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Effects of MOS and Heat Activated Sodium Bentonite as Aflatoxin Absorbents on Antibody Titers Against Newcastle Disease and Infectious Bursal Disease Viruses in Broiler Chickens

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Abstract: In a completely randomized design, preventive effects of mannan-oligosaccharide (MOS, 0.05 and 0.1% dry matter of diet) and heat activated sodium bentonite (1.5 and 3% dry matter of diet) on aflatoxin detoxification were evaluated and compared. Chicks were fed on different dietary treatments at four replicates from 1 to 42 d of age. Birds were vaccinated against Newcastle disease and infectious bursal disease viruses and sera antibody contents of three birds per replicates measured at different ages. Results of this study clearly indicated that MOS and sodium bentonite addition to diet of broilers effectively diminished the adverse effects of aflatoxin on the investigated titers ($P<0.05$). The lower dietary concentration of heat activated sodium bentonite and the higher concentration of MOS were found more effective than the other concentrations against the adverse effects of aflatoxin on the antibody titers of Newcastle disease and infectious bursal disease ($P<0.05$).

Key words: Aflatoxin · Broilers · Antibody · Newcastle disease · Infectious bursal disease

INTRODUCTION

Aflatoxins (AFs) potent mycotoxins produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, are a major concern in poultry production. AF contamination causes different problems, especially increased susceptibility to environmental and microbial stresses, reduction of immune function and antibody titers [1-3].

Different approaches such as the use of mold inhibitors, fermentation, physical separation, irradiation, ammoniation and ozone degradation were used to prevent food contamination by aflatoxins. Unfortunately, most of these procedures are expensive, time-consuming and only partial effective. Currently, the most promising and practical approach has been the addition of adsorbents (Mycozorb and aluminosilicates) to contaminated feeds to selectively bind the aflatoxin molecule. Adsorbent and the bound aflatoxins cannot be absorbed from the animal's digestive tract, finally excreted in the faeces [2].

So far, the effects of aflatoxin adsorbents have not been evaluated on antibody titers of chickens. The main purpose of this study was to evaluate the effects of sodium bentonite and MOS on antibody titers against Newcastle disease and Infectious bursal disease viruses in chickens fed ration contaminated with aflatoxin.

MATERIALS AND METHODS

Aflatoxin Production: High levels of aflatoxins were produced on rice as a natural substrate by toxigenic *A. parasiticus* according to the method of Shotwell *et al*. [4]. Aflatoxin was extracted and measured in rice powder using high performance liquid chromatography (HPLC) based on the procedure described by Wilson and Romer [5], using Mycosep multifunctional cleanup column. Aflatoxin containing rice powder was mixed with ration to obtain level of 200 ppb.

Birds and Rearing Conditions: Two hundred and forty one day-old male broilers (Ross 308), obtained from a commercial hatchery, were used in this study. Individually weighed chicks were divided at random into six groups. There were four replications of 10 broiler chicks for each dietary treatment. Chicks were housed in floor pen,

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maintained on a 23 h light: 1 h dark cycle at 22-28°C and allowed free access to feed and tap water until they were six weeks old.

Experimental Diet: Basal diet was prepared and formulated to contain National Research Council [6] requirements of all nutrients (ME; 3200 kcal/kg, CP, 20%). The experimental design consisted of six dietary treatments. 1) negative control: basal diet; 2) positive control or AF diet: basal diet plus 200 ppb AF; 3) AF diet containing 1.5% heat activated sodium bentonite; 4) AF containing 3% heat activated sodium bentonite; 5) AF diet containing 0.05% mannan-oligosaccharide and 6) AF diet containing 0.1% mannan-oligosaccharide. In this study, mycotoxin binder or MOS (named in trademark as BioTox) was used that consisted of various silicates combined with extracts of the cell walls of *Saccharomyces cerevisiae*.

Vaccination and Serology: Birds were vaccinated against Newcastle disease and infectious bursal disease. Randomly, blood samples of three birds per replicate were collected at days 21, 28, 35 and 42 of age. Samples were kept at room temperature for two hours, then overnight at 4°C in refrigerator and centrifuged at 1500 × g for 15 min. Serum was inactivated at 56°C for 30 min and stored at -20°C until analyses of antibody titers. The titers of antibody against Newcastle disease virus were measured by haemagglutination-inhibition test [7]. The titers of antibody against infectious bursal disease virus were measured using commercial ELISA kit (IDEXX Lab., CA, USA).

Statistical Analysis: Data were analyzed as a completely randomized design using the General Linear models procedure of SAS Institute [8]. Means for treatments showing significant differences in the analysis of variance were compared using Duncan's multiple range tests. All statements of significance are based on the probability level of 0.05.

RESULTS

The geometric means of antibody titers of chicks against Newcastle and infectious bursal diseases and bursal body weight ratio are in Tables 1 and 2. There was a significant difference for the relative bursal weight between positive control and negative control groups, but not among negative control and other treatments. Serum antibody titers showed progressive changes even in birds fed positive control. For both antibody titers, the highest

Table 1: Geometric mean titer of broiler chicks against Newcastle disease

	Geometric mean titer			
Treatments	21st day	28th day	35th day	42nd day
Negative control	6.69[a]	7.55[a]	8.75[a]	9.25[a]
Positive control	2.91[c]	4.29[d]	4.63[d]	4.79[d]
HASB 1.5%	4.25[b]	6.71[b]	6.75[b]	8.05[b]
HASB 3%	4.09[b]	6.69[b]	5.74[c]	6.77[c]
MOS 0.05%	4.37[b]	6.24[c]	6.13[c]	6.58[c]
MOS 0.1%	3.92[b]	7.35[a]	6.54[b]	6.32[c]
SEM	0.287	0.199	0.228	0.359

HASB, heat activated sodium bentonite; MOS, mannan-oligosaccharide; SEM, standard error of means

Means with the different superscripts within column are differ (P<0.05)

Table 2: Geometric mean titer of broiler chicks against infectious bursal disease

	Geometric mean titer			
Treatments	21st day	28th day	35th day	42nd day
Negative control	365[a]	1039[a]	1800[a]	2390[a]
Positive control	306[c]	567[c]	1569[c]	2189[c]
HASB 1.5%	364[a]	1010[a]	1600[bc]	2250[bc]
HASB 3%	329[b]	689[b]	1589[bc]	2201[c]
MOS 0.05%	350[a]	1019[a]	1620[bc]	2309[ab]
MOS 0.1%	362[a]	1030[a]	1651[b]	2390[a]
SEM	12.3	27.8	40.5	64.4

HASB, heat activated sodium bentonite; MOS, mannan-oligosaccharide; SEM, standard error of means

Means with the different superscripts within column are differ (P<0.05)

(P<0.05) means were observed in negative control group and the lowest (P<0.05) mean were observed in positive control group. Inclusion of heat activated sodium bentonite or mannan-oligosaccharide increased (P<0.05) antibody titers of both Newcastle disease and infectious bursal disease compared to positive control. Birds in groups fed ration supplemented with sodium bentonite 0.05 or 0.1% mannan-oligosaccharide showed the higher (P<0.05) Newcastle titer than other treatments. Those fed ration supplemented with mannan-oligosaccharide showed the highest (P<0.05) infectious bursal titers compared to positive control and sodium bentonite treatments.

DISCUSSION

The results of this study indicate that aflatoxin severely inhibited the immune system of the birds. The aflatoxin causes the regression of the bursa of Fabricius, therefore the low antibody titers against the Newcastle disease and infectious bursal disease may be attributed to the regression of this organ (data not shown). Our results

are in agreement with Virdi *et al*. [9], who reported a significant decrease in antibody response to injected sheep red blood cells and weight losses to the extent of 25-38% in the bursa of Fabricius and thymus in chicks fed an aflatoxin diet for three weeks. Giambrone *et al*. [10] reported a significant decrease in concentrations of serum IgG and IgA in chicks given dietary aflatoxin from hatching to 4 weeks of age. Recently, Tessari *et al*. [11] reported that chicks receiving mycotoxin treated ration had lower geometrical mean antibody titers against Newcastle disease at day 35.

Zaghini *et al*. [12] reported mannan-oligosaccharide could adsorb and degrade aflatoxins, reducing gastrointestinal absorption of aflatoxins and its levels in tissues. Shashidhara and Devegowda [13] reported that antibody responses against infectious bursal disease virus were significantly higher in the group fed diet supplemented with mannan-oligosaccharide. In their study, maternal antibody titers in progeny were also influenced significantly by mannan-oligosaccharide supplementation.

The results of this study are in line with Ibrahim *et al*. [14] who reported that addition of sodium bentonite was significantly effective in ameliorating the negative effect of AF on the percentage and mean of phagocytosis. The presence of AF alone in the diet depressed the immune response of chicks as measured by haemagglutination inhibition (HI) test. Sodium bentonite was also effective in ameliorating the suppressive effect of AF on the HI-titer in chicks vaccinated against Newcastle disease.

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