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CHEMICAL DETOXIFICATION OF AFB1 IN EXPERIMENTAL QUAILS USING COMMERCIALLY AVAILABLE TOXIN BINDERS

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ABSTRACT

AFB1 causes public health hazards by affecting 25% of world's food crops. For optimum production performance from poultry, proper detoxification of AFB1 in feed is mandatory. This study was designed to analyze the chemical detoxification potential of four commercially available toxin binders (activated charcoal, kaolin, vitamin E and selenium, myco-AD) in experimental quails fed with AFB1 added diets. For this purpose, 360 quail birds (divided in 6 groups) were reared in experimental conditions. Positive and negative control groups were fed basal diet and basal diet with 0.5mg/kg AFB1, respectively. Treatment groups were fed 4 different experimental diets with 0.5 mg/kg of AFB1 contamination and chemical binders added according to recommended dose rate. Growth parameters (feed intake, body weight gain &feed conversion ratio), hematology (hemoglobin, hematocrit, erythrocyte sedimentation rate, total leukocyte count), immune response and histopathology of soft organs (liver, kidney, lungs) of all the experimental birds were weekly recorded for 6 weeks and results were analyzed by Repeated Measure of ANOVA and Duncan Multiple Range Test. Results showed significant reduction in all the deleterious effects of AFB1 in all the tested parameters during the course of study. All the toxin binders brought significant changes ($P < 0.05$) in tested parameters. The active ingredient of Myco AD (Hydrated sodium calcium aluminosilicate, HSCAS) and Vitamin E and selenium were found as better detoxifying agent among the toxin binders used in this study. This study reports the success of commercially available toxin binders as chemical detoxification agent for the quails, an emerging protein source in thickly populated developing countries.

Key words: Detoxification, Histopathology, Toxin binders, Quails.

INTRODUCTION

Mycotoxins are toxic metabolites of fungi, produced due to improper storage conditions of feed ingredients or feed (Rao *et al*., 2016). Approximately, 25% of food crops get affected by mycotoxins annually, all around the globe (Shahzad *et al*., 2014). Among reported mycotoxins, aflatoxin (AF) is the most toxic and studied mycotoxin (Rashid *et al*., 2012). These are produced by *Aspergillus parasiticus and Aspergillusflavus* which effect various feed ingredients, stored improperly (Filazi andSireli, 2013). According to literature, aflatoxin B1 (AFB1) is the most prevalent and potent naturally occurring carcinogenic, mutagenic and teratogenic compound which causes immune-suppression (Pizzolitto *et al*, 2013., Pleadin *et al*., 2014). Continuous low level intake of toxins causes a variety of metabolic disorders causing economic losses due to poor growth performance and productivity (Bryden *et al*., 2012). Aflatoxins cause a variety of effects in poultry including decreased weight gain, poor feed efficiency, reduced egg production and egg weight, increased liver fat, changes in organ weights, reduction in serum protein levels, carcass bruising, poor pigmentation, liver damage, decreased activities of several enzymes involved in the digestion of starch, protein, lipids, and nucleic acids and immunosuppression (Devegowda and Murthy, 2005). Evidence suggests that immunosuppression caused by AF results in many disease outbreaks, vaccination failures, and poor antibody titers (Devegowda and Murthy, 2005). At necropsy, livers are usually pale and enlarged as a result of aflatoxicosis. Histologically, liver lesions include congestion of the hepatic sinusoids, focal hemorrhages, centrilobular fatty cytoplasmic vacuolation and/or necrosis, biliary hyperplasia and nodular lymphoid infiltration (Leeson *et al*., 1995). Aflatoxicosis is also associated with biochemical, haematological, pathological and immune functions changes (Sur and Celik, 2005).Ability of certain food crops to uptake AFB1 from contaminated soil has also been reported (Hariprasad *et al*., 2015). Due to all stated problems, need of efficient detoxification methods is unavoidable to improve productivity of livestock and poultry. Recent approaches intended to deal with mycotoxins contaminated feed stuff have been directed towards the prevention of their absorption in gastrointestinal tract (Verheecke *et al*., 2016). The most important feature of the adsorption is the physical structure of the adsorbent, i.e. the total charge, charge distribution, the accessible surface area and pore size (Huwig *et al*., 2001).Due to

ever rising increase in meat demand in developing South Asian countries, Japanese Quail (*Coturnix Japonica*) has a pivotal role as a meat source to fulfill needs of growing human population (Ayyub *et al*., 2014). In Pakistan, quail meat is the second most consumed poultry meat after broiler meat, even then it is the most neglected components of poultry sector (Jatoi *et al*., 2013). To date, no data exist on the efficacy of chemical absorbents in experimental quails, fed with AFB1 contaminated feed despite significant increase in quail farming in country and prevalence of AFB1 in finished feed samples as high as 60% (Anjum *et al*., 2012). So, this study was conducted to treat this threat which if not taken seriously may push country a step deeper in food insecurity. During this debut study, we evaluated the detoxification potential of four commercially available toxin binders (Activated charcoal, Kaolin, Vitamin E + Selenium and Myco AD) in experimental quails, fed with AFB1 added feed along with different toxin binders.

MATERIALS AND METHODS

The research model was based on completely randomized design (CRD). This study was designed to analyze the efficacy of four commercially available toxin binders viz: Activated Charcoal, Kaolin, Vitamin E + Selenium and Myco AD {hydrated sodium calcium aluminosilicate (HSCAS)} for detoxification of AFB1 (Casarin *et al*., 2005) in experimental quails, fed with 0.5mg/kg AFB1 added diet. Post treatment analysis of hematological profile (Mangoli *et al*. 2011), growth performance (Kasmani *et al* 2012), immune response (Shewita and Taha, 2011) and histopathology of liver, kidney and lungs (Bancroft and Gamble, 2008) were performed on weekly basis for 6 weeks.

Experimental Site: This study was conducted in June to July, 2014 at Avian Research and Training Centre (ARTC), University of Veterinary and Animal Sciences Lahore, Pakistan. Synopsis of this experiment was planned taking into consideration all the national legislation for protection of animal welfare and guidelines of the Advanced Studies and Research Board (ASRB), UVAS were followed keenly.

Experimental Birds and Management: Japanese quails (n=360) were procured from Avian Research and Training Centre, UVAS Lahore and were kept in clean experimental sheds. Floor, drinkers and feeders were disinfected with Baloran. Fresh and clean water was offered *ad libitum*.

Experimental Design: Efficacy of four commercially available toxin binders were evaluated in experimental quails. For the purpose 360 quail birds were divided into six groups (60 each) viz; A to F.

Group A was kept as negative control and was fed basal diet without any toxin or toxin binder, while group B was given AFB 1 (0.5mg/kg) added basal diet, without any toxin binder (positive control). Group Cwas fed AFB1 added basal di*et al*ong with 5% Activated Charcoal, group D was given 5% Kaolin added in AFB1 contaminated basal diet, group E was offered AFB1 added basal diet with 1 mg Selenium and 200 mg Vitamin E, while, group F was fed with AFB1 (0.5mg/kg) added basal diet with 2.50gm Myco ADas summarized in table 1. Each group was further divided in two subgroups; one subgroup was vaccinated with NDV vaccine, while other subgroup was not vaccinated with NDV vaccine. Quails were observed for next 42 days to evaluate post math of performance parameters (feed intake, body weight gain and feed conversion ratio), blood profile (Hb, PCV, TLC, ESR) and humoral immune response (mean antibody titre). Histopathologicalalterations in liver, kidney and lungs were also quantified using scoring method (Bovo *et al*., 2015)

Statistical analysis: Data regarding growth performance parameters, hematology, immunology and histopathology were recorded and significance*($P < 0.05$)* between groups was analyzed using "Repeated Measure of Analysis of Variance (ANOVA)" throughStatistical Product and Service Solutions (SPSS) version 20.0.Experimental groups found significantlydifferent were further compared using"Duncan Multiple Range Test"(Khan *et al*., 2012).

RESULTS

Growth Performance Parameters: The results for feed intake (gms)during experimental period are presented in table 2. After first week, the feed intake was non-significantly different ($P > 0.05$) from the control group and treated groups. Feed intake after second week of control group (71±0.32) and group F (71±0.03) was non-significantly different ($P > 0.05$). The feed intake of groups B, C, D and E were 46±0.31, 45±0.63, 45.6±0.4 and 71.48±0.14 respectively and significantly different ($P < 0.05$) from the control group. Whereas, the feed intake of groups E (91.7±0.08) and F (91.2±0.024) were non-significantly different ($P > 0.05$) from the control group (91±0.24gms). The same pattern of results for feed intake was observed after fourth, fifth and sixth weeks.

Results for body weight gain (gms) of quails reared on different chemical binders in the presence of aflatoxin B1 containing feed in comparison to basal feed are presented in Table 2. After first week, the body weight gain of control group (30±0.08), group B(30.3±0.17), group C(30.6±0.11), group D (30.4±0.17), group E (30.6±0.42) and group F (30±0.33) were non-significantly different ($P > 0.05$). At second week, the

body weight gain of group F (0. 5mg AFB1/kg feed + 2.5 gmMycoAD) was non-significantly different (P > 0.05) from the control group A (basel diet). The mean body weight gain of Group B(0.5mgAFB1/kg feed), Group C(0.5mg AFB1/kg feed + 0.5% Activated Charcoal), group D (0.5mg AFB1/kg feed+ 0.5% Kaolin) and group E (0.5mg AFB1/kg feed + 1mg Se plus 200mg Vit.E) were significantly different (P > 0.05) from the control group. After fourth week, the mean body weight gain of groups E (69.6±0.50gms) and F (71.1±0.53gms) were non-significantly different (P > 0.05) from the control group A (70±0.70gms). The body weight gain of groups B (43.7±0.77gms), C (47.3±0.11gms) and D (50.4±0.93gms) were significantly different (P < 0.05) from the control group. Similar results were recorded during following fifth and sixth weeks.

The feed conversion ratio of control group at first week was non-significantly different (P > 0.05) from the treated groups. At second week, the FCR of group F (1.5±0.017) was non-significantly different (P > 0.05) from the control group (1.5±0.017). FCR of groups B, C, D and E were significantly different (P < 0.05) from the control group. The similar pattern of change in FCR was noted during following third, fourth, fifth and sixth weeks.

Hematological Parameters: Hemoglobin concentration (g/dl) of control group (12.8±0.003), group B (12.8 ± 0.005), C (12.8 ±0.003), D (12.8 ± 0.003), E (12.8 ± 0.002) and F (12.7±0.13) were non-significantly different (P > 0.05). After second week, group B (12.8±0.008), C(12.7±0.006) and D(12.7±0.008) had significant difference (P < 0.05) with control group (12.9±0.012), while groups E(12.9±0.004) and F(12.9±0.015) had non-significant difference (P > 0.05). After third week, the Hb. content of groups B (12.7±0.006) and C (12.8±0.005) were significantly different (P < 0.05) from control group and other treated groups (D, E and F). Same trend was observed till sixth week.

Packed Cell Volume (%) after first week showed non-significant difference (P > 0.05) from the control group (38±0.005) and treated groups B(38±0.005), C(38±0.005), D(38.4 ± 0.007), E (38.4 ± 0.009) and F (38±0.009). After second week, the PCV of group B (38±0.004) and C (38±0.004) were significantly different (P < 0.05) from the control group and treated groups D , E and F. Whereas, in third week, the groups B (38.2±0.04), C (38±0.016%) and D(38±0.02%)were significantly different (P < 0.05) from the control group(38±0.006%). Similar results were recorded during fourth, fifth and sixth weeks.

After first week, the mean total leukocyte count ($x10^3/mm^3$) of control group (4.6±0.007) and treated groups B (4.6±0.007), C (4.6±0.009), D (4.6±0.007), E (4.6±0.007) and F (4.6±0.004) were non-significantly different (P > 0.05), while during second week, the TLC of groups B (4.6±0.004), C (4.5±0.004) and D (4.5±0.004) were significantly different (P < 0.05) as compared to control group(4.6±0.004) and other treated groups (E and F). Same trend in all groups was recorded during rest of the study period.

Erythrocyte Sedimentation rate (mm/hr) at first week shown by control group (3.5±0.004) was non-significantly different (P > 0.05) from treated groups. After second week, the ESR of group B(3.5±0.002) and group C (3.53±0.004) were significantly different (P < 0.05) from the control group A (3.5±0.004) and other treated groups D(3.5±0.004), E(3.5±0.004) and F (3.5±0.004). Similar results were found in all groups from third week to fifth week, while after sixth week, the ESR of groups B (3.7±0.02), C (3.6±0.01), D(3.6±0.02) and E (3.5±0.004)were significantly different (P < 0.05) from control group (3.5±0.002) and MycoAD treated group (3.5±0.003).

Immunology: During 1st week, the mean antibody titer of all groups had non-significant difference (P > 0.05). After 2nd week, the mean antibody titer of group C (5.4±0.24) was significantly lower (P < 0.05) than the control group (6.4 ± 0.24). During 3rd week, the mean antibody titer of group C (5±0.32) was significantly different (P < 0.05) from the control group (6.4±0.4) and other treated groups E(6.4±0.40) and F (6.4±0.40). The mean antibody titer of group D (5.4±0.24) was non-significantly different (P > 0.05) from the control group and group treated with activated Charcoal. At 4th week, the mean antibody titer of group B (4.8±0.20), C (5±0.00) and D (6±0.32) were significantly different (P < 0.05) from the control group (6.8±0.37) and other treated groups E (7.2±0.20) and F (7±0.32). Similar trend in mean antibody titre was found during 5th and 6th weeks.

Histopathology: During first week, group B (0.6±0.24) exhibited significant histological lesions in liver. After second week, the groups B (1.6±0.24), C (1.4±0.24) and D (0.8±0.37) had significant difference from the control group (0.00±0.0) and groups E and F. Similar pattern in recordings were found in all groups from third to sixth week (table 5). The histological lesions were observed in the kidneys of treated groups. During opening week, the group B (0.6±0.24) and C (0.6±0.24) showed significant (P< 0.05) histological lesions as compared to control group, while in second week, similar results were found in all experimental groups. After third week, histological lesion scores of groups B (2.2±0.20), C (1.2±0.20) and D (1±0.32) had significant difference (P < 0.05) as compared to control group. Similar findings were observed at fourth, fifth and sixth weeks (Table 5).

During first week, the group B (0.8±0.4) and group C (0.6±0.24) showed significant histological lesions (P < 0.05) as compared to control group. After second week, the histological lesion scores of groups B(1.4±0.4), C(1±0.32) and D(0.4±0.24) were significant

($P < 0.05$) as compared to control group and other treated groups. Similar trend in findings were observed during rest of study period (Table 5).

Table 1. Experimental Design

Group	Feed	No. of birds	Immune status
A	Basal Diet	60	NDV vaccine (30)
			No NDV vaccine (30)
B	Basal diet + 0.5mg/kg AFB1	60	NDV vaccine (30)
			No NDV vaccine (30)
C	Basal diet + 0.5mg/kg AFB1+ 5% activated charcoal	60	NDV vaccine (30)
			No NDV vaccine (30)
D	Basal diet + 0.5mg/kg AFB1+ 5% kaolin	60	NDV vaccine (30)
			No NDV vaccine (30)
E	Basal diet + 0.5mg/kg AFB1+ 1 mg Selenium +200 mg Vitamin E	60	NDV vaccine (30)
			No NDV vaccine (30)
F	Basal diet + 0.5mg/kg AFB1+ 2.50gm MycoAD	60	NDV vaccine (30)
			No NDV vaccine (30)

Table 2. Effect of toxin binders on the growth performance parameters of Quails

Groups	***Weekly Mean Body Weight Gain (g)***					
	1	**2**	**3**	**4**	**5**	**6**
A (Basal diet)	30±0.08[a]	48±0.5 [a]	60.7±0.53 [a]	70±0.70 [a]	78±1.06 [a]	83.6±1.6 [a]
B (0.5mgAFB1/kg feed)	30.3±0.17 [a]	45.2±0.04 [b]	45.4±0.51 [d]	43.7±0.77 [d]	37.4±1.4 [d]	32.4±0.92 [d]
C (0. 5mg AFB1/kg feed + 0.5% Activated Charcoal)	30.6±0.11 [a]	45±0.63 [b]	46.7±0.50 [d]	47.3±0.11 [c]	47.6±0.51 [c]	49±0.84 [c]
D (0. 5mg AFB1/kg feed+ 0.5% Kaolin)	30.4±0.17 [a]	45.6±0.4 [b]	48.6±0.40 [c]	50.4±0.93[b]	59.4±1.63 [b]	61.4±2.2 [b]
E (0. 5mg AFB1/kg feed + 1mg Se plus 200mg Vit.E)	30.6±0.42 [a]	46±0.31 [b]	55.2±0.86 [b]	69.6±0.50 [a]	76.5±1.7 [a]	81.6±0.93 [a]
F (0. 5mg AFB1/kg feed + 2.5 gmMyco AD)	30±0.33 [a]	47.8±0.6 [a]	61.2±0.86 [a]	71.1±0.53 [a]	79.5±0.50 [a]	84.2±1.11 [a]
	Weekly Feed Intake (g)					
A (Basal diet)	35±0.07 [a]	71±0.32 [b]	91±0.24[a]	116±0.05 [a]	146±0.07 [a]	146±0.15 [a]
B (0.5mgAFB1/kg feed)	35.2±0.05 [a]	69.5±0.02 [d]	81±0.45[c]	97±1.6 [c]	94±2.8 [c]	85.6±1.6 [c]
C (0.5mg AFB1/kg feed + 0.5% Activated Charcoa)l	35.3±0.05 [a]	70±0.45 [c]	84.5±1.71[b]	105.8±3.1 [b]	118.4±4.7 [b]	130.6±1.7 [b]
D (0.5mg AFB1/kg feed+ 0.5% Kaolin)	35.2±0.06 [a]	71.2±0.73 [a]	81.4±0.4[c]	112±4.3 [a b]	124±3.1 [b]	146±0.18 [a]
E (0. 5mg AFB1/kg feed + 1mg Se plus 200mg Vit.E)	35.2±0.2 [a]	71.48±0.14 [a]	91.7±0.08[a]	116.7±0.18 [a]	146±0.18 [a]	146.3±0.05 [a]
F (0. 5mg AFB1/kg feed + 2.5 gmMycoAD)	35.2±0.07 [a]	71±0.03 [b]	91.2±0.024[a]	116±0.03 [a]	146.4±0.19 [a]	146±0.29 [a]
	Weekly Feed Conversion Ratio					
A (Basal diet)	1.2±0.008 [a]	1.5±0.015 [a]	1.5±0.013 [a]	1.7±0.016 [a]	1.9±0.03 [a b]	1.8±0.03 [a]
B (0.5mg AFB1/kg feed)	1.2±0.008 [a]	1.5±0.001 [b]	1. 8±0.03 [c]	2.2±0.07 [b]	2.5±0.09 [c]	2.7±0.12 [c]
C (0.5mg AFB1/kg feed + 0.5% Activated Charcoal)	1.2±0.004 [a]	1.6±0.022 [b]	1.8±0.05 [c]	2.2±0.06 [b]	2.5±0.12 [c]	2.7±0.07 [c]
D (0. 5mg AFB1/kg feed+ 0.5% Kaolin)	1.2±0.008 [a]	1.6±0.012 [b]	1.7±0.02 [b]	2.2±0.07 [b]	2.1±0.10 [b]	2.4±0.08 [a]
E (0. 5mg AFB1/kg feed + 1mg Se and 200mg Vit.E)	1.2±0.011 [a]	1.6±0.008 [b]	1.7±0.03 [b]	1.7±0.01 [a]	1.9±0.5 [a b]	1.8±0.02 [a]
F (0.5mg AFB1/kg feed +2.5 g MycoAD)	1.2±0.013 [a]	1.5±0.017 [a]	1.5±0.02 [a]	1.6±0.012 [a]	1.8±0.013 [a]	1.7±0.02 [a]

Means with same superscripts differ non-significantly ($P \geq 0.05$) whereas with different superscripts differ significantly ($P \leq 0.05$) in same column.

Table No. 3: Effect of toxin binders on hematology of Quails

Groups	***Weekly Mean Hemoglobin Contents (g/dl)***					
	1	**2**	**3**	**4**	**5**	**6**
A (Basal diet)	12.8±0.003 [a]	12.9±0.012 [a]	12.8±0.004 [a]	12.8±0.003 [a]	12.8±0.004 [a]	12.8±0.003 [a]
B (0.5mgAFB1/kg feed)	12.8±0.005 [a]	12.8±0.008 [b]	12.7±0.006 [c]	12.7±0.012 [c]	12.5±0.005 [c]	12.3±0.06 [d]
C (0.5mg AFB1/kg feed + 0.5% Activated Charcoal)	12.8±0.003 [a]	12.7±0.006 [b]	12.8±0.005 [c]	12.7±0.008 [b]	12.6±0.04 [b]	12.6±0.012 [c]
D (0.5mg AFB1/kg feed+ 0.5% Kaolin)	12.8±0.004 [a]	12.7±0.008 [b]	12.8±0.004 [a]	12.8±0.013 [b]	12.6±0.05 [b]	12.7±0.007 [b]
E (0.5mg AFB1/kg feed + 1mg Se plus 200mg Vit.E)	12.8±0.002 [a]	12.9±0.004 [a]	12.8±0.003 [a]	12.8±0.003 [a]	12.8±0.004 [a]	12.8±0.004 [a]
F (0.5mg AFB1/kg feed + 2.5 gmMycoAD)	12.7±0.13 [a]	12.9±0.015 [a]	12.8±0.02 [a]	12.8±0.01 [a]	12.8±0.01 [a]	12.8±0.01 [a]
Weekly Packed Cell Volume						
A (Basal diet)	38±0.005[a]	38±0.005 [a]	38±0.006 [a]	38±0.006 [a]	38.5±0.005 [a]	38.5±0.005 [a]
B (0.5mgAFB1/kg feed)	38±0.005 [a]	38±0.004 [b]	38.2±0.04 [c]	38±0.03 [c]	37.9±0.02 [d]	37.9±0.02 [c]
C (0.5mg AFB1/kg feed + 0.5% Activated Charcoal)	38±0.005 [a]	38±0.004 [b]	38±0.016[bc]	38±0.007 [b]	38±0.024 [c]	38.3±0.02 [b]
D (0.5mg AFB1/kg feed+ 0.5% Kaolin)	38±0.007 [a]	38.4±0.008 [a]	38±0.02 [b]	38.2±0.02 [b]	38.4±0.008 [b]	38.4±0.007 [b]
E (0.5mg AFB1/kg feed + 1mg Se plus 200mg Vit.E)	38±0.009 [a]	38±0.009 [a]	38±0.006 [a]	38±0.006 [a]	38.5±0.003 [a]	38.5±0.003 [a]
F (0. 5mg AFB1/kg feed + 2.5 gmMycoAD)	38±0.009 [a]	38±0.009 [a]	38±0.009 [a]	38±0.009 [a]	38.5±0.007 [a]	38.5±0.007 [a]
Weekly Total Leukocyte Count (X 10^3/mm^3)						
A (Basal diet)	4.6±0.007[a]	4.6±0.004 [a]	4.6±0.005 [a]	4.6±0.002 [a]	4.6±0.003 [a]	4.57±0.004[a]
B (0.5mgAFB1/kg feed)	4.6±0.007 [a]	4.5±0.005 [c]	4.5±0.004 [c]	4.5±0.002 [c]	4.47±0.007 [c]	4.39±0.01 [c]
C (0.5mg AFB1/kg feed + 0.5% Activated Charcoal)	4.6±0.009 [a]	4.5±0.004 [b]	4.5±0.002 [b]	4.5±0.004 [b]	4.5±0.007 [b]	4.49±0.004 [b]
D (0.5mg AFB1/kg feed+ 0.5% Kaolin)	4.6±0.007 [a]	4.5±0.004 [b]	4.5±0.002 [b]	4.5±0.004 [b]	4.5±0.004 [b]	4.49±0.002 [b]
E (0.5mg AFB1/kg feed + 1mg Se plus 200mg Vit.E)	4.6±0.007 [a]	4.6±0.004 [a]	4.6±0.004 [a]	4.6±0.002 [a]	4.6±0.002 [a]	4.57±0.002 [a]
F (0.5mg AFB1/kg feed + 2.5 gmMycoAD)	4.6±0.004 [a]	4.6±0.004 [a]	4.6±0.004 [a]	4.6±0.002 [a]	4.6±0.002 [a]	4.56±0.002 [a]
Weekly Erythrocyte Sedimentation Rate (mm/Hr.)						
A (Basal diet)	3.5±0.004 [a]	3.5±0.004 [a]	3.5±0.005 [a]	3.5±0.007 [a]	3.5±0.009 [a]	3.5±0.002 [a]
B (0.5mgAFB1/kg feed)	3.5±0.002 [a]	3.5±0.002 [b]	3.6±0.01 [c]	3.6±0.007 [b]	3.6±0.01 [c]	3.7±0.02 [d]
C (0.5mg AFB1/kg feed + 0.5% Activated Charcoal)	3.5±0.004 [a]	3.53±0.004 [b]	3.5±0.005 [b]	3.6±0.03 [b]	3.6±0.009 [b]	3.6±0.01 [b]
D (0.5mg AFB1/kg feed+ 0.5% Kaolin)	3.5±0.004 [a]	3.5±0.004 [a]	3.5±0.004 [a]	3.5±0.008 [a]	3.5±0.004 [a]	3.6±0.02 [b]
E (0.5mg AFB1/kg feed + 1mg Se plus 200mg Vit.E)	3.5±0.004 [a]	3.5±0.004 [a]	3.5±0.005 [a]	3.5±0.004 [a]	3.5±0.002 [a]	3.5±0.004 [a b]
F (0.5mg AFB1/kg feed + 2.5 gm MycoAD)	3.5±0.011 [a]	3.5±0.004 [a]	3.5±0.009 [a]	3.5±0.007 [a]	3.5±0.005 [a]	3.5±0.003 [a]

Means with same superscripts differ non-significantly (P≥0.05) whereas with different superscripts differ significantly (P≤0.05) in same column.

Table No. 4: Effect of toxin binders on the Mean Antibody Titer of Quails.

Groups	*Weekly Mean Antibody Titer*					
	1	**2**	**3**	**4**	**5**	**6**
A (Basal diet)	6±0.00 [a]	6.4±0.24 [a]	6.4±0.4 [a]	6.8±0.37 [a]	7.4±0.24 [a]	7.6±0.24 [a]
B (0.5mgAFB1/kg feed)	5.2±.037 [a]	5.2±0.37 [a]	4.6±0.24 [a]	4.8±0.20 [c]	4.6±0.24 [c]	4±0.32 [c]
C (0.5mg AFB1/kg feed + 0.5% Activated Charcoal)	5.4±0.24 [a]	5.4±0.24 [b]	5±0.32 [b]	5±0.00 [c]	5±0.32 [c]	5±0.32 [c]
D (0.5mg AFB1/kg feed+ 0.5% Kaolin)	5.6±0.24 [a]	6.2±0.2 [a]	5.4±0.24 [a b]	6±0.32 [b]	6.2±0.4 [b]	5.6±0.24 [b]
E (0.5mg AFB1/kg feed + 1mg Se plus 200mg Vit.E)	5.6±0.24 [a]	6.4±0.24 [a]	6.4±0.40 [a]	7.2±0.20 [a]	7.4±0.24 [a]	7.6±0.24 [a]
F (0.5mg AFB1/kg feed + 2.5 gmMycoAD)	5.4±0.24 [a]	6.2±0.24 [a]	6.4±0.40 [a]	7±0.32 [a]	7.4±0.24 [a]	7.6±0.24 [a]

Means with same superscripts differ non-significantly (P≥0.05) whereas with different superscripts differ significantly (P≤0.05) in same column.

Table 5. Effect of toxin binders on the Histopathology of Liver of Quails

Groups	*Weekly histological scoring of liver*					
	1	**2**	**3**	**4**	**5**	**6**
A (Basal diet)	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
B (0.5mgAFB1/kg feed)	0.6±0.24 [b]	1.6±0.24 [c]	2.8±0.20 [c]	3±0.00 [c]	3.4±0.24 [c]	3.6±0.24 [c]
C (0.5mg AFB1/kg feed + 0.5% Activated Charcoal)	0.2±0.200 [a]	1.4±0.24[bc]	1.2±0.37 [b]	1.6±0.51 [b]	2±0.32 [b]	2.4±0.24 [b]
D (0.5mg AFB1/kg feed+ 0.5% Kaolin)	0.2±0.200 [a]	0.8±0.37 [b]	1±0.45 [b]	1.2±0.5 [b]	1.2±0.5 [b]	1.8±0.5 [b]
E (0. 5mg AFB1/kg feed + 1mg Se plus 200mg Vit.E)	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.2±0.200 [a]	0.2±0.200 [a]	0.2±0.200 [a]
F (0.5mg AFB1/kg feed + 2.5 gm MycoAD)	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.2±0.200 [a]	0.2±0.200 [a]	0.2±0.200 [a]
Weekly Histological Scoring of Kidney						
A (Basal diet)	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
B (0.5mgAFB1/kg feed)	0.6±0.24 [b]	1.4±0.24 [c]	2.2±0.20 [c]	2.6±0.24 [c]	3.2±0.20 [c]	3.4±0.24 [c]
C (0.5mg AFB1/kg feed + 0.5% Activated Charcoal)	0.6±0.24 [b]	0.8±0.20 [b]	1.2±0.20 [b]	1.6±0.24 [b]	2±0.32 [b]	2.4±0.40 [b]
D (0.5mg AFB1/kg feed+ 0.5% Kaolin)	0.2±0.200 [a]	0.2±0.200 [a]	1±0.32 [b]	1.4±0.40 [b]	1.4±0.6 [b]	2±0.45 [b]
E (0.5mg AFB1/kg feed + 1mg Se plus 200mg Vit.E)	0.2±0.200 [a]	0.00±0.00 [a]	0.2±0.200 [a]	0.2±0.20 [a]	0.20±0.20 [a]	0.2±0.20 [a]
F (0.5mg AFB1/kg feed + 2.5 gmMycoAD)	0.2±0.200 [a]	0.00±0.00 [a]	0.2±0.200 [a]	0.2±0.200[a]	0.20±0.200[a]	0.20±0.20 [a]
Weekly Histological Scoring of Lung						
A (Basal diet)	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00[a]	0.00±0.00 [a]	0.00±0.00 [a]
B (0.5mgAFB1/kg feed)	0.8±0.4 [b]	1.4±0.4 [c]	1.6±0.24 [c]	2±0.00 [c]	2.4±0.24 [c]	2.8±0.20 [b]
C (0.5mg AFB1/kg feed + 0.5% Activated Charcoal)	0.6±0.24 [b]	1±0.32[bc]	1±0.32[bc]	1.4±0.40 [b]	1.8±0.5 [b c]	2±0.6 [b]
D (0.5mg AFB1/kg feed+ 0.5% Kaolin)	0.00±0.00 [a]	0.4±0.24[ab]	0.6±0.4[ab]	1±0.45 [b]	1.4±0.4 [b]	1.8±0.5 [b]
E (0.5mg AFB1/kg feed + 1mg Se plus 200mg Vit.E)	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.2±0.20 [a]	0.2±0.200 [a]
F (0.5mg AFB1/kg feed + 2.5 gmMycoAD)	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00[a]	0.2±0.20 [a]	0.20±0.200[a]

Means with same superscripts differ non-significantly (P≥0.05) whereas with different superscripts differ significantly (P≤0.05) in same column.

DISCUSSION

Decontamination of feed and feed stuffs from one of the most potent toxin "AFB1" is a matter of public health and needs special effort to design effective decontamination procedures to avoid spoilage of feed and feed stuff. Several methods have been used to overcome detrimental effects of mycotoxins from contaminated feedstuffs. These include the thermal inactivation and irradiating as physical method, treatment with acid/base solutions, ozonation, and ammoniation as chemical method (Kong *et al*., 2014). A detoxification method must be economically practical to remove traces of AF without leaving harmful residues and must not impair the nutritional quality of the feed stuff (Kubena *et al*., 1998).It is an urgent requirement to design commercially acceptable method for detoxifying AFB1 for application in the food industry.

Four commercially toxin binders were incorporated into the AFB1 contaminated diet. In current study, best toxin binding ability was shown by hydrated sodium calcium aluminosilicates, which is active ingredient of Myco AD and then by Vitamin E and selenium. During a study, the lymphocyte proliferation decreased in ducks fed contaminated maize diets, which might have resulted from oxidative stress and greater production of reactive oxygen species. They could promote the release of glucocorticoids and inhibit immune cell proliferation. Dietary addition of vitamin E and Se tended to alleviate the reduction in lymphocyte proliferation. Similar findings were observed in weanling pigs (Yuan *et al*., 2007) and cattle (Reddy *et al*., 1987) receiving oxidative stress, which is in line with the findings of this study (He *et al*., 2013). Findings of the present study are coherent with the previous reports of retarded growth during aflatoxicosis (Rashid *et al*., 2012). AFB1 ingestion has been reported to decrease feed intake and weight gain in broiler birds (Kaoud, 2012), which is in line with the findings of this study. During detoxification, Guo *et al*.,(2012) reported protective effect of selenium to significantly ameliorate AFB_1 negative effect on ducklings' growth performance and the immune organs' development. This can be explained as vitamin E supports the integrity of long-chain polyunsaturated fatty acids in the membranes of cells and hence maintains their signaling molecules which could be altered by oxidative stress (Traber., 2007). Kaolin is simple stratified clay consisting of the mineral kaolinite. In systemic mineralogy, kaolinite ranks among the phyllosilicates, which are minerals formed by a net of tetrahedral and octahedral layers (Gilani *et al*., 2016). In a study in Iran, koalin was reported to alleviate hepatic changes induced by AFB1 and made fatty changes in liver disappear in 45 days which is in line with the findings of current study. Furthermore, it has been indicated that zeolite, bentonite, and kaolin at a1.5% level in the diet significantly improved the growth rate of broiler chickens (Gilani *et al*., 2016). In present study Hb content was reduced significantly which was also reported, which indicates anemia (Fapohunda *et al*., 2012). This study reports decrease in PCV and ESR, which is in agreement with the findings of Umar *et al*. (2012), which might have occurred due to abnormalities in protein synthesis. Edrington *et al*.,(1997) supplemented super activated charcoal (0.5%) to AF contaminated (4 ppm) broiler diet and active charcoal alleviated the toxic effects of toxicities on growth performance, hematology and biochemistry of treated birds. These findings are in agreement with the findings of this study. Activated charcoal is non-adsorbable carrier that adsorbs to toxic molecules, thereby eliminating their absorption from the intestinal tract (Edmunds *et al*., 2016).Kaolin and activated charcoal (0.5%) to when added to AF contaminated (30 ppb) broiler diet, ameliorated the toxic effects of AF on performance but did not reduce the histopathological changes associated with aflatoxicosis. Furthermore, activated charcoal moderately alleviated the toxic effects of aflatoxin on growth performance, hematology and biochemistry of treated birds, which is in agreement with the findings of this experiment (Hesham *et al*.,2004). Generally, the absorption properties of activated charcoal AC are strictly dependent on the source materials and physico-chemical parameters, such as surface area and pore size distribution. Its adsorption property was found effective against AFB1 and ochratoxinA up to 95% and 91%, respectively (Galvano *et al*., 2001). The information on the amount of activated charcoal to be added to the feed and possibly long term effects on adsorption of essential nutrients are scanty. Charcoal at 2% level had shown beneficial effects on GIT tract histology during in vivo studies (Adebiyi *et al*., 2015). Activated charcoal has been proven an effective adsorbent of deoxynivalenol (DON), ZON, AFB1, fumonisin B1 (FB1) and OTA (Devreese *et al*., 2012).Nevertheless, its unspecific binding is the major drawback in the practical use of activated charcoal as a feed additive. It diminishes nutrient absorption, such as vitamins and minerals, and consequently impairs the nutritional value of feed (Avantaggiato *et al*., 2004 and Devreese *et al*., 2013).

Yunus *et al*., (2011) determined the immunotoxicity of AFB_1.The results indicated that AFB_1 dietary exposure (0.4 to 2%) decreases humoral immunity while inducing an inflammatory response. These impairments in the immune response could curtail the success of vaccination protocols, increasing susceptibility to infections ingesting AFB_1. Similar findings were found in current study, which showed immunosupression in quails fed with AFB_1

The findings of the present study are in agreement with the results reported by Casarin *et al*., 2005 who used Myco-AD to remove the toxic effects of

aflatoxin B1 and ochratoxin in broiler chicks. This study of chemical detoxification of AFB1 concludes the use of commercially available chemical toxin binders in preventing the negative effects of AF in experimental quails. This study proves the use of toxin binders in quail diet to alleviate AFB1 toxicity in quails which is the competitive source of protein when compared to broiler birds in developing countries including Pakistan.

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