Feeding of Clay Materials as Mycotoxin Binder to Aflatoxin Fed Broiler Chicks

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ABSTRACT

Aflatoxin (AF) (0.5ppm) and Clay Material as Mycotoxin Binder (CMMB) (0.5, 0.75 and 1%) were tested in an in vivo study forming 8 dietary treatments each with three replicates on a total of 336 on broiler chicks up to five weeks. Results showed that chicks receiving AF contaminated feed had suppressed body weight which significantly improved with inclusion of CMMB. Supplementation of CMMB at 0.75 and 1% to the diets containing AF significantly (9.97 and 9.15% respectively) improved the feed consumption. Efficiency of feed utilization which has decreased significantly with addition of 0.5 ppm AF, were improved with inclusion of CMMB. The relative weights of liver (19.56%) and kidney (18.38%) which increased significantly with addition of 0.5ppm AF were improved with dietary inclusion of 0.75 and 1% CMMB. Compared with control, relative weights of gizzard and pancreas were not affected either in AF fed or control groups. The relative thymus (38.99%) and bursa (31.36%) weights were significantly lower at 0.5ppm inclusion of AF. The thymus and bursa weights were not altered by supplementation of CMMB compared to control diets. Relative weight of spleen was not affected either in AF fed or CMMB supplemented groups. The serum antibody titers against ND and IBD vaccination which were significantly depressed by AF, were restored with the inclusion of 1% CMMB. The serum concentration of total protein (38.37%) uric acid and albumin were not affected either in AF fed or CMMB supplemented groups. The activity of serum GGT significantly increased in AF fed group and the addition of CMMB did not show significant reduction in activity of serum GGT. Compared with control, activity of serum ALT was not affected either in AF, control or CMMB supplemented groups.

Key words: Aflatoxin, broilers, performance.

Introduction

Cereal grains and associated by-products constitute important sources of energy for poultry. There is increasing evidence that global supplies of cereal grains for animal feedstuffs are commonly contaminated with mycotoxins. Aflatoxins are secondary toxic metabolites produced by certain strains of fungi, e.g. Aspergillus flavus and Aspergillus parasiticus species. Aflatoxin B1 (AFB1), the most toxic of all aflatoxins (AFB1, AFB2, AFG1 and AFG2), is produced by certain strains of fungi in greater quantities than in others. In poultry, aflatoxin ingestion leads to “Aflatoxicosis” syndrome which is characterized by retardation of growth, feed consumption, feed conversion efficiency, bruising, immunosuppression and mortality. Co-contamination of cereal grains with mycotoxins produced by different fungal genera, including Fusarium and Aspergillus has been reported to increase the toxicity symptoms in poultry [11].

At present, one of the more encouraging approaches is the addition of non-nutritive and natural adsorbent materials to contaminated feed in order to selectively bind the mycotoxin during the digestive process and make it harmless to the feed. The major advantages of these adsorbents include low cost, safety and the ease with which they can be added to animal feed. Layered amino silicates such as sodium bentonite found to be effective in counteracting mycotoxins [31,28,12,25,24,8]. However, the ability of bentonite to bind mycotoxins depends on pH, molecular arrangements and its geographic region of origin [33]. Considering all these facts, the present study has been undertaken to study the ability of graded levels of Clay Material as Mycotoxin Binder obtained from mines of Kutch area of Gujarat to counteract the toxic effects of aflatoxin broilers.

Materials And Methods

Experimental Animals and Design:

Three hundred and thirty six, unsexed one-day old commercial broiler chicks were wing banded, weighed and assigned to a 4X2 factorial arrangement
of two levels of Aflatoxin AF (0 and 0.5ppm) and four levels of Clay Material as Mycotoxin Binder (CMMB) (0, 0.5, 0.75 and 1%) in a Completely Randomized Design manner, forming a total of 8 dietary treatments each with 3 replicates.

Experimental Housing, Management And Test Diet:

Each replicate group of chicks was housed in an independent pen in an open sided deep litter conventional house. Chicks in all the replicate groups were reared up to five week of age under uniform standard conditions throughout the study. Brooding was done till three weeks of age using incandescent bulbs. Each pen was fitted with an automatic bell type drinker and a hanging tubular feeder. Chicks were provided continuous light throughout the study. Aflatoxin was produced on using the pure culture of Aspergillus parasiticus MTCC 411 grown on potato dextrose agar. Then toxin produced on rice was then extracted as described by Romer [23] and quantified by thin layer chromatography (TLC) as described by A.O.A.C. [1]. The experimental diets were prepared by the addition of required quantities of rice containing aflatoxin to arrive at the levels of 0 and 0.5ppm of aflatoxin B$_1$. To each of these diets, CMMB (a natural raw material claimed to posses high adsorption capacity due to high surface area) was added at 0, 0.5, 0.75 and 1% levels. Basal diet was formulated and compounded to meet the nutrient requirements of commercial broilers during the starter (0-3 wks) and finisher (4-5 wks) phases. Chicks were provided ad libitum supply of feed and water throughout the study. Feeding of test diets commenced at zero day of age and continued till the termination of experiment at five weeks of age. Chicks were vaccinated against Newcastle Disease (ND) on 7th day using F1 strain (Ventri’s Biologicals, Bangalore) and against Infectious Bursal Disease (IBD) on 14th day using intermediate strain (Ventri’s Biologicals, Bangalore). Both the vaccines were given by ocular route.

Data collection:

At the end of the trials, body weight, feed consumption and mortality, if any were recorded and gain in weight and feed efficiency were calculated. Six birds from each replicate were sacrificed by cutting the jugular vein at the end of the trial and the weight of internal organs such as liver, kidney, gizzard, pancreas, spleen, thymus and bursa were recorded and expressed as grams per kilogram body weight. Blood was collected in non-heparinized tubes from six birds in each treatment (3 males and 3 females) by puncturing the brachial vein during 5th week of age. Serum was separated after 8 to 10 hours as per the standard procedures [3] and was stored at –20 ºC for subsequent analysis. The individual serum samples were analyzed for total protein, serum albumin, uric acid and the activities of gamma glutamyl transferase (GGT) and alanine amino transferase (ALT) using automatic analyzer (Boehringer Mannhein Hitachi 704 automatic analyzer, Japan), antibody titers against Newcastle disease (ND) and Infectious Bursal Disease (IBD) using ELISA technique.

Statistical Analysis:

The experimental data were analyzed statistically by using the General Linear Model procedure of Statistical Analysis System (SAS®) software [26]. Overall data analyzed by repeated measurement design. Duncan multiple range test was employed for comparison of the means [7]. The result of this study was subjected to one way ANOVA test.

Results And Discussion

The body weight, feed consumption, feed conversion ratio and mortality data of broilers fed control and different experimental diets at fifth week of age are presented in Table1. Results showed that chicks receiving AF contaminated feed had significantly (P<0.05) suppressed body weight, feed consumption and efficiency of feed utilization compared to chicks fed control diet. CMMB supplementation at 0.75 and 1.00 per cent to the diets containing AF significantly (P<0.05) improved the body weight and feed consumption when compared to toxin control diet and it remained non significant with control diet. Efficiency of feed utilization which was decreased significantly with addition of 0.5ppm AF, were improved with inclusion of 0.75 and 1.00 per cent CMMB. High mortality rate of 14.20 per cent was observed in group fed with diet containing 0.5ppm AF. Mortality rate was reduced considerably in the groups supplemented with 0.5, 0.75 and no mortality in 1.00 per cent CMMB fed chicks. The decreased body weight, feed consumption and increased feed conversion ratio due to AF are consistent with the findings of [29,21,2,9]. The Growth depression effects of AF may be due to their inhibitory action on protein synthesis and nutrient utilization [17]. Addition of CMMB at graded levels (0.5, 0.75 and 1 per cent) to control diet did not affect body weight and feed consumption in broilers. Feed conversion ratio was significantly (P<0.05) superior in birds given either 0.75 or 1 per cent CMMB. The results indicated that the naturally occurring sorbent used in the study is inert and non toxic. Similar results were reported by Kurnick and Reid [16]. The results suggest a beneficial effect of addition of CMMB in the presence of AF on growth performance. The relative weights of various organs expressed as grams per kilogram body weight are shown in Table2. The AF showed significant (P<0.05) increase in sizes of liver, kidney. This could
be attributed to increased lipid deposition in liver due to impaired fat metabolism [27]. Supplementation of 0.75 and 1.00 per cent CMMB to AF containing diets significantly (P<0.05) reduced the relative weights of liver and kidney which were comparable to control group indicating the reversal of aflatoxicosis. The increase in liver and kidney weights were in accordance with the findings of [21,2,9,19,18]. The protective effects of bentonite against AF induced organ weight increase in broilers was earlier observed by [4]. The relative weights of pancreas and gizzard were not altered from those of the control in all treatments. Feeding AF significantly (P<0.05) reduced relative weights of bursa of fabricius and thymus as compared to control. Thymus weights appeared to be more sensitive than that of bursa of fabricius to the adverse effects AF. Aflatoxins are known to cause immuno suppression in broilers and concomitantly decrease the relative size of bursa and thymus responsible for immunological competence. A similar results were reported by Pier and McLaughlin, [20]; Devegowda et al., [6]; Raju and Devegowda, [22]; Perozo and Rivera, [19] and Miazzo et al., [18]. The supplementation of graded level of CMMB to control and diets containing toxin did not show any significant differences in relative weights of bursa of fabricius, thymus and spleen as compared to control diet. The relative weight of spleen was non-significantly differing from those of the control in all treatments. Increased weight of spleen observed in accordance with findings of trials conducted by Kubena et al., [14], Girish et al., [9] and Miazzo et al., [18]. The effect of CMMB supplementation to the diets containing AF on the antibody titers against New Castle Disease (ND) and Infectious Bursal Disease (IBD), serum protein, serum albumin, uric acid, the activities of gamma glutamyl transferase (GGT) and alanine amino transferase (ALT) are presented in Table3. A significant (P<0.05) decrease in antibody titer values against ND and IBD vaccine were observed upon feeding AF. This depression in titer values are clear indication of immuno depressing effects of AF on humoral antibody response. These findings agree with the previous reports [32,6,29,13,14,10]. The reduction of antibody titers could be due to inhibition of DNA and protein synthesis by aflatoxin through impairment of amino acid transport and m-RNA transcription resulting in lowered level of antibody production [30]. Addition of graded levels of CMMB alone to control diets did not alter antibody titers against ND and IBD at fifth week of age as compared to control, whereas addition of CMMB to diets containing AF significantly (P<0.05) improved the antibody titers against ND and IBD vaccine when compared to their respective controls. The results clearly demonstrated the protective effects of CMMB at 1.00 per cent inclusion to AF diet in chickens. The findings of present study were comparable to the reports of Daoud, 2002. The serum concentration of total protein which was significantly (P<0.05) decreased by AF, was elevated to normal level with the inclusion of 1.00 per cent CMMB. Compared with control, serum concentration of uric acid and albumin were not affected either in AF fed group or CMMB supplemented groups. The activity of serum GGT significantly (P<0.05) increased in AF fed group. The addition of CMMB to AF containing diet did not show significant reduction in the activity of serum GGT. Compared with control, activity of serum ALT was not affected either in AF fed group or control, CMMB supplemented groups.

It maybe concluded that CMMB is partially effective in counteracting the adverse effects of aflatoxin in broilers. Among the various levels of Clay Material as Mycotoxin Binder, 1.00 per cent had shown the best level against the aflatoxicosis in broilers.

### Table 1: Effect of Clay Material as Mycotoxin Binder on fifth week body weight, feed consumption, feed conversion ratio and mortality of broilers fed aflatoxin.

<table>
<thead>
<tr>
<th>AF (ppm)</th>
<th>CMMB (%)</th>
<th>Body weight (g)</th>
<th>Feed consumption (g/bird)</th>
<th>Feed Conversion Ratio</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1313±4.88*</td>
<td>2513.1±6.07*</td>
<td>1.91±0.00*</td>
<td>4.70</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>1180±7.49*</td>
<td>2306±2.92*</td>
<td>2.09±0.005*</td>
<td>14.20</td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>1314±11.11*</td>
<td>2505±10.3*</td>
<td>1.90±0.008*</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0.75</td>
<td>1326±4.91*</td>
<td>2501±8.13*</td>
<td>1.85±0.006*</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>1.0</td>
<td>1339±10.03*</td>
<td>2495±14.5*</td>
<td>1.86±0.003*</td>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
<td>0.75</td>
<td>1202±8.81*</td>
<td>2305±2.89*</td>
<td>2.05±0.006*</td>
<td>7.10</td>
</tr>
<tr>
<td>0.5</td>
<td>1.0</td>
<td>1240±6.35*</td>
<td>2517±14.63*</td>
<td>2.03±0.005*</td>
<td>4.70</td>
</tr>
<tr>
<td>0.75</td>
<td>0</td>
<td>1274±7.39*</td>
<td>2536±7.73*</td>
<td>1.99±0.005*</td>
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</tr>
</tbody>
</table>

Means bearing at least one common superscript in a column do not differ significantly (P<0.05)

### Table 2: Effect of CMMB on relative weights of organs (grams per kg body weight) in broilers fed aflatoxin.

<table>
<thead>
<tr>
<th>AF (ppm)</th>
<th>CMMB (%)</th>
<th>Liver</th>
<th>Kidney</th>
<th>Gizzard</th>
<th>Pancreas</th>
<th>Spleen</th>
<th>Bursa</th>
<th>Thymus</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>27.60±0.76*</td>
<td>8.16±0.16*</td>
<td>24.53±0.6*</td>
<td>5.10±0.17*</td>
<td>1.57±0.28*</td>
<td>1.69±0.02*</td>
<td>4.36±0.21*</td>
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<tr>
<td>0.5</td>
<td>0</td>
<td>33.00±0.57*</td>
<td>9.66±0.16*</td>
<td>25.67±1.20*</td>
<td>4.66±0.33*</td>
<td>1.66±0.16*</td>
<td>1.16±0.16*</td>
<td>2.66±0.33*</td>
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<tr>
<td>0</td>
<td>0.5</td>
<td>27.67±0.42*</td>
<td>8.33±0.16*</td>
<td>25.73±0.52*</td>
<td>4.16±0.03*</td>
<td>1.51±0.16*</td>
<td>1.69±0.03*</td>
<td>4.03±0.12*</td>
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<tr>
<td>0.5</td>
<td>0.75</td>
<td>28.63±0.23*</td>
<td>8.16±0.16*</td>
<td>25.97±0.21*</td>
<td>4.66±0.26*</td>
<td>1.57±0.16*</td>
<td>1.68±0.04*</td>
<td>4.06±0.40*</td>
</tr>
</tbody>
</table>
Table 3: Effect of CMMB on the antibody titers against New Castle Disease (ND) and Infectious Bursal Disease (IBD), serum protein, serum albumin, uric acid, the activities of gamma glutamyl transferase (GGT) and alanine amino transferase (ALT) in broilers fed aflatoxin.

<table>
<thead>
<tr>
<th>AF (ppm)</th>
<th>CMMB (%)</th>
<th>ND titer</th>
<th>IBD titer</th>
<th>Serum protein (g%)</th>
<th>Serum Albumin (g%)</th>
<th>Uric acid (µg/dl)</th>
<th>GGT (IU/L)</th>
<th>ALT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>4297.7±17.05ab</td>
<td>4281.0±8.80bc</td>
<td>2.72±0.18a</td>
<td>1.28±0.17b</td>
<td>647.9±7.54ba</td>
<td>9.53±1.15ba</td>
<td>28.17±0.60ba</td>
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<tr>
<td>0.5</td>
<td>0</td>
<td>3204±106.3c</td>
<td>1349±69.72d</td>
<td>1.67±0.15c</td>
<td>1.10±0.18c</td>
<td>600.4±7.32bc</td>
<td>17.8±1.72ac</td>
<td>25.83±1.36bc</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
<td>4018±119.2c</td>
<td>4252±21.79bc</td>
<td>2.43±0.23cd</td>
<td>1.23±0.06cd</td>
<td>610.6±0.69ae</td>
<td>11.65±0.73be</td>
<td>25.07±1.47be</td>
</tr>
<tr>
<td>0.5</td>
<td>0.75</td>
<td>4305±93.19bc</td>
<td>4329±25.48c</td>
<td>2.51±0.20c</td>
<td>1.26±0.07c</td>
<td>629.9±2.02bc</td>
<td>11.65±0.14bc</td>
<td>28.6±1.62bc</td>
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<tr>
<td>0</td>
<td>1.0</td>
<td>4418±56.72bc</td>
<td>4378±26.74bc</td>
<td>2.72±0.15c</td>
<td>1.36±0.06bc</td>
<td>653.6±3.01bc</td>
<td>10.6±0.96bc</td>
<td>29.67±2.34bc</td>
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<td>3582±30.19ab</td>
<td>3352±73.59bc</td>
<td>1.66±0.11cd</td>
<td>1.14±0.17ed</td>
<td>614±34.09bc</td>
<td>22.57±2.16bc</td>
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</tr>
<tr>
<td>0.5</td>
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<td>3797±10.73ae</td>
<td>3694±73.64bc</td>
<td>1.61±0.15de</td>
<td>1.19±0.17de</td>
<td>610.6±3.00bc</td>
<td>17.47±2.25bc</td>
<td>28.67±0.14bc</td>
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<td>0</td>
<td>1.0</td>
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<td>4046±182.3bc</td>
<td>2.57±0.22bc</td>
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<td>28.87±0.49bc</td>
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</table>

Means bearing at least one common superscript in a column do not differ significantly (P<0.05).

References