

## Full Length Research Paper

# Efficacy of esterified glucomannan, sodium bentonite and humic acid to counteract experimental aflatoxicosis on antibody titers against Newcastle disease in broilers

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A study was conducted on the impact of aflatoxin (AF) and sodium bentonite, esterified glucomannan and humic acid, on immunization against Newcastle disease (ND) in broiler feed with naturally contaminated diet with aflatoxin. Seven-day-old chicks were randomly assigned to nine dietary treatments in four replicates of 12 chicks. Treatments were 1) Control; 2) naturally contaminated diet with aflatoxin; 3, 4, 5, 6 and 7) naturally contaminated diet with aflatoxin supplemented with 0.2, 0.4, 0.6, 0.8 and 1.0% humic acid, respectively; 8 and 9) naturally contaminated diet supplemented with 0.5% sodium bentonite and 0.1% esterified glucomannan, respectively. The measured aflatoxin in contaminated diet, confirmed by thin layer chromatography (TLC), was 254 ppb. Blood sample was taken from each bird and the titers of antibody against ND were measured by haemagglutination-inhibition test. Compared to the control diet, the antibody titers against ND was significantly ( $P < 0.01$ ) lower in 254 ppb aflatoxin fed chicks from 28 to 35 days of age. The addition of esterified glucomannan, sodium bentonite and humic acid to the AF-containing diet ameliorated the adverse effects of aflatoxin on ND antibody titers, but humic acid proved to be more effective in the amelioration of the adverse effect of AF on humeral immunity against ND.

**Key words:** Esterified glucomannan, sodium bentonite, humic acid, aflatoxicosis, Newcastle disease.

## INTRODUCTION

Aflatoxin  $\beta_1$ , a metabolite of the fungus *Aspergillus flavus* and *Aspergillus Parasiticus*, is an extremely hepatotoxic compound that frequently contaminates poultry feeds at low levels (Aravind et al., 2003). The United States Food and Drug Administration (FDA) have set regulatory level of 20 ppb AF for poultry feeds (Sklan et al., 2001). One of the myriad effects of mycotoxins is the ability to impair

the immune system in fowl. Aflatoxin (AF) 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 turkeys 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). The inhibitory effect was demonstrated against Newcastle disease (ND) vaccination in broiler chickens (El-zanaty et al., 1989) and layer-breeders (Boulton et al., 1980). The effects of mycotoxins are related to both dose and time of exposure. Almost all studies have examined the effects of

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**Abbreviations:** ND, Newcastle disease; HI, humoral immunity; TLC, thin layer chromatography; HA, humic acid; NCD, naturally contaminated with mycotoxins; SB, sodium-bentonite; E-GM, esterified glucomannan; HI, haemagglutination-inhibition; FH, Farmagulatör DRY<sup>TM</sup> humate.

**Table 1.** Composition of experimental diets (7 - 21day).

Ingredient	Diets								
	1*	2	3	4	5	6	7	8	9
Corn grain	53.64	53.64	53.26	52.88	52.51	52.13	51.64	52.7	53.54
Fish meal	4.58	4.58	4.55	4.52	4.5	4.46	4.45	4.52	4.58
Soybean meal	33.49	33.49	33.57	33.66	33.73	33.81	33.99	33.7	33.49
Salt	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41	0.41
Dicalcium Phosphate	1.09	1.09	1.1	1.1	1.1	1.11	1.11	1.1	1.09
Oyster shell	1	1	1	1	1	1	1	1	1
Corn oil	4.94	4.94	5.06	5.18	5.3	5.42	5.54	5.18	4.94
Vitamin Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
L- Lysine	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
DL- Methionine	0.25	0.25	0.25	0.25	0.25	0.26	0.26	0.25	0.25
FH**	-	-	0.2	0.4	0.6	0.8	1	-	-
SB	-	-	-	-	-	-	-	0.5	-
E-GM	-	-	-	-	-	-	-	-	0.1
Total	100	100	100	100	100	100	100	100	100
<b>Calculated values***</b>									
ME, Kcal/Kg	3150	3150	3150	3150	3150	3150	3150	3150	3150
Crude protein (%)	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5	22.5
Lysine	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35	1.35
Methionine	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.64	0.64
Calcium	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Available Phosphor	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45

1\* = Control; 2 = NCD; 3 = NCD+0.2%FH; 4 = NCD+0.4%FH; 5 = NCD+0.6%FH; 6 = NCD+0.8%FH; 7 = NCD+1.0%FH; 8 = NCD+0.5%SB; 9 = NCD+0.1%E-GM; \*\*FH = % Farmagulator DRY™ humate; SB = sodium bentonite; E-GM = esterified glucomannan; \*\*\*based on NRC (1994) feed composition tables.

doses of 1,000 ppb or higher when fed for periods of up to 3 weeks (Jelinek et al., 1989; Ibrahim et al., 1998). The scenario under practical feeding conditions is prolonging exposure of chicken to lower dietary mycotoxin levels. 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 (Miazzi 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. In recent years, it was reported that humic acid is effective in reducing aflatoxicosis at the inclusion rate of 0.35% in broiler diets (Van Rensburg et al., 2006). Humate or humic acid (HA), a class of compounds formed due to the decomposition of organic matter and particularly plants, is a natural constituent of drinking water. This compound inhibits bacterial and fungal growth and thus decreases levels of mycotoxins in feed (Islam et al., 2005). The purpose of the present study was to further investigate the ameliorative effect of dietary sodium bentonite, esterified glucomannan and humic acid on antibody production against Newcastle disease in broiler feed with naturally contaminated diet

with aflatoxin.

## MATERIALS AND METHODS

### Experimental design, bird and data collection

A total of 500 one-day-old broiler chicks (not sexed) were adapted for a seven day period before commencement of the trial. During this period, the birds were subjected to conventional broiler chicken management and housed in floor pens in an environmentally controlled broiler house with litter floors. They received a commercial broiler starter and grower diets, (similar to the negative control diet in the respective experiments, Table 1, 2) formulated to meet or exceed the nutritional requirements of broilers, as recommended by the NRC (1994). This diet, as well as basal diets used subsequently, was analyzed and tested negative for AF. Lighting was provided for 23 h/d. At seven days of age, 432 chicks of similar weights were randomly assigned to 36 clean pens in the same broiler house used for the adaptation period. The birds were assigned to the following treatment groups (in 4 replicates) of 12 chicks: 1) Basal feed free of mycotoxins (control), 2) diet naturally contaminated with mycotoxins (NCD), 3) NCD supplemented with 0.2% HA, 4) NCD supplemented with 0.4% HA, 5) NCD supplemented with 0.6% HA, 6) NCD supplemented with 0.8 HA, 7) NCD supplemented with 1% HA, 8) NCD supplemented with 0.5% Na-bentonite (SB) and 9) NCD supplemented with 0.1% esterified glucomannan (E-GM). Each kilogram of humic acid contained 160 mg polymeric

**Table 2.** Composition of experimental diets (21-35day).

Ingredient	Diets								
	1*	2	3	4	5	6	7	8	9
Corn grain	56.52	56.52	56.17	55.82	55.47	55.12	54.77	55.62	56.52
Soybean meal	34.43	34.43	34.47	34.5	34.54	34.57	34.61	34.6	34.43
Salt	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34	0.34
Dicalcium Phosphate	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13	1.13
Oyster shell	1.42	1.42	1.42	1.42	1.42	1.42	1.42	1.42	1.42
Corn oil	5.58	5.58	5.69	5.81	5.92	6.04	6.15	5.81	5.58
Vitamin Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL –Methionine	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
FH**	-	-	0.2	0.4	0.6	0.8	1.0	-	-
SB	-	-	-	-	-	-	-	0.5	-
E-GM	-	-	-	-	-	-	-	-	0.1
Total	100	100	100	100	100	100	100	100	100
<b>Calculated values***</b>									
ME, Kcal/Kg	3200	3200	3200	3200	3200	3200	3200	3200	3200
Crude protein (%)	20	20	20	20	20	20	20	20	20
Lysine	1.07	1.07	1.07	1.07	1.07	1.07	1.07	1.07	1.07
Methionine	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
Calcium	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Available Phosphor	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35

1\* = Control; 2 = NCD; 3 = NCD+0.2%FH; 4 = NCD+0.4%FH; 5 = NCD+0.6%FH; 6 = NCD+0.8%FH; 7 = NCD+1.0%FH; 8 = NCD+0.5%SB; 9 = NCD+0.1%EGM; \*\*FH = % Farmagulator DRY™; humate; SB = sodium bentonite; E-GM = esterified glucomannan; \*\*\*based on NRC (1994) feed composition tables.

polyhydroxy acid (humic, fulvic, ulmic and humatomelanic acids), 663.3 mg SiO<sub>2</sub> and other minerals (Mn, 50 mg; Zn, 60 mg; Fe, 60 mg; Cu, 5 mg; Co, 0.2 mg; I, 1 mg; Se, 0.5 mg and Al, Na, K, Mg and P in trace amounts.

### Mycotoxin quantification and diet preparation

Individual feed ingredients and the finisher diet were analyzed and screened for AF content. AF was extracted according to Romer (1975) and was quantified by 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 254 ppb AF (detection limit: 1 µg/kg diet). The AF composition consisted of 78.6% aflatoxin B1 (200ppb), 8% aflatoxin B2, 11% aflatoxin G1 and 2.4% aflatoxin G2 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. Levels of AF in the contaminated diet ranged from 278 to 285 µg/kg.

### Vaccination and serology

Prior to the division of chicks (1-day old) into various 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). At 10 days, all chicks were vaccinated with Hitcher B<sub>1</sub> ND vaccine by eye dropper and bivalent killed vaccine (ND plus AI) by inoculation according to the recommendation of the manufacturer. 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 lasted for 35 days. Data are expressed as mean ± SEM and were evaluated with ANOVA for a complete randomized design, using the general linear models procedure of SAS software (SAS Institute, 1985). The treatment means with significant differences were compared by using Duncan's new multiple range tests.

## RESULTS

The maternal antibody titer was 6.80 ± 0.25. The effects of dietary treatments on antibody production against ND in broilers from day 7 to 35 are presented in Table 3. On the 7th day of the study, there was no difference among

**Table 3.** Effect of AF-contaminated diet, sodium bentonite (SB), esterified glucomannan (E-GM) and Farmagulator DRY™ humate (FH) on ND antibody titers in broiler chicks fed mycotoxin contaminated feed from 7 to 35 days of age.

Treatment				Antibody titers				
AF	SB (%)	E-GM (%)	FH (%)	7th day	14th day	21st day	28th day	35th day
-	-	-	-	5.765 ± 0.23	4.565 ± 0.25	4.620 ± 0.38	6.810 <sup>ab</sup> ± 0.45	6.311 <sup>a</sup> ± 0.22
+	-	-	-	6.051 ± 0.25	4.400 ± 0.42	4.558 ± 0.26	4.975 <sup>c</sup> ± 0.56	4.393 <sup>c</sup> ± 0.33
+	-	-	0.2	5.753 ± 0.37	4.590 ± 0.26	4.528 ± 0.37	5.508 <sup>bc</sup> ± 0.45	4.940 <sup>bc</sup> ± 0.41
+	-	-	0.4	5.888 ± 0.46	4.510 ± 0.36	4.638 ± 0.31	7.400 <sup>a</sup> ± 0.46	5.698 <sup>ab</sup> ± 0.38
+	-	-	0.6	5.633 ± 0.26	4.568 ± 0.42	4.550 ± 0.43	5.455 <sup>bc</sup> ± 0.53	5.500 <sup>ab</sup> ± 0.34
+	-	-	0.8	5.908 ± 0.33	4.645 ± 0.37	5.053 ± 0.46	6.248 <sup>abc</sup> ± 0.34	5.678 <sup>ab</sup> ± 0.47
+	-	-	1.0	5.763 ± 0.35	4.568 ± 0.31	5.048 ± 0.37	6.878 <sup>ab</sup> ± 0.38	6.430 <sup>a</sup> ± 0.32
+	0.5	-	-	5.770 ± 0.27	4.632 ± 0.38	5.043 ± 0.33	6.975 <sup>ab</sup> ± 0.45	5.216 <sup>bc</sup> ± 0.38
+	-	0.1	-	6.193 ± 0.33	4.653 ± 0.28	4.808 ± 0.46	5.998 <sup>abc</sup> ± 0.59	5.280 <sup>bc</sup> ± 0.50

AF = Aflatoxin; SB = sodium bentonite; E-GM = esterified glucomannan; FH = Farmagulator DRY™ humate; <sup>a-c</sup> values within a column with no common superscript differ significantly ( $P < 0.01$ ); each value represents the mean ± SEM of 4 replicates with 12 birds per replicate.

antibody titers of experimental groups. The feeding of AFB<sub>1</sub> at a level of 200 ppb in the ration, reduced the antibody production against ND in broilers from 28 to 35 days of age ( $p < 0.01$ ). The addition of Na-bentonite to the AF-containing diet ameliorated the adverse effect of AF on antibody production ( $p < 0.01$ ). Addition of 0.4, 0.6, 0.8 and 1.0% of Farmagulator DRY™ humate (FH) to the AF-containing diet, significantly ameliorated the adverse effect of AF on antibody production against ND in broiler from 28 to 35 days of age ( $p < 0.01$ ). Moreover, the addition of FH (0.2%) to the AF-containing diet did not affect the investigated value at 35th day ( $P > 0.05$ ). E-GM supplementation of the contaminated diet did not diminish the effect of AF on the antibody titers; however, an increase in antibody titers against ND was observed compared to the NCD alone (Group 2 at 28 and 35 days of age).

## DISCUSSION

Ingestion of aflatoxin contaminated feed significantly decreased the antibody titers in chickens immunized against ND compared to the control ( $P < 0.01$ ). This was similar to the results in other studies (Ghosh and Chauhan, 1991; Hegazy et al., 1991; Gabal and Azzam, 1998) showing the immunotoxic effects of AF with 100 to 2500 ppb AF in the diet. It is important to consider poor humoral immunity (HI) in chicks caused by this AF-level (200 ppb) in the present 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) through its 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, while no significant differences were seen in 50 ppb AF group compared to the control group ( $P < 0.05$ ). It is clear from the result of the HI test that feeding AF caused a depressed response of the experimental chicks to ND vaccine, as indicated from the lower ND antibody titers obtained from chicks fed AF-contaminated diet alone.

It is reported that using Na-bentonite up to 0.6% to the diet is effective in reducing aflatoxicosis in chicken (Araba and Wyatt, 1991, Dale and Wyatt, 1995, Ibrahim et al., 1998). In the present study the addition of Na-bentonite was effective in ameliorating the negative effect of AF on antibody production against ND. This effect could be attributed to the role of Na-bentonite as a sequestering agent against AF present in the diet through reducing its bioavailability in the gastrointestinal tract (Araba and Wyatt, 1991). Our study agree with previous findings by Ibrahim et al. (2000), who reported that the presence of AF in the diet depressed the immune response of chicks as measured by HI test. They have also reported that Na-bentonite is effective in ameliorating the suppressive effect of AF on the HI-titer in chicks vaccinated against Newcastle disease and the best result was obtained when Na-bentonite was added at a rate of 0.4% of feed to the AF-containing diets (Ibrahim et al., 2000). Present findings show that humic acid significantly ( $P < 0.01$ ) ameliorated the adverse effect of AF on the humoral immunity against ND. Humic acid has been known to bind to the AF molecules in gastrointestinal tract and precluding their absorption that can alleviate the toxicity of AF in poultry (Van Rensburg et al., 2006). In this study, humic acid proved to be more effective in the amelioration

of aflatoxicosis in broiler than the E-GM. Van Rensburg et al. (2006) showed that humic acid, but not E-GM could alleviate some of the toxic effects of aflatoxin in growing broiler. According to Van Rensburg, humic acid was able to adsorb (*in vitro*) about 10.3, 7.4 and 11.9 mg of AFB<sub>1</sub> /g of oxihumate at pH 3, 5 and 7. These results clearly demonstrate that 254 ppb AF-treatment significantly affects the HI against ND and simultaneous addition of Na-bentonite (0.5%) and humic acid (0.4%) to the AF-containing diet provide significant reduction to the immunotoxic effects of AF.

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