

# Aflatoxicosis in Broilers: Efficacy of a Commercial Mycotoxin Binder on Performance and Immunity Parameters

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**Abstract** Experimental mycotoxicosis was induced into broiler chickens by feeding 0.6ppm aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) and 0.2% commercial mycotoxin binder (BINDER) from 0 to 42 days of age using 240 day old chicks to evaluate the body weight, feed consumption, FCR and antibody response against ND, IB and AI. AF fed birds showed a clear indication of aflatoxicosis by reduces in body weight, feed consumption and increase in FCR almost at all weeks. The antibody titers are also affected by incorporation of AF into the diet at 42 days of age. The addition of binder could significantly alter the adverse effects of AF and in absence of AF, when binder alone was fed to chicks; the better performance was recorded, when compared with control group. The study revealed that AF and binder in combination could act cumulatively and adversely affect the health of broiler chicken.

**Keywords** Aflatoxin B<sub>1</sub>, Mycotoxin binder, Body weight, Feed consumption, FCR, Antibody titers, Broilers

## 1. Introduction

The poultry industry has gained much interest as an important economic activity across the globe. Mycotoxins are the toxic metabolites produced by certain fungi. They are always a hazard to man and domestic animals and had come to public interest since the past 30 years. Among them, Aflatoxins are the most dangerous toxin produced by *Aspergillus flavus* and *Aspergillus parasiticus*, species of fungi on foods and feeds. The disease which aflatoxin causes is called aflatoxicosis. The factors influencing occurrence of aflatoxins are certain environmental conditions; hence the extent of contamination will vary with geographic location, farming methods and the susceptibility of commodities to fungal invasion during pre-harvest, storage, and processing periods (Wan *et al.*, 2013; Manafi and Khosravinia, 2013). Many countries have attempted to limit exposure to aflatoxins by imposing regulatory limits on commodities intended for use as food and feed. The mycotoxins are known to have strong hepatotoxic and carcinogenic effects and are regulated by feed/food law in at least 100 countries. Numerous reports on effects of aflatoxins on bird performance and serum chemistry have been previously reviewed by many scientists (fig. 1). There is a general agreement that dietary aflatoxins reduce weight gain, feed

intake, and increase feed conversion ratio. A study by Dersjant-Li *et al.* (2003) reported that each ppm of AFB<sub>1</sub> in diet would decrease the growth performance of broilers by 5%. However, the data presented in last decade is not consistent with this general term. For instance, Raju and Devegowda (2002) reported a 21% decrease in body weight of broilers fed 300ppb AFB<sub>1</sub> in their diet. Contrary to this, Tedesco *et al.* (2004) noted a reduction at the rate of only 10% in weight gain of broilers at 28 days fed 0.8ppm AFB<sub>1</sub>. In higher levels of 3ppm AFB<sub>1</sub>, only 11% reduction in final body weight was reported by Valdivia *et al.* (2001). From all these reports, it is obvious that both the level and length of AFB<sub>1</sub> exposure affect the amount of reduction in weight gain of broilers. Next to this, effects on liver, the immunosuppressive nature of AFB<sub>1</sub> is the best recognized area of its toxicity. Recent data indicates high correlation between outbreaks of Newcastle disease (ND) and aflatoxin contamination in broilers (Yunus *et al.*, 2008). Commonly, the immune-toxic dose of AFB<sub>1</sub> is considered as less than the dose required provoking a reduction in bird performance. The edge dose of AFB<sub>1</sub> may be generalized to be 0.4 and 1mg/kg for the negative effects on cell mediated and humoral immunity, respectively (Galvano *et al.*, 2005).

In order to avoid mycotoxicosis, several strategies have been investigated which can be divided into pre and post-harvest technologies and biological, chemical and physical methods. However, these strategies are of limited opportunity because of their range of activity and have not gained much importance. Therefore, there has been increasing interest to replace synthetic preservatives with

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natural, effective and nontoxic compounds. Those are extracts and essential oils (EOs) of spices and herbs (Newmana and Cragg, 2007). As natural foodstuffs, spices and herbs are considered as a safe synthetic food additives. Several reports have been published on the antimicrobial activities of plant extracts against different types of microbes, including foodborne pathogens (Gurib-Fakim, 2006; Aggarwal *et al.*, 2003). It has been reported that spices owe their antimicrobial properties mostly to the presence of alkaloids, phenols, glycosides, steroids, essential oils, coumarins and tannins (Shen and Ji, 2007). As reviewed by López-Malo *et al.* (2006), some of antimicrobial components that have been identified in spices and herbs are eugenol from cloves, thymol from thyme and oregano, carvacrol from oregano, vanillin from vanilla, allicin (an organosulfur compound obtained from garlic), cinnamic aldehyde from cinnamon, allyl isothiocyanate (AITC) from mustard, etc. (Matur *et al.*, 2010).

Turmeric (*Curcuma longa*) is a plant of open forests with a wide range of positive effects. Turmeric is a spice that comes from the root of *Curcuma longa*, a member of the ginger family, Zingiberaceae. In traditional medicine, turmeric has been used for its medicinal properties for various indications and through different routes of administration, including topically, orally, and by inhalation. Turmeric, also known as curcuma, produces a root that is used to produce the vibrant yellow spice used as a culinary spice so often used in curry dishes.

Scientists have established a regular diet including spices such as turmeric reverses damage caused by aflatoxin (produced by a fungus) poisoning and its complications.

Indeed, spices have been shown to protect the liver against aflatoxin poisoning. It has been found that food additives such as turmeric and its active ingredient curcumin (diferuloylmethane), asafoetida (flavoring agent), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and ellagic acid inhibited the mutagenesis induced by aflatoxin B<sub>1</sub> (Li *et al.*, 2012).

With increased mycotoxin concentrations in feedstuffs, inclusion of binders in diets will increase. Some classes of mycotoxin sequestering agents may render some minerals and vitamins unavailable for absorption and metabolism. Therefore, providing a highly bioavailable source of trace minerals in a complex form is further warranted.

Diatomaceous earth, a clay mineral of the smectite group is formed of highly colloidal. Clays, composed of mainly montmorillonite, and is produced by *in situ* diversification of volcanic ash (Parker and McGraw-Hill, 1988). It may also contain feldspar, biotite, kaolinite, illite, cristobalite, pyroxene, zircon and crystalline quartz (Parker and McGraw-Hill, 1988). It has the unique characteristic of swelling to several times its original volume when placed in water and of forming thixotropic gels with water even though the amount of clay is relatively less.

Eraslan *et al.* (2005) reported a moderate increase in the albumin: globulin ratio of broilers by addition of 0.3 per cent hydrated sodium bentonite in aflatoxin mixed feed of

broilers. They also reported that histo-pathological finding in liver sections of broiler fed aflatoxin plus hydrated sodium bentonite indicated a non-protective effect of this adsorbent. Due to their montmorillonite content, bentonites swell and form thixotropic gels, as result of their ion exchange capabilities, they are widely used as mycotoxin sequestering agent (Duarte and Smith, 2005). Eraslan *et al.* (2006) reported the effectiveness of sodium bentonite in relieving the damages due to the presence of aflatoxins (1ppm) in 45 day old broiler chickens.

The aims of the current study were to determine the activity of the commercial mycotoxin binder containing curcuminoid and minerals and to evaluate the protective effects of the binder on performance and immune status of broilers fed with Aflatoxin B<sub>1</sub>.

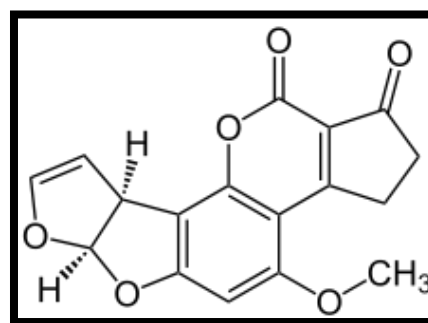


Figure 1. Chemical structure of aflatoxin B<sub>1</sub>

## 2. Materials and Methods

This experiment was planned and carried out in the Department of Animal Science, Faculty of Agricultural Sciences, Malayer University, Malayer, Iran with objective of evaluating the performance and immune response of broilers fed with aflatoxin B<sub>1</sub> and a mycotoxin binder.

### 2.1. Experimental Design, Housing, Management and Test Diet

240 day-old unsexed Ross 308 strain of broiler chicks were wing banded, weighed and randomly spread in a completely randomized experimental design with four treatments and three replications of twenty chicks in each. Each replicate group of chicks was housed in an independent pen, conventional deep litter house. Chicks in all the replicates were kept up to six weeks of age under uniform standard conditions. Brooding was done till three weeks of age. Each pen was fitted with an automatic bell type drinker and a hanging tubular feeder. Chicks were provided *ad libitum* feed and water throughout the study. Feeding of test diets commenced at first day of age and continued till the termination of experiment at six weeks of age. The temperature was maintained at 30±1°C in the first week and reduced by 2.5°C per week to 21°C. From day one until day 4, the lighting schedule was 24 hour. At days 14-42 the dark time was gradually increased to 4 hour. Diets were prepared to meet the nutrient requirements of commercial broilers during the starter (0-2 weeks), grower (2-4 weeks)

and finisher (4-6 weeks) periods. The composition of diets was adopted from NRC, (1994) and is presented in Table 1. The basal diet was formulated using commonly available feed ingredients which were screened for AF prior to the formulation of diets and it is found to be around 280ppb in normal feed. The Aflatoxin B<sub>1</sub> was procured from Sigma Aldrich, USA and diluted to reach to the required level of administration. The experimental diets were prepared by adding required quantity of aflatoxin to arrive at the level of 600ppb of AFB<sub>1</sub>. Diets were prepared without addition of aflatoxin and binder as Control (group 1); 600 ppb Aflatoxin B<sub>1</sub> (group 2); 0.2% of binder (group 3) and 600ppb Aflatoxin B<sub>1</sub> + 0.2% of binder (group 4). Nilttox, the mycotoxin binder used in this study is a unique composition of minerals (extra purified clay containing diatomaceous earth mineral), antioxidants (Curcuminoids extracted from Turmeric) and enzymes (Epoxidase and Esterase), a property product of Zeus Biotech Limited, Mysore, India. It is claimed that incorporation of this product in poultry diets would effectively prevent DNA adduct formation and cellular damages in the biological systems through degrading peroxides, amides and lacto rings in non-polar toxins such as aflatoxins. This study was undertaken to evaluate the efficacy of a mycotoxin binder for counteracting AFB<sub>1</sub> in experimentally contaminated broiler breeder diets.

**Table 1.** Ingredients and composition of the basal diets (as-fed basis, %)

Ingredients (%)	Starting diet (0-2wk)	Growing diet (2-4wk)	Finishing diet (4-6wk)
Corn	59.00	67.36	72.01
Soybean meal	33.74	28.63	24.46
Soybean oil	1.56	0.65	0.56
Calcium carbonate	0.60	0.67	0.63
Dicalcium phosphate	1.41	1.02	0.84
Oyster shell	0.66	0.66	0.63
Common salt	0.30	0.30	0.30
Vit. And Min. Premix <sup>1</sup>	0.50	0.50	0.50
DL-Methionine	0.13	0.06	0.02
Lysine – HCL	0.09	0.14	0.05
Calculated analysis			
ME (Kcal/kg)	2900	2950	3000
Crude protein (%)	20.84	18.43	16.87

<sup>1</sup>The vitamin and mineral premix provide the following quantities per kilogram of diet: vitamin A, 10,000 IU (all-trans-retinal); Vit. D3 (cholecalciferol), 2,000 IU; vitamin E, 20 IU ( $\alpha$ -tocopherol); vitamin K3, 3.0 mg; riboflavin, 18.0 mg; niacin, 50 mg; D-calcium pantothenic acid, 24 mg; choline chloride, 450 mg; vitamin B12, 0.02 mg; folic acid, 3.0 mg; manganese, 110 mg; zinc, 100 mg; iron, 60 mg; copper, 10 mg; iodine, 100 mg; selenium, 0.2 mg and antioxidant, 250 mg.

## 2.2. Vaccination Schedule

The local office of Iranian Veterinary Organization has proposed the required vaccination which is modulated by the

veterinarian of Department of Animal Science, Malayer University, as below:

vaccination for Newcastle Disease (ND) virus happened three times: first spray at one days old of chicken in breeder farm, second on the 13<sup>th</sup> day as B<sub>1</sub>, BRONHOPEST B<sub>1</sub> SPF (VETERINA<sup>®</sup>, Zagreb, Croatia) and (CEVA<sup>®</sup>, Libourne, France) in drinking water and their booster on 20<sup>th</sup> day as clone-30 (HIPRAVIAR<sup>®</sup> CLON, Amer, Spain) through drinking water. Vaccination against Bronchitis virus happened in two times as the following: first spray at commencement of the experiment and it's booster in drinking water on the 10<sup>th</sup> day, both as H-120 (CEVA<sup>®</sup>, Libourne, France). Vaccination against Infectious Bronchitis (IB) virus happened in two times: first on day 16 and the second on the 23<sup>th</sup> day, both as Gambo-1 (CEVA<sup>®</sup>, Libourne, France) in drinking water. The sera were applied to HI test in 28 days, to determine Ab to NDV. In titers lower than 5, the booster B<sub>1</sub>, BRONHOPEST B<sub>1</sub> SPF (VETERINA<sup>®</sup>, Zagreb, Croatia) was administrated in drinking water for broilers.

## 2.3. Studied Parameters

### Performance parameters

Body weight and cumulative feed consumption were recorded and feed conversion ratio (FCR) were calculated week wise. All chickens were weighed individually at the end of each week till week VI, by digital electronic top pan balance with 0.01g accuracy to record body weight. Feed consumption was recorded replicate-wise each week in all pens till 6 weeks of age and feed consumption per bird was calculated. Weekly FCR was calculated up to 6 weeks, as feed consumed per unit body weight gain.

### 2.4. Immunity Parameters

At the end of the trials, upon obtaining the permission of Ethical Committee of the University, six birds from each replicate were sacrificed by cutting the jugular vein and blood samples were individually collected in 10-mL heparinized tubes and stored on ice for hematology analysis. Blood was centrifuged @4000 rpm for 10 min and serum separated after 8 to 10 hours as per the standard procedures (Calnek *et al.*, 1992) and was stored at -20 °C for subsequent analysis. The individual serum samples were analyzed for antibody titers against Newcastle disease (ND), Infectious Bronchitis (IB) and Avian Influenza (AI) by ELISA technique (bio check<sup>®</sup>) using an automatic analyzer (Boehringer Mannheim Hitachi 704 automatic analyzer, Japan). Treatment-wise means of titers were computed.

## 2.5. Statistical Analysis

The total experimental data were statistically analyzed using the General Linear Model procedure of the Statistical Analysis System (SAS<sup>®</sup>) software (SAS Institute, USA, 2000). Overall data were analyzed using one way ANOVA test. Duncan multiple comparisons range test with 0.05 significance level was employed for comparison of the means (Duncan, 1955).

### 3. Results and Discussion

The effects of aflatoxin and mycotoxin binder on broilers have been shown in Table 2. It is found that, at the time of initiation of trial, all chicks were in almost similar range and uniform. At the end of first week, there was a significant reduction in body weight of broilers fed AF. In group fed binder, the body weight has shown no significant changes, when compared with control group. In AF+BINDER group, adverse effects of aflatoxin which was seen in AF group, could not significantly ( $P<0.05$ ) alleviated. At day 14, the AF fed group had significantly ( $P<0.05$ ) lower body weight, compared with control group. The binder fed group has shown the best body weight among all treatments and addition of binder to AF could significantly ( $P<0.05$ ) increase the body weight of broilers. At the end of 21 days, the AF fed group brought down the body weight significantly ( $P<0.05$ ) up to 69 grams and addition of binder could increase the body weight for 22 grams. The maximum BW seen in group fed binder alone. At day 28 of trial, the gap between the AF and control group has been increased and addition of binder could significantly ( $P<0.05$ ) restore the BW of broilers. At the end of week 6, the final BW of broilers fed AF was found to be 2052g and compared with that of control group (2207g), there was a significant decrease in AF fed groups. Addition of toxin binder to AF contaminated diet had significantly ( $P<0.05$ ) increased the BW of broilers at 42 days and reached much closed to control group. The binder alone fed group has shown the

highest BW (2275g) which stands for the best BW among all treatment groups.

The effects of cumulative feed consumption of broilers fed different dietary treatments are shown in Table 3. At the end of first week, the feed intake was significantly ( $P<0.05$ ) increased in AF fed group and binder alone could restore partially the adverse effects of AF. At day 14, the AF fed group showed a higher feed consumption and addition of binder to the AF fed group could significantly ( $P<0.05$ ) lower this parameter. The feed intake of broilers fed AF+BINDER was the minimum in this week. At the end of third and fourth week, the same trend was followed and the maximum feed consumption was found in AF group. The addition of binder in to AF group could significantly ( $P<0.05$ ) lower the consumption, however, feed intake the binder alone group was found to be significantly ( $P<0.05$ ) lesser than control group. At the end of week 5, the trend is changed. The feed consumption was increased by addition of AF into the diet, when compared with control group, however addition of binder could not restore the adverse effects of AF only in this week and binder alone group also had shown increased consumption, when compared with control group. At day 42, the feed consumption was decreased due to AF addition and addition of binder could increase its consumption. In this week, in binder alone group, the consumption was significantly ( $P<0.05$ ) lesser than control group.

**Table 2.** Body weight (g) of chicks fed *Aflatoxin B<sub>1</sub>* and *Mycotoxin Binder* (Mean±SE)

Treatment groups	Week 0 (day 1)	Week I (day 7)	Week II (day 14)	Week III (day 21)	Week IV (day 28)	Week V (day 35)	Week VI (day 42)
CON <sup>1</sup>	45.10±0.25 <sup>a</sup>	202.93±0.77 <sup>a</sup>	428.06±0.35 <sup>b</sup>	766.56±0.85 <sup>b</sup>	1284.43±0.49 <sup>b</sup>	1775.26±0.72 <sup>b</sup>	2207.93±0.53 <sup>b</sup>
AF <sup>2</sup>	45.02±0.76 <sup>a</sup>	184.10±0.96 <sup>b</sup>	387.20±0.39 <sup>d</sup>	707.10±0.46 <sup>d</sup>	1217.80±0.37 <sup>d</sup>	1643.10±0.23 <sup>d</sup>	2052.60±0.18 <sup>d</sup>
BINDER <sup>3</sup>	45.60±0.96 <sup>a</sup>	201.75±0.77 <sup>a</sup>	440.20±0.04 <sup>a</sup>	807.01±0.39 <sup>a</sup>	1311.65±0.75 <sup>a</sup>	1808.54±0.71 <sup>a</sup>	2275.49±0.94 <sup>a</sup>
AF+BINDER <sup>4</sup>	44.28±0.12 <sup>a</sup>	186.93±0.37 <sup>b</sup>	402.24±0.37 <sup>c</sup>	729.73±0.73 <sup>c</sup>	1228.71±0.27 <sup>c</sup>	1710.55±0.31 <sup>c</sup>	2163.67±0.71 <sup>c</sup>

Mean values within a row with different superscript letters (a to d) were significantly different ( $P<0.05$ ). <sup>1</sup>CON (Control), <sup>2</sup>AF (Aflatoxin B<sub>1</sub> at 600ppb level), <sup>3</sup>BINDER (mycotoxin binder at 0.2% level) and <sup>4</sup>AF+BINDER (Aflatoxin B<sub>1</sub> and mycotoxin binder at 600ppb and 0.2% levels, respectively). SEM: Standard Error of the Means

**Table 3.** Cumulative Feed Consumption (g/bird) of chicks fed *Aflatoxin B<sub>1</sub>* and *Mycotoxin Binder* (Mean±SE)

Treatment groups	Week I (day 7)	Week II (day 14)	Week III (day 21)	Week IV (day 28)	Week V (day 35)	Week VI (day 42)
CON <sup>1</sup>	167.53±0.96 <sup>d</sup>	516.70±0.33 <sup>c</sup>	1148.93±0.48 <sup>c</sup>	2041.06±0.92 <sup>b</sup>	3062.32±0.38 <sup>d</sup>	4345.25±0.49 <sup>a</sup>
AF <sup>2</sup>	185.05±0.84 <sup>a</sup>	535.00±0.19 <sup>a</sup>	1226.11±0.29 <sup>a</sup>	2139.67±0.17 <sup>a</sup>	3164.61±0.23 <sup>b</sup>	4127.77±0.31 <sup>d</sup>
BINDER <sup>3</sup>	182.18±0.26 <sup>b</sup>	529.61±0.28 <sup>b</sup>	1187.21±0.38 <sup>b</sup>	1935.99±0.93 <sup>d</sup>	3106.56±0.22 <sup>c</sup>	4300.67±0.27 <sup>b</sup>
AF+BINDER <sup>4</sup>	170.00±0.95 <sup>c</sup>	486.71±0.65 <sup>d</sup>	1180.70±0.74 <sup>b</sup>	2006.48±0.19 <sup>c</sup>	3226.09±0.08 <sup>a</sup>	4221.32±0.37 <sup>c</sup>

Mean values within a row with different superscript letters (a to d) were significantly different ( $P<0.05$ ). <sup>1</sup>CON (Control), <sup>2</sup>AF (Aflatoxin B<sub>1</sub> at 600ppb level), <sup>3</sup>BINDER (mycotoxin binder at 0.2% level) and <sup>4</sup>AF+BINDER (Aflatoxin B<sub>1</sub> and mycotoxin binder at 600ppb and 0.2% levels, respectively). SEM: Standard Error of the Means

**Table 4.** Feed conversion ratio (FCR) of chicks fed *Aflatoxin B<sub>1</sub>* and *Mycotoxin Binder* (Mean±SE)

Treatment groups	Week I (day 7)	Week II (day 14)	Week III (day 21)	Week IV (day 28)	Week V (day 35)	Week VI (day 42)
CON <sup>1</sup>	0.825±0.08 <sup>d</sup>	1.207±0.02 <sup>bc</sup>	1.498±0.12 <sup>c</sup>	1.589±0.18 <sup>c</sup>	1.724±0.01 <sup>c</sup>	1.968±0.18 <sup>b</sup>
AF <sup>2</sup>	1.005±0.27 <sup>a</sup>	1.381±0.28 <sup>a</sup>	1.734±0.28 <sup>a</sup>	1.757±0.22 <sup>a</sup>	1.926±0.29 <sup>a</sup>	2.010±0.72 <sup>a</sup>
BINDER <sup>3</sup>	0.903±0.12 <sup>c</sup>	1.203±0.14 <sup>c</sup>	1.471±0.34 <sup>d</sup>	1.476±0.35 <sup>d</sup>	1.717±0.49 <sup>c</sup>	1.890±0.11 <sup>d</sup>
AF+BINDER <sup>4</sup>	0.954±0.21 <sup>b</sup>	1.210±0.29 <sup>b</sup>	1.618±0.28 <sup>b</sup>	1.633±0.15 <sup>b</sup>	1.886±0.13 <sup>b</sup>	1.951±0.24 <sup>c</sup>

Mean values within a row with different superscript letters (a to d) were significantly different ( $P<0.05$ ). <sup>1</sup>CON (Control), <sup>2</sup>AF (Aflatoxin B<sub>1</sub> at 600ppb level), <sup>3</sup>BINDER (mycotoxin binder at 0.2% level) and <sup>4</sup>AF+BINDER (Aflatoxin B<sub>1</sub> and mycotoxin binder at 600ppb and 0.2% levels, respectively). SEM: Standard Error of the Means

**Table 5.** Antibody titers of broilers fed *Aflatoxin B<sub>1</sub>* and *Mycotoxin Binder* at 42 days (Mean±SE)

Treatment groups	ND	IB	AI
CON <sup>1</sup>	3.66±0.62 <sup>c</sup>	8105.45±0.82 <sup>c</sup>	3.00±0.63 <sup>c</sup>
AF <sup>2</sup>	4.27±0.44 <sup>b</sup>	10297.16±0.21 <sup>b</sup>	4.28±1.05 <sup>b</sup>
BINDER <sup>3</sup>	5.05±0.37 <sup>ab</sup>	8100.92±0.34 <sup>c</sup>	4.92±0.29 <sup>a</sup>
AF+BINDER <sup>4</sup>	5.37±0.64 <sup>a</sup>	16058.17±0.92 <sup>a</sup>	4.31±0.69 <sup>b</sup>

Mean values within a row with different superscript letters (a to d) were significantly different ( $P<0.05$ ).

<sup>1</sup>CON (Control), <sup>2</sup>AF (Aflatoxin B<sub>1</sub> at 600ppb level), <sup>3</sup>BINDER (mycotoxin binder at 0.2% level) and <sup>4</sup>AF+BINDER (Aflatoxin B<sub>1</sub> and mycotoxin binder at 600ppb and 0.2% levels, respectively). SEM: Standard Error of the Means. ND: Newcastle disease; IB: Infectious Bronchitis and AI: Avian Influenza. SEM: Standard Error of the Means

The effects of aflatoxin, binder and combination of AF+BINDER along with control group on FCR parameter of broilers was shown in Table 4. Results showed that at the end of first week, FCR was the maximum in AF group and significantly ( $P<0.05$ ) higher, compared with control. Addition of binder to AF, could significantly ( $P<0.05$ ) improve FCR. The binder alone had shown a good FCR in broilers compared with AF fed group. At day 21, the higher FCR values were found in AF group, followed by AF+BINDER, BINDER and control groups. The same trend is followed in days 28 and 35 and at the end of trial, the FCR in AF fed group was found to be 2.010 and was significantly ( $P<0.05$ ) higher than control group (1.968). Addition of binder to AF group could reduce the FCR values significantly ( $P<0.05$ ) (1.951). The binder alone group showed 1.890 FCR values and was significantly ( $P<0.05$ ) better, when compared with control group.

The data on immunity of broilers fed aflatoxin and mycotoxin binder are shown in Table 5. Results at 42 days showed that the antibody titers against Newcastle Disease had significantly ( $P<0.05$ ) increased in AF group. Inclusion of binder alone and in combination with AF had increased the ND values significantly ( $P<0.05$ ), when compared with control group. In case of Infectious Bronchitis, the titer was increased significantly ( $P<0.05$ ) in AF group and addition of binder into AF group had more increased the value. The binder alone group showed no significant changes, when compared with control group. For Avian Influenza, the value was increased in AF, BINDER and AF+BINDER groups, when compared with control group.

Aflatoxin B<sub>1</sub> is widely believed to result in mal-absorption

syndrome regarding macro nutrients and also in reduced activity of digestive enzymes (Devegowda and Murthy, 2005). It also is famous as hepatotoxic and it might result in more thoughtful negative effects in poultry with more effective nutrient conversion demanding faster hepatic metabolism. However, a clear difference in the susceptibility between the different poultry has been already predicted to be due to differences in metabolic rate of these bird types and sensitivity of analytical methods available at the time of previous and present studies.

Previously, it was believed that the dosage above 1.25 ppm AFB<sub>1</sub> in diet has negative impact on growth performance; however, recent literature revealed that administration of lower dosage (0.02 mg/kg diet) of AF in the diet can show their harmful effects. The justification for these differences in earlier and recent reports could be the difference in the performance of broilers available at the time of study. New generations of broiler is known to gain more weight by utilizing less feed in shorter time. The dose of AF used in this study (0.6ppm) had shown a direct negative impact on broilers body weight, feed intake and FCR directly. This is mainly due to this fact that generally, the immune-toxic dose of AFB<sub>1</sub> is considered as less than the dose required eliciting a reduction in bird performance.

Decreased feed intake during mycotoxicosis has been reported earlier by Hoehler and Marquardt (1996) for T-2 and Kubena *et al.* (1997), Raju and Devegowda (2005) and Girish and Devegowda (2004), for combined mycotoxicosis.

Significant reduction in feed efficiency with AF was reported earlier by several workers (Kubena *et al.*, 1998; Raju and Devegowda, 2000; Girish and Devegowda, 2004).

In agreement to the observations recorded in this trial, significant reduction in feed efficiency was reported earlier (Hoehler and Marquardt, 1996; Raju and Devegowda, 2002; Arvind *et al.*, 2003).

The responses on antibody of birds fed AF (humoral immunity) are clearly indicated in current study. The enquiry about immune-toxicity sensitivity of new generations of commercial broilers remains unanswered. Furthermore, there is proof about biphasic nature of AFB<sub>1</sub> on humoral immunity. It is worthy to mention that humoral immune feedback of birds might rise and decline depends upon the dose and duration of aflatoxin exposure (Manafi *et al.*, 2012).

The increase in titers against ND, IB and AI on AF fed broilers of current trial might be based on mechanisms for temporary increase in humoral immune response which are not yet fully understood.

To tell the truth, the precise mechanisms of even immunosuppression during aflatoxicosis are not clearly understood despite more than 50 years of research on the field of toxins. However, it is believed that the susceptibility of the immune system to mycotoxin immunosuppression comes from the vulnerability of the continually proliferating and differentiating cells that subsidize in immune-mediated activities and regulate the multifaceted communication chain between cellular and humoral components. This immunosuppression effect may be established as depressed T- or B-lymphocyte activity, suppressed antibody production and impaired macrophage / neutrophil-effector activities. The immune system is primarily responsible for defense against attacking organisms. Inhibited immune activity by mycotoxins may ultimately decrease resistance to infectious diseases, reactivate chronic infections and/or decrease vaccine and drug efficacy (Corrier, 1991; Surai and Dvorska, 2001).

The effects on liver, the immunosuppressive nature of AFB<sub>1</sub> is the best recognized area of its toxicity. The high correlation between outbreaks of Newcastle disease (ND) and presence of aflatoxin in the diet of broilers have been reported.

Based on the reports of Tung *et al.* (1970) it was assumed that any impaired function of FAE might result in serious shortages in both cellular and antibody responsiveness of the chicken immune system. This is due to this fact that FAE of bursal follicles play a major role in antigen production to the lymphoid cell population. Besides the effects on lymphocytes, non-specific effects of the toxin on protein synthesis by embarrassment of RNA polymerase, lipid peroxidation and liver damage are also deliberated to result in reduced immunoglobulin production.

Aflatoxin is believed to be converted into its epoxide and this derivative produces DNA adducts which will lead to DNA stand breaks and mutation (Soni *et al.*, 1992). To overcome this, antioxidants were reported to inhibit the AF induced DNA adduct formation.

The current toxin binder used in this study contains Curcumin from Turmeric, Turmeric (*Curcuma longa*) and its

active ingredient (curcumin) has been already shown to scavenge the free radicals and act as a suitable antioxidants. Also, due to its non-toxicity, the use of turmeric in preventing the AF induced liver damage in the duckling has been reported (Ebana, *et al.*, 1991).

The enzymes are believed to break the functional atomic group of the mycotoxin molecule and thereby render them nontoxic (Kumar *et al.*, 1993). Enzymes viz., carboxylesterase present in the microsomal fraction of the liver, esterase and epoxidase are being tried for their practical applicability in the field conditions (Pasteiner, 1997).

Curcumin can reportedly suppress the tumorigenic activity of a wide variety of carcinogens in cancers of the colon, duodenum, esophagus, fore stomach, stomach, liver, breast, leukemia, oral cavity, and prostate.

A yellow pigment present in turmeric (curry powder) has been shown to exhibit plentiful activities. It binds to a variety of proteins and inhibits the activity of various kinases. Accumulating proof suggests that curcumin has a diverse range of molecular targets, which supports the notion that curcumin influences numerous biochemical and molecular cascades. Among its molecular targets are transcription factors, growth factors and their receptors, cytokines, enzymes, and genes regulating cell proliferation and apoptosis. Curcumin regulates the expression of inflammatory enzymes, cytokines, adhesion molecules, and cell survival proteins. Several animal studies suggest that curcumin has potential as an antiproliferative, antiinvasive and antiangiogenic agent, as a mediator of chemoresistance and radio resistance; as a chemo preventive agent; and as a therapeutic agent in wound healing (Dorman and deans, 2000).

The other ingredient of the toxin binder used in this study is clay containing diatomaceous earth minerals. The ability of minerals to effectively diminish the adverse effect of AF could be due to adsorption of toxin during digestive process which renders most of the toxins unavailable for absorption from the gastro intestinal tract. Improved performance of broilers fed AF with clay materials was supported by the results of Kubena *et al.* (1999); Santurio *et al.* (1999) and Rosa *et al.* (2001).

The reduction of antibody titers could be due to mycotoxin inhibiting DNA and protein synthesis through impairment of amino acid transport and m-RNA transportation resulting in lower level of antibody production. The possible mechanism of the counteraction of AF by minerals from clays was binding of AF in GIT leading to excretion of AF without affecting the immune system. Improvement of antibody titers against IBD and ND diseases on clay-product supplementation was earlier reported by Kubena *et al.* (1997) and Rosa *et al.* (2001).

## 4. Conclusions

It could be concluded that aflatoxin B<sub>1</sub> in the broiler diet can influence the performance and immune response and

addition of a mycotoxin binder containing curcumin, enzymes and minerals could significantly reestablish these harmful effects on broilers. Nevertheless, there is lack in the proof of its beneficial impacts in nutrient digestibility and gut function of broilers.

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## REFERENCES

- [1] Aggarwal, B.B., Kumar, A. and Bharti, A.C. 2003. Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res*, 23:363–98.
- [2] Arvind, K.L., Patil, V.S., Devegowda, G., Umakantha, B. and Ganpule, S.P., 2003. Efficacy of Esterified glucomannan to counteract Mycotoxicosis in naturally contaminated feed on performance, serum biochemical and hematological parameters in broilers. *Poult. Sci.*, 82: 571–576.
- [3] Corrier, D.E. 1991. Mycotoxicosis: mechanisms of immunosuppression. *Veterinary Immunology and Immunopathology*, 30: 73–87.
- [4] Devegowda, G. and Murthy, T.N.K. 2005. Mycotoxins: Their effects in poultry and some practical solutions. In *The Mycotoxin Blue Book*; Diaz, D.E., Ed.; Nottingham University Press: Nottingham, UK. pp. 25–56.
- [5] Dersjant-Li, Y., Verstegen, M.W.A. and Gerrits, W.J.J. 2003. The impact of low concentrations of aflatoxin, deoxynivalenol or fumonisin in diets on growing pigs and poultry. *Nutr. Res. Rev.* 16, 223–239.
- [6] Dorman, H.J.D. and Deans, S.G., 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. App. Microbiol.* 88: 308–316.
- [7] Duarte, E.D. and Smith, T.K., 2005. *Mycotoxin blue book*. Nottingham University press. pp. 323–339.
- [8] Duncan, D.B. 1955. Multiple range and multiple F tests. *Biometrics*. 11:1–42.
- [9] Ebana, R.U.B., Madunagu, B.E., Ekpe, E.D. and Otung, I.N., 1991. Microbiological exploitation of cardiac glycosides and alkaloids from *Garcinia kola*, *Borreria ocyroides*, *Kola nitida* and *Citrus aurantifolia*. *J. App. Bacteriol.* 71:398–401.
- [10] Eralsan, G., Essz, D., Akdogan, M., Sahindokuyucu, F. and Altrintas, L., 2005. The effects of aflatoxin and sodium bentonite and alone on some blood electrolyte levels in broiler chickens. *Turk Veterinerlik ve Hayvanclık Dergisi*. 29: 601–605.
- [11] Galvano, F., Ritieni, A., Piva, G. and Pietri, A. 2005. Mycotoxins in the human food chain. In *The Mycotoxin Blue Book*; Diaz, D.E., Ed.; Nottingham University Press: Nottingham, UK, pp. 187–224.
- [12] Girish, C.K., and Devegowda, G., 2004. Evaluation of modified glucomannan (Mycosorb) and HSCAS to ameliorate the individual and combined toxicity of aflatoxin and T-2 toxin in broiler chickens. In: *Aust. Poult. Sci. Symp.* 16: 126–129. Sydney, Australia.
- [13] Gurib-Fakim A. 2006. Medicinal plants: traditions of yesterday and drugs of tomorrow. *Mol Aspects Med*, 27:1–93.
- [14] Kubena, L.F., Edrington, T.S., Harvey, R.B., Buckley, S.A., Phillips, T.D., Rottinghaus, G.E. and Caspers, H.H., 1997. Individual and combined effects of Fumonisin B<sub>1</sub> present in *Fusarium moniliforme* culture material and deoxynivalenol in broiler chicks. *Poult. Sci.*, 76: 1239–1247.
- [15] Kubena, L.F., Harvey, R.B., Buckley, S.A. Bailey, R.H. and Rottinghaus, G.E., 1999. Effects of long-term feeding of diets containing moniliformin, supplied by *Fusarium fujikuroi* culture material, and fumonisin, supplied by *Fusarium moniliforme* culture material, to laying hens. *Poult. Sci.*, 78: 1499–1505.
- [16] Kumar, A.A., Chanda, S.K., Rao, A.T., Biscoi, P.C. and Mishra, P.K., 1993. Clinico-pathological changes in experimental aflatoxicosis in quails. *Indian J. Poult. Sci.*, 28:150–153.
- [17] Li, Y., Liu, Y.H., Yang, Z.B., Wan, X.L. and Chi, F., 2012. The efficacy of clay enterosorbent to ameliorate the toxicity of aflatoxin B<sub>1</sub> from contaminated corn (*Zea mays*) on hematology, serum biochemistry, and oxidative stress in ducklings. *J. Appl. Poult. Res.* 21:806–815.
- [18] López-Malo, A., Barreto-Valdivieso, J., Palou, E. and Martin, F.S., 2006. *Aspergillus flavus* growth response to cinnamon extract and sodium benzoate mixtures. *Food Control* 18: 1358–1362.
- [19] Manafi, M. and Khosravinia, H., 2013. Effects of Aflatoxin on the Performance of Broiler Breeders and Its Alleviation by Herbal Mycotoxin Binder. *J. Agri. Sci. Tech.*, 15: 55–63.
- [20] Manafi, M., Murthy, H.N.N. and Narayana Swamy, H.D., 2012. Evaluation of Different Mycotoxin Binders on Broiler Breeders Induced With Aflatoxin B<sub>1</sub>: Effects on Visceral Organ Weight and Organ Lesions Parameters. *Am. Eur. J. Agri. Env. Sci.*, 12 (5):574–578.
- [21] Matur, E., Ergul, E., Akyazi, I., Eraslan, E. and Cirakli, Z.T., 2010. The effects of *Saccharomyces cerevisiae* extract on the weight of some organs, liver, and pancreatic digestive enzyme activity in breeder hens fed diets contaminated with aflatoxins. *Poult. Sci.* 89:2213–2220.
- [22] Newman, D.J. and Cragg, G.M. 2007. Natural products as sources of new drugs over the last 25 years. *J Nat Prod*, 70: 461–77.
- [23] NRC, National Research Council. 1994. *Nutrient Requirements of Poultry*. 9th ed. Natl. Acad. Sci., Washington.
- [24] Parker, S.P. and McGraw-HILL., 1988. *Encyclopedia of the geological sciences*, 2nd ed. New York, McGraw-Hill, pp 32–33, 69–72, 400–401.
- [25] Pasteiner, S. 1997. Copying with mycotoxin contaminated feed stuffs. *Feed Int.*, 18: 12–16.
- [26] Raju, M.V.L.N. and Devegowda, G., 2002. Esterified glucomannan in broiler chicken diets contaminated with aflatoxin, ochratoxin and T-2 toxin: Evaluation of its binding

- ability (in vitro) and efficacy as immunomodulator. *Asian-Aust. J. Anim. Sci.*, 15: 1051-1056.
- [27] Rosa, C.A.R., Miazoo, R., Magnoli, C., Salvano, M., Chicchiera, S.M., Ferrero, S., Saenz, M., Carvalho, E.C.Q., and Dalcero, A., 2001. Evaluation of the efficacy of bentonite from the south Argentina to ameliorate the toxic effects of aflatoxin in broilers. *Janerio Institute de Veterrinaria, Brazil*.
- [28] Santurio, J.M., Mallmannl, C.A., Rosa, A.P., Appel, G., Heer, A., Dageforde, S. and Bottcher, M. 1999. Effect of sodium bentonite on the performance and blood variables of broiler chickens intoxicated with aflatoxin. *Br. Poult. Sci.*, 40: 115-119.
- [29] SAS. Institute. 2000. SAS® User's Guide: Statistics. Version & Edition. SAS Institute Inc., Cary, NC, USA.
- [30] Shen, L. and Ji, H.F. 2007. Theoretical study on physicochemical properties of curcumin. *Spectrochim Acta A Mol Biomol Spectrosc*, 67:619-23.
- [31] Soni, K.B., Rajan, A. and Kuttan, R., 1992. Reversal of aflatoxin induced liver damage by turmeric and curcumin. *Cancer Letters*, 66: 115-121.
- [32] Surai, P.F. and Dvorska, J.E. 2001. Dietary organic selenium and egg: from improvement in egg quality to production of functional food. *Proceedings of the IX Symposium on the Quality of Eggs and Egg Products, Kusadasi, Turkey*, pp. 163-160.
- [33] Tedesco, D., Steidler, S., Galletti, S., Tameni, M., Sonzogni, O. and Ravarotto, L. 2004. Efficacy of silymarin-phospholipid complex in reducing the toxicity of aflatoxin B<sub>1</sub> in broiler chicks. *Poult. Sci.*, 83, 1839-1843.
- [34] Tung, H.-T., Donaldson, W.E., Hamilton, P.B, 1970. Effects of aflatoxin on some marker enzymes of lysosomes. *Biochim. Biophys. Acta Gen. Subj.* 222, 665-667.
- [35] Valdivia, A., Martinez, A., Damian, F., Quezada, T., Ortiz, R., Martinez, C., Llamas, J., Rodriguez, M., Yamamoto, L. and Jaramillo, F. 2001. Efficacy of N-acetylcysteine to reduce the effects of aflatoxin B<sub>1</sub> intoxication in broiler chickens. *Poult. Sci.*, 80: 727-734.
- [36] Wan, X.L., Yang, Z.B., Yang, W.R., Jiang, S.Z., Zhang, G.G., Johnston, S.L., and Chi, F. 2013. Toxicity of increasing aflatoxin B<sub>1</sub> concentrations from contaminated corn with or without clay adsorbent supplementation in ducklings. *Poult. Sci.*, 92: 1244-1253.
- [37] Yunus, A.W., Nasir, M.K., Farooq, U. and Böhm, J. 2008. Prevalence of poultry diseases in district Chakwal and their interaction with mycotoxicosis: 1. Effects of age and flock size. *J. Anim. Plant Sci.*, 18, 107-113.