Full Length Research Paper

Ameliorative effect of glucomannan-containing yeast product (Mycosorb) and sodium bentonite on performance and antibody titers against Newcastle disease in broilers during chronic aflatoxicosis

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Accepted 31 October, 2011

The aim of this study was to determine the impact of aflatoxin (AF), yeast glucomannan (YG) and sodium bentonite (SB) on performance and immunization against Newcastle disease (ND) in broiler feed with naturally contaminated diet with aflatoxin. For this experiment, 300 7-day-old Ross 308-strain broiler chickens were chosen and randomly assigned to ten dietary treatments in three replicates of ten chicks. Treatments were: 1) diet free from aflatoxin (positive control group); 2) naturally contaminated diet with aflatoxin (negative control group); 3, 4, 5, 6, 7, 8, 9 and 10) naturally contaminated diet with aflatoxin supplemented with 1.5% SB, 3% SB, 0.05% YG, 0.1% YG, 1.5% SB + 0.05% YG, 1.5% SB + 0.1% YG, 3% SB + 0.05% YG and 3% SB + 0.1% YG, respectively. Chicken in each replicate were weighed weekly and feed intake, weight gain and feed conversion ratio were measured. Blood sample was taken from each bird and the titers of antibody against ND were measured by haemagglutination inhibition test. Aflatoxin in contaminated diet was measured and confirmed by thin layer chromatography (TLC) to be 250 ppb. Compared to the positive control diet, growth parameters were significantly (P<0.05) lower in 250 ppb aflatoxin fed chicks during experiment and also the antibody titer against ND was significantly (P<0.05) lower in the negative control diet from 35 to 42 days of age. The addition of yeast glucomannan and sodium bentonite, individually and in combination to the AF-containing diet ameliorated the adverse effects of aflatoxin, but 0.1% yeast glucomannan supplementation to the contaminated diet with aflatoxin proved to be much more effective in the amelioration of the adverse effect of AF on performance and humeral immunity against ND.

Key word: Aflatoxin, broilers, 0.1% yeast glucomannan, performance, Newcastle disease.

INTRODUCTION

Aflatoxins (AF) are a group of closely related biologically active mycotoxins produced by Aspergillus flavus and Aspergillus parasiticus. They commonly occur as natural contaminants of poultry feeds (Edds and Bortell, 1983). One of the myriad effects of this mycotoxin is the ability to impair the immune system in fowl. Aflatoxin is the best-known mycotoxin for its ability to impair reticulo-endothelial activity (Michael et al., 1973), primary immune response (Thaxton et al., 1974), complementary system (Richard and Thurston, 1973), phagocytic activity of leukocytes and alveolar macrophages (Richard and Thurston, 1973; Chang and Hamilton, 1997). Aflatoxin can also cause vaccine failure against fowl cholera in turkey and chickens (Pier and Heddleston, 1970). The inhibitory effect of aflatoxin on antibody production has also been demonstrated in chicks (Thaxton et al., 1974) and turkeys (Pier and Heddleston, 1970; Thaxton et al.,

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The inhibitory effect was demonstrated against Newcastle disease vaccination in broiler chickens (El-Zanaty et al., 1989), and layer-breeders (Boulton et al., 1980). In chickens, aflatoxin causes a dose-related regression in the size of both the thymus and bursa, which are the primary determinants of immunity (Thaxton et al., 1974; Ibrahim et al., 1998).

The other effects of aflatoxin in poultry including listlessness, anorexia with lowered growth rate, poor feed utilization, decreased weight gain, decreased egg weight and production, increased susceptibility to environmental and microbial stresses, and increased mortality (Leeson et al., 1995; Miazza et al., 2000; Zaghini et al., 2005; Pasha et al., 2007).

Since the beginning of the 1990s, adsorbents-based studies have been performed for removing AF from contaminated feed and minimizing the toxicity of AF in poultry (Ibrahim et al., 2000). Zeolites (Miazza et al., 2000), bentonites (Ogaz and Kurtoglu, 2000) and esterified glucomannan (Aravind et al., 2003) were preferred because of their reducing effect on AF absorption from the gastrointestinal tract. A natural organic product, esterified glucomannan - a cell wall derivative of Saccharomyces cerevisiae, has shown considerable binding ability with several commonly occurring mycotoxins and is also found beneficial as a low-inclusion binder in minimizing the adverse effects of aflatoxins present in contaminated livestock and poultry feeds (Raju and Devegowda, 2000; Aravind et al., 2003; Girish and Devegowda, 2006).

The purpose of this study was to further investigate the ameliorative effect of dietary glucomannan-containing yeast product (Mycosorb) and sodium bentonite on performance and antibody production against Newcastle disease in broiler feed with naturally contaminated diet with aflatoxin.

**MATERIALS AND METHODS**

**Chickens and diet**

A total of 325 one-day old Ross-308-strain male broiler chickens were provided from a commercial broiler producer. Chickens were acclimatized for a 7-day period prior to commencement of the study. During this period, the birds were maintained with conventional broiler chicken management and housed in floor pens in an environmentally controlled broiler house with litter floors. At seven days of age, 300 chicks of similar weights were randomly assigned to 30 clean pens in the same broiler house used for the acclimatization period. They were fed with a commercial feed starter [maize and soybean based; 20.84% crude protein (CP) and 2950.29 metabolizable energy (ME)] up to 21 days and thereafter a grower diet (19.68% CP, 3150 ME) up to 42 days; they had access to feed and water ad libitum from 1 to 42 days of age. In addition, birds were inspected daily and any health related problems were recorded.

The basal diet was supplemented with amino acids, mineral and vitamins at levels recommended by National Research Council (NRC, 1994), without added antibiotics, coccidiostats or growth promoters. Lighting was provided for 23 h/day.

**Experimental design**

The birds were assigned to the following treatment groups (in 3 replicates) of 10 chicks: 1) positive control diet, basal diet without additive (control); 2) negative control diet, diet naturally contaminated with mycotoxins (NCD); 3) NCD supplemented with 1.5% SB; 4) NCD supplemented with 3% SB; 5) NCD supplemented with 0.05% YG; 6) NCD supplemented with 0.1% YG; 7) NCD supplemented with 1.5% SB + 0.05% YG; 8) NCD supplemented with 1.5% SB + 0.1% YG; 9) NCD supplemented with 3% SB + 0.05% YG; and 10) NCD supplemented with 3% SB + 0.1% YG.

**Mycotoxin quantification and diet preparation**

Individual feed ingredients were analyzed and screened for AF content. AF was extracted according to Romer (1975) and was quantified by thin-layer chromatography (TLC). The basal control diet was formulated and compounded to meet the nutritional requirements of commercial broilers (NRC, 1994) during the starter and grower period. The basal diet did not contain detectable levels of AF (< 1 µg/kg diet). The maize obtained from a private feed mill (that was naturally contaminated with mold) was stored in 20% moisture during two months for increase of mold growth. The naturally contaminated maize which had been rejected due to severe mold growth was obtained from a private feed mill. The presence of aflatoxin in the maize was confirmed by TLC. The contaminated diet treatments were formulated by replacing aflatoxin-free maize with naturally contaminated maize. The samples were selected by quartering technique as aliquot of the whole sample. Upon analysis, the contaminated diet contained 250 ppb AF (detection limit: 1 µg/kg diet). The AF composition consisted of 84.72% AFB1, 5.50% AFB2, 8.20% AFG1 and 1.58% AFG2 based on total AF in the contaminated diet. During the experimental period, the control and contaminated diet were analyzed for AF. The found levels of AF in control diet were below the detection limits.

**Performance parameters**

On weekly basis, birds were weighed by pen and total feed consumption recorded for each pen. Average feed consumption (FC) and body weight gain (BW) were corrected for mortality while calculating feed conversion ratio (FCR) for each pen.

**Vaccination and serology**

Before chicks (one-day-old) were separated into groups, blood sample was taken from each bird and the titers of maternal antibody against ND were measured by haemagglutination inhibition (HI) test (Allan and Gough, 1974). All chicks were vaccinated by eye dropper and bivalent killed vaccine (ND plus AI) by inoculation according to the recommendation of the manufacturer. All chicks were vaccinated at day 21 with clone 30 ND vaccine by eye dropper and bivalent killed vaccine (ND plus AI) by inoculation according to the recommendation of the manufacturer. All chicks were vaccinated at day 21 with clone 30 ND vaccine administered in drinking water. Blood samples were collected every week from the wing veins of individual chickens in all groups and their sera were separated and inactivated at 56°C for 30 min and kept at -20°C until analysis for the level of ND antibody (Allan and Gough, 1974).

**Statistical analysis**

The experiment was performed as a completely randomized design.
with 3 replicates of 10 chickens assigned to each of ten dietary treatments. Data were subjected to statistical analysis using the general linear models procedure of statistical analysis software (SAS) (SAS Institute, 1996). The treatment means showing significant differences in the one-way ANOVA were compared using Duncan’s multiple-range test. All statements of significance were based on the 0.05 level of probability.

RESULTS

The effects of dietary treatments on performance are presented in Tables 1 and 2. FCR (kg of feed per kg of gain), FC and BW gain during the experiment were affected by treatments (P<0.05). When compared with treatment 1 and other treatments, the chickens fed with the negative control diet had lower feed consumption, with no statistically significant differences between treatments 1 and 2. During day 7 to 21, chickens fed with treatment 4 diet had higher feed consumption than those receiving treatments 2, 3, 9 and 10 (P<0.05). Treatments 1, 5, 6, 7 and 8 were not different from treatment 4 (P>0.05). Moreover, treatments 4, 6, 8, 7, 5, 3, 9 and 10 increased the FC by 18, 12, 10, 9, 6, 2 and 0.6%, respectively, compared to the treatment 2. During days 22 to 42, treatment 6 had the highest feed consumption (3397.3); there was no statistically significant difference among treatments (P>0.05). There were 7.4, 6.2, 5.8, 5.1, 4.2, 3.1, 2.2 and 1.5% increase in weight gain for treatments 6, 7, 4, 9, 10, 8, 5 and 3, respectively, during day 7 to 42, as compared with the treatment 2.

For day 7 to 21, birds in treatment 2 had significantly lower BW gain (30%) than the positive control group (P<0.05), and also had differences between treatment 2 and treatments 6, 7 and 10. However, treatments 3, 4, 5, 8 and 9 were not different from the negative control group (P>0.05). Treatments 6, 10, 8, 5, 4, 9, 7 and 3 increased the BW gain by 45, 28, 24, 22, 20, 19 and 18%, respectively, compared to the treatment 2. During day 22 to 42, birds in treatment 1 had higher BW gain (14%) than the positive control group. There was no significant difference (P>0.05) among treatments in BW gain; there were 7.4, 6.2, 5.8, 5.1, 4.2, 3.1, 2.2 and 1.5% increase in weight gain for treatments 6, 7, 4, 9, 10, 8, 5 and 3, respectively, as compared to the negative control group. During the experiment at day 7 to 42, birds in treatment 2 had significantly lower BW gain (18%) than the positive control group (P<0.05). There were no differences among treatments 3, 4, 5, 6, 7, 8, 9, 10 and treatment 1. There were 7.4, 6.2, 5.8, 5.1, 4.2, 3.1, 2.2 and 1.5% increase in feed consumption for treatments 6, 7, 4, 9, 10, 8, 5 and 3, respectively, during day 7 to 42, as compared with the treatment 2.

Furthermore, for day 7 to 21, treatment 1 had a better feed conversion ratio (24%) than the negative control
Table 2. Effect of AF-contaminated diet, sodium bentonite (SB) and yeast glucomannan (YG) on BW gain and FCR in broiler chicks fed mycotoxin contaminated feed from 7 to 42 days of age.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 7 to 21</th>
<th>Day 22 to 42</th>
<th>Day 7 to 42</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BW gain (g)</td>
<td>FCR (kg)</td>
<td>BW gain (g)</td>
</tr>
<tr>
<td>1</td>
<td>583.96 ± 20.80^a</td>
<td>1.35 ± 0.04^c</td>
<td>1766.12 ± 50.00</td>
</tr>
<tr>
<td>2</td>
<td>410.58 ± 11.20^c</td>
<td>1.79 ± 0.06^a</td>
<td>1522.00 ± 93.79</td>
</tr>
<tr>
<td>3</td>
<td>458.66 ± 12.35^abc</td>
<td>1.60 ± 0.04^abc</td>
<td>1670.91 ± 114.97</td>
</tr>
<tr>
<td>4</td>
<td>500.00 ± 16.90^bc</td>
<td>1.73 ± 0.04^abc</td>
<td>1657.66 ± 44.24</td>
</tr>
<tr>
<td>5</td>
<td>502.08 ± 21.15^abc</td>
<td>1.60 ± 0.07^abc</td>
<td>1620.00 ± 53.66</td>
</tr>
<tr>
<td>6</td>
<td>595.84 ± 35.90^a</td>
<td>1.39 ± 0.03^c</td>
<td>1797.66 ± 66.57</td>
</tr>
<tr>
<td>7</td>
<td>490.12 ± 111.50^abc</td>
<td>1.64 ± 0.04^abc</td>
<td>1565.33 ± 97.58</td>
</tr>
<tr>
<td>8</td>
<td>509.33 ± 38.95^abc</td>
<td>1.59 ± 0.11^abc</td>
<td>16944.66 ± 39.37</td>
</tr>
<tr>
<td>9</td>
<td>494.00 ± 8.48^abc</td>
<td>1.51 ± 0.08^abc</td>
<td>1723.33 ± 57.11</td>
</tr>
<tr>
<td>10</td>
<td>525.33 ± 41.64</td>
<td>1.41 ± 0.17^abc</td>
<td>1685.00 ± 98.37</td>
</tr>
</tbody>
</table>

Significance: ** NS ** ** **

^a,b,cMeans presented in a column with different superscripts differ significantly (P<0.05). *1) Positive control diet, basal diet without additive (control); 2) negative control diet, diet naturally contaminated with mycotoxins (NCD); 3) NCD supplemented with 1.5% SB; 4) NCD supplemented with 3% SB; 5) NCD supplemented with 0.05% YG; 6) NCD supplemented with 0.1% YG; 7) NCD supplemented with 1.5% SB + 0.05% YG; 8) NCD supplemented with 1.5% SB + 0.1% YG; 9) NCD supplemented with 3% SB + 0.05% YG; and 10) NCD supplemented with 3% SB + 0.1% YG. SB, Sodium bentonite; YG, yeast glucomannan; NS, not significant, ** (P<0.05). FCR, Feed conversion ratio; BW, body weight gain.

Discussion

AFs are important to the poultry industry because of their toxicity and frequency of occurrence in food stuffs (Tessari et al., 2006). The toxicity with AFs in poultry has...
It is reported that adding Na-bentonite and yeast glucomannan to the diet is effective in reducing AF-contaminated diet. However, it is important to consider poor humoral immunity against ND is significantly decreased in the negative control group (NCD) as compared to positive control diet, basal diet without additive (control); 2) negative control diet, diet naturally contaminated with mycotoxins (NCD); 3) NCD supplemented with 1.5% SB; 4) NCD supplemented with 3% SB; 5) NCD supplemented with 0.05% YG; 6) NCD supplemented with 0.1% YG; 7) NCD supplemented with 1.5% SB + 0.05% YG; 8) NCD supplemented with 1.5% SB + 0.1% YG; 9) NCD supplemented with 3% SB + 0.05% YG; and 10) NCD supplemented with 3% SB + 0.1% YG. SB, Sodium bentonite; YG, yeast glucomannan; NS, not significant; ** (P<0.05).

Boden and Jensen (1985) stated that the nutritional deficiency induced by AF could have disrupted the activity of the digestive enzymes and the absorption of essential nutrients. The immunotoxic effects of AF in poultry have been well-documented by other researchers (Michael et al. 1973; Richard and Thurston, 1973; Chang and Hamilton, 1997). Therefore, we particularly aimed to assess the impact of 250 ppb AF, which naturally occurred in field conditions, on antibody production against ND in the present study. The results of this study indicate that the impact of 250 ppb AF, which naturally occurred in field conditions, on antibody production against ND in the present study. The results of this study indicate that the immunotoxic effects of AF with 100 to 250 ppb AF in the diet. It is important to consider poor humoral immunity (HI) in chicks caused by this AF-level (250 ppb) in this group from 7 to 42 days of age (P<0.05). Also, the addition of yeast glucomannan and Na-bentonite were effective on BW gain and the highest result was significantly obtained compared with the negative control group during day 7 to 42 (P<0.05) when yeast glucomannan was added at of 0.1% of feed to the AF-containing diets. In addition, the best FCR was observed in 0.1% yeast glucomannan compared with 1.5% Na-bentonite during the experiment. Higher food intake (7.4%), body weight gain (24%) and better feed conversion ratio (13.7%) were obtained with 0.1% yeast glucomannan compared with 1.5% Na-bentonite during the experiment which showed a better performance than with other treatments during starter and finisher phases. These results were in agreement with previous reports (Raju and Devegowda, 2000; Aravind et al., 2003; Karaman et al., 2005; Girish and Devegowda, 2006; Kamaizadeh et al., 2009).

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study. This level of AF can be found in broiler feed in field conditions without showing significant clinical signs in boilers during the rearing period (Oguz et al., 2000). The immunosuppressive effect of AF has been related to its direct inhibition of protein synthesis (Oguz et al., 2000) including those with specific function such as immunoglobulin G (IgG) and A (IgA), inhibition of migration of microphages (Ibrahim et al., 2000), interference with the haemolytic activity of complement, reduction of number of lymphocytes (Ghosh and Chauhan, 1991), toxic effect on the bursa of Fabricius (Ortatatli and Oguz, 2001) and impairment of cytokines formation by lymphocytes (Gabal and Azzam, 1998). Our study agrees with previous findings by Oguz et al. (2003), who reported that ND titers were significantly lower (P < 0.05) in 100 ppb AF fed chicks compared to the control group (P < 0.05).

It was also observed that 1.5% Na-bentonite and 0.1% yeast glucomannan significantly ameliorated the adverse effect of AF on the humoral immunity against ND at day 42 compared with other treatments (P<0.05). This finding is in agreement with other studies performed by previous researchers (Ibrahim et al., 2000; Raju and Devegowda, 2000; Girish and Devegowda, 2006). The addition of 0.1% yeast glucomannan was more effective in ameliorating the suppressive effect of AF on the HI-titer in chicks vaccinated against Newcastle disease than 1.5% Na-bentonite, and the best result was obtained on day 42 of age (P < 0.05) when yeast glucomannan was added at of 0.1% of feed to the AF-containing diets.

**Conclusion**

The performance and humeral immunity against ND were significantly affected by AF (250 ppb) treatment. The addition of yeast glucomannan and sodium bentonite, individually and in combination to the AF-containing diet ameliorated the adverse effects of aflatoxin, but 0.1% yeast glucomannan supplementation to the contaminated diet with aflatoxin proved to be much more effective in the amelioration of the adverse effect of AF on growth performance and antibody production against ND.

**Abbreviations**

AF, Aflatoxin; YG, yeast glucomannan; SB, sodium bentonite; ND, Newcastle disease; NCD, naturally contaminated with mycotoxins; TLC, thin layer chromatography; HI, haemagglutination inhibition; CP, crude protein; ME, metabolizable energy; feed consumption (FC) and BW, body weight gain; FCR, feed conversion ratio.

**REFERENCES**


