
SEm	2.234	11.99	13.04
Stat	P<0.01	P<0.01	P<0.01

^{abcde}Values in a column with different superscripts differ significantly.

Table 4. Feed conversion ratio in different growth phases of broilers fed different dietary treatments

Treatment	0–3	wks 4–6	wks 0–6wks
1	1.638^{a}	2.076^{a}	1.928^{a}
2	1.808^{c}	2.259^{c}	2.099^{c}
3	1.694^{ab}	2.164^{b}	2.004^{b}
4	1.679^{ab}	2.169^{b}	2.000^{b}
5	1.676^{ab}	2.174^{b}	2.005^{b}
6	1.686^{ab}	2.156^{b}	2.001^{b}
7	1.684^{ab}	2.153^{b}	1.997^{b}
8	1.697^{b}	2.145^{b}	1.996^{b}
9	1.697^{b}	2.150^{b}	1.998^{b}
10	1.685^{ab}	2.145^{b}	1.995^{b}
11	1.689^{ab}	2.147^{b}	1.995^{b}
12	1.655^{ab}	2.142^{b}	1.980^{b}
SEm	0.007	0.007	0.006
Stat	P<0.01	P<0.01	P<0.01

^{abc}Values in a column with different superscripts differ significantly.

T_2 (300 ppb AFB_1), T_8 (0.5% zeolite) and T_9 (1% zeolite). During 4–6 weeks or overall (0–6 weeks) growth phase, FCR remained statistically higher (P<0.01) in any binder fed group. Poor feed conversion efficiency is a common feature in broilers exposed to aflatoxins. Raju and Devegowda (2000) observed poor FCR in broilers fed diets with 300 ppb level of dietary AFB_1. Similarly, others also reported a dose dependent significant (P<0.05) reduction in feed efficiency due to presence of aflatoxin in diet (Reddy *et al.* 1982, Verma 1994, Rosa *et al.* 2001). The adverse effect was more after 3 weeks of age, which might be attributed to chronic exposure of low levels of aflatoxin. Inclusion of DE at 0.5% and 1% level in the 300 ppb AFB_1 contaminated diet, improved FCR significantly. Modirsanei *et al*. (2008) obtained a similar result. Administration of 0.5 and 1% SB with 300 ppb AFB_1 contaminated diet also resulted in improvement in FCR significantly. Some previous studies (Rosa *et al*. 2001, Pasha *et al.* 2007) also indicated similar results. Addition of 0.5 and 1% zeolite in the diet with 300 ppb of AFB_1 improved feed-utilization efficiency in the present study. Safameher (2008) also reported that addition of zeolite at 2% level in diet with 0.5 mg of AFB_1/kg showed increased FCR.

Energy and protein efficiency: The data pertaining to energy and protein efficiency at different growth phases are given in Table 5. During 0–3 weeks of age, energy and protein efficiency of AFB_1 fed group was poorer (P<0.01) than control, which improved on addition of binders. At 4–6 and 0–6 weeks of growth phases, the energy efficiency of all the treatments followed similar pattern. Though all the binders effectively reduced the AFB_1 effect on energy and protein efficiency but could not eliminate the entire adverse effect of AFB_1. The mycotoxins cause severe damage to the gut epithelium (Schiefer and Beasley 1989, Hoerr 2003) and liver (Wyatt 1991) resulting in poor absorption of nutrients and thus depressed energy and protein efficiency.

Table 5. Energy and protein efficiency in different growth phases of broilers fed different dietary treatments

Treatment	Energy efficiency			Protein efficiency		
	0–3 wks	4–6 wks	0–6 wks	0–3 wks	4–6 wks	0–6 wks
1	4.73^{a}	6.21^{a}	5.71^{a}	0.35^{a}	0.39^{a}	0.42^{a}
2	5.22^{c}	6.75^{c}	6.21^{c}	0.39^{c}	0.43^{c}	0.40^{c}
3	4.89^{b}	6.47^{b}	5.93^{b}	0.36^{b}	0.41^{b}	0.39^{b}
4	4.85^{ab}	6.48^{b}	5.92^{b}	0.36^{ab}	0.41^{b}	0.40^{b}
5	4.84^{ab}	6.50^{b}	5.94^{b}	0.36^{ab}	0.41^{b}	0.39^{b}
6	4.87^{ab}	6.45^{b}	5.93^{b}	0.36^{ab}	0.41^{b}	0.39^{b}
7	4.86^{ab}	6.44^{b}	5.91^{b}	0.36^{ab}	0.41^{b}	0.39^{b}
8	4.90^{b}	6.41^{b}	5.91^{b}	0.37^{b}	0.41^{b}	0.39^{b}
9	4.90^{b}	6.43^{b}	5.92^{b}	0.37^{b}	0.41^{b}	0.39^{b}
10	4.87^{ab}	6.41^{b}	5.91^{b}	0.36^{ab}	0.41^{b}	0.39^{b}
11	4.88^{ab}	6.42^{b}	5.91^{b}	0.36^{ab}	0.41^{b}	0.39^{b}
12	4.78^{ab}	6.40^{b}	5.86^{b}	0.36^{ab}	0.41^{b}	0.39^{b}
SEm	0.02	0.02	0.02	0.002	0.001	0.001
Stat	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01	P<0.01

^{ab}Values in a column with different superscripts differ significantly.

It may be concluded that addition of 300 ppb aflatoxin B_1 (AFB_1) in the diet impaired body weight gain, feed intake and utilization efficiency of feed, energy and protein in broiler chickens during 0–6 weeks of age. Sodium bentonite, diatomaceous earth or zeolites either at 0.5 or 1% level, alone or in combination (DE+Z, SB +Z or DE+SB+Z), were partially efficacious in ameliorating the adverse effects of aflatoxin in broiler chickens. Among all the 3 binders tested, diatomaceous earth appeared to be the least efficaceous in ameliorating the adverse effect caused by aflatoxin in broiler chickens.

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