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RESEARCH ARTICLE

Aflatoxicosis and Ochratoxicosis in Broiler Chicks and their Amelioration with Locally Available Bentonite Clay

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ABSTRACT

The present study was designed to investigate the ameliorative ability of locally available bentonite clay (BN) against aflatoxin B1 (AFB1) and ochratoxin A (OTA) toxicity in broiler chicks. In the first experiment the broiler chicks were kept on feeds contaminated with AFB1 (0.1, 0.2 and 0.6 mg/kg) alone and in combination with bentonite clay (3.7 and 7.5 g/kg). In the second experiment OTA (0.15, 0.3 and 1.0 mg/kg) alone and concurrently with bentonite clay (3.7, 7.5 and 15 g/kg) was fed to the broiler chicks. The dietary addition of AFB1 and OTA alone exerted deleterious effects on the performance, sero-biochemical and oxidative status of the broiler chicks. A concurrent dietary incorporation of BN (3.7 and 7.5 g/kg) ameliorated the adverse effects of 0.1 and 0.2 mg/kg AFB1 on body weight, FCR, serum ALT and urea concentration. Serum creatinine, total protein and albumin concentrations were mostly significantly lower in combination groups suggesting a partial or no amelioration. Dietary incorporation of BN at all levels only partially alleviated the deleterious effects of 0.15 and 0.3 mg/kg dietary OTA on performance, serobiochemical and oxidative stress, but amelioration was absent when the concentration of OTA in the feed was 1 mg/kg. Results suggested that the dietary incorporation of BN (3.7 and 7.5 g/kg) ameliorated the adverse effects of 0.1 and 0.2 mg/kg of AFB1 on various parameters. However, BN at all levels did not alleviate the deleterious effects of OTA.

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INTRODUCTION

Aflatoxins (AF) are the important secondary fungal metabolites of *Aspergillus flavus* and *Aspergillus parasiticus*. Ochratoxins (OT) are mainly produced from *Aspergillus ochraceus* (Klich *et al*., 2009). The warm and humid conditions promote the growth of these fungi render them as ubiquitously natural contaminants of agricultural commodities used as animal feed ingredients, particularly cereals and peanuts (Tedesco *et al*., 2004; El Miniawy *et al*., 2014). Among different aflatoxins, AFB1 is the most potent hepatotoixc, hepatocarcinogenic, immune-suppressive, mutagenic and teratogenic. In recent years, OT has drawn considerable attention because it not only affects the health and performance of animals but also exerts deleterious effects on human health. These AF and OT not only induced disease in chicken, but these may enter into the food chain and exert its deleterious effects in human populations (Khan *et al*., 2013). Therefore, most research has been focused on these mycotoxins and efforts are made to protect the poultry birds from the deleterious effects of these mycotoxins. At present, different strategies have been adopted to minimize the losses associated with mycotoxins in poultry feeds (Makati *et al*., 2001; Kumar *et al*., 2015).

Silicate materials or clay minerals like bentonite have been found effective to ameliorate the toxic effects of mycotoxins (Indresh *et al*., 2013; Saleemi *et al*., 2015). The binding ability of the mycotoxin is not only dependent on the crystalline structure and physical nature of the adsorbent but also on the physiochemical properties of the mycotoxins (Kabak *et al.*, 2006). In Pakistan, bentonite clays are available in different regions, mainly Potohar plateau, Kashmir etc. These bentonite clays are being used for decontamination of vegetable oils to improve color transparency and flavor. However, there is little use of these bentonite clays to decontaminate the poultry feeds from mycotoxins. The present experimental studies elaborate the amelioration of AFB1 and OTA using locally available bentonite clay.

MATERIALS AND METHODS

Production of aflatoxin and ochratoxin: AFB1 and OTA were produced using the pure cultures of *Aspergillus flavus*, link Fries. A (NRRL 6540 and CECT 2687) and *Aspergillus ochraceus*, link Fries. A (CECT: 2948), respectively. AFB1 was produced following the methods of Shotwell *et al*. (1966) while Trenk *et al.* (1971) was followed for the production of OTA. The extracted mycotoxins were quantified by HPLC. BN was procured from the Potohar plateau region of Pakistan.

Birds housing and feed: A total of 840, broiler chicks were procured from a commercial hatchery and were kept on rice husk litter material under standard management conditions. The basal broiler feed comprising of corn and soy meal having 22% total protein contents and 3100 Kcal/kg metabolize able energy was prepared without addition of any toxin binder and antibiotics. After two days of acclimatization period the birds were divided into groups having 30 birds in each and used in two experiments.

Experimental design: The layouts of experiments 1 and 2 have been presented in Table 1. In experiment 1, broiler birds were divided into 12 groups and three levels of AFB1 (0.1, 0.2 and 0.6 mg/kg) and two levels of BN (3.7 and 7.5 g/kg) were administered in the feed alone and concurrently in different combinations. Similarly, in experiment 2, 480 day old broiler chicks were divided into 16 groups and three levels of both OTA (0.15, 0.3 and 1.0 mg/kg) and BN (3.7, 7.5 and 15 g/kg) were fed to the birds alone and concurrently in different combinations.

Parameters studied:

Body weight and feed conversion ratio: Body weight and feed conversion ratio (FCR) were determined at the end of the experiment.

Serum biochemical parameters: The blood collected from the wing vein of the birds prior to killing on the 42nd day of each experiment was allowed to clot to separate serum. The collected serum was used to determine serum alanine aminotransferase (ALT), urea and creatinine concentrations using commercially available kits (Merck). Serum total proteins and albumin concentrations were determined by Biuret method and bromocresol green dye-binding method, respectively (Anonymous, 1984). The serum globulin concentration was determined by subtracting the serum albumin concentration from that of total proteins.

The total antioxidant capacity: The total antioxidant capacity (TAS) was determined in plasma, liver, kidney and muscle of the broiler chicks following the method as described by Erel (2004).

Statistical analysis: The data obtained was subjected to the statistical analysis by analysis of variance. The means of different groups were compared by Duncan's multiple range test. The level of significance was 0.05 or lower. The MSTATC statistical software package was used for this purpose.

Table 1: Layout of experiments 1 and 2

Sr. No.	Groups	Different Combinations of bentonite and AFB1 added to feed (Experiment 1)
1	Control	Control
2	A1	AFB1 (100 ng/g)
3	A2	AFB1 (200 ng/g)
4	A3	AFB1 (600 ng/g)
5	B1	Bentonite (3.7 g/kg)
6	B2	Bentonite (7.5 g/kg)
7	A1B1	AFB1(100 ng/g) + Bentonite (3.7 g/kg))
8	A2B1	AFB1(200 ng/g) + Bentonite (3.7 g/kg)
9	A3B1	AFB1(600 ng/g) + Bentonite (3.7 g/kg)
10	A1B2	AFB1(100 ng/g) + Bentonite (7.5 g/kg)
11	A2B2	AFB1(100 ng/g) + Bentonite (7.5 g/kg)
12	A3B2	AFB1(100 ng/g) + Bentonite (7.5 g/kg)
Different combinations of ochratoxin A and bentonite added to feeds (Experiment 2)		
1	Control	Control
2	O1	OTA (150 ng/g)
3	O2	OTA (300 ng/g)
4	O3	OTA (1000 ng/g)
5	B1	Bentonite (3.7 g/kg)
6	B2	Bentonite (7.5 g/kg)
7	B3	Bentonite (15 g/kg)
8	O1B1	OTA (150 ng/g) + Bentonite (3.7 g/kg)
9	O2B1	OTA (300 ng/g) + Bentonite (3.7 g/kg)
10	O3B1	OTA (1000 ng/g) + Bentonite (3.7 g/kg)
11	O1B2	OTA (150 ng/g) + Bentonite (7.5 g/kg)
12	O2B2	OTA (300 ng/g) + Bentonite (7.5 g/kg)
13	O3B2	OTA (1000 ng/g) + Bentonite (7.5 g/kg)
14	O1B3	OTA (150 ng/g) + Bentonite (15.0 g/kg)
15	O2B3	OTA (300 ng/g) + Bentonite (15.0 g/kg)
16	O3B3	OTA (1000 ng/g) + Bentonite (15.0 g/kg)

RESULTS

Experiment 1 (Aflatoxicosis): The body weights (Fig. 1) of groups A1, A2, A3, A2B1, A3B1 and A3B2 at day 42 of the experiment were significantly lower than that the of control group. FCR values (Fig. 2) of groups A1, A2, A3 A3B1 and A3B2 were higher than that of the control group. Groups B1, B2, A1B1, A2B1 and A2B2 had lower FCR than those of groups fed AFB1 alone and also in close proximity of the control group.

Serum biochemical parameters of broiler chicks kept on different dietary levels of AFB1 and BN have been presented in Table 2. The serum ALT concentration of groups A2, A3, A3B1 and A3B2 was significantly higher than the corresponding value of the control group. Serum urea concentration had significantly higher value in groups A1, A2, A3, A1B1, A2B1, A3B1 and A3B2 in comparison to the control group. The concentration of serum total protein of control group was significantly higher from all other experimental groups. Serum albumin concentration of the control group had non-significant difference from groups B1, B2 and A1B2. The concentration of serum globulin had significantly lower value in groups A2, A3 and A3B1. The total antioxidant capacity (Table 3) in plasma, liver, kidney and muscle of the control group had a non-significant difference from groups B1 and B2.

Experiment 2 (Ochratoxicosis): The body weight (Fig. 3) of groups B1 and B2 at day 42 had non-significant difference from control while all other groups had significantly lower values than that of control.

The FCR values (Fig. 4) of group B3 and all the groups fed OTA alone or in combination with different levels of BN had higher values than control while the groups B1 and B2 had FCR values in close proximity to that of the control group.

Table 2: Serum biochemical parameters (Mean±SD) of broiler chicks given different dietary levels of AFB1 and Bentonite Clay

Group	ALT (IU/µL)	Urea (mg/100 ml)	Creatinine (mg/100 ml)	Total protein (g/100 ml)	Albumin (g/100 ml)	Globulin (g/100 ml)
Control	19.81±3.97e	11.30±1.58g	0.46±0.05h	4.78±0.27a	3.46±0.18a	1.31±0.15a
A1	23.74±3.94de	16.17±2.58de	0.99±0.03d	4.27±0.09cd	3.16±0.20bc	1.12±0.15abc
A2	27.05±4.28bcd	19.93±2.21bc	1.54±0.05c	4.11±0.05d	3.15±0.11bc	0.96±0.15bc
A3	36.19±3.41a	29.62±2.79a	2.14±0.04a	3.62±0.05e	2.77±0.35d	0.85±0.36c
B1	20.09±3.31e	11.90±1.47fg	0.54±0.04g	4.77±0.16b	3.36±0.14ab	1.15±0.23ab
B2	22.29±4.67de	13.19±1.30fg	0.61±0.03f	4.49±0.16b	3.36±0.10ab	1.14±0.17ab
A1B1	23.99±3.35de	14.69±1.69ef	0.62±0.04f	4.43±0.25bc	3.23±0.13bc	1.20±0.29ab
A2B1	24.93±4.62cde	17.77±2.21cd	0.79±0.04e	4.33±0.09bc	3.19±0.07bc	1.14±0.12ab
A3B1	31.20±4.40b	21.21±2.92b	1.72±0.07b	4.08±0.14d	3. 08±0.09c	1.00±0.14bc
A1B2	22.45±2.39de	13.10±1.87efg	0.55±0.05g	4.40±0.11bc	3.29±0.14abc	1.11±0.09abc
A2B2	24.17±3.87de	12.75±1.59efg	0.82±0.04e	4.32±0.24bc	3.25±0.10bc	1.08±0.24abc
A3B2	29.19±3.69bc	18.25±4.25cd	1.52±0.06c	4.24±0.12cd	3.19±0.19bc	1.06±0.22abc

Values in each column followed by different letters are significantly (P<0.05) different.

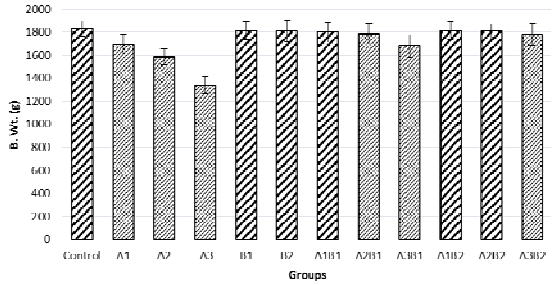

Fig. 1: Body weight (g) of broiler chicks given different dietary levels of AFB1 and Bentonite Clay (Mean±SD). Bar column bearing different filling pattern are significantly different (P<0.05).

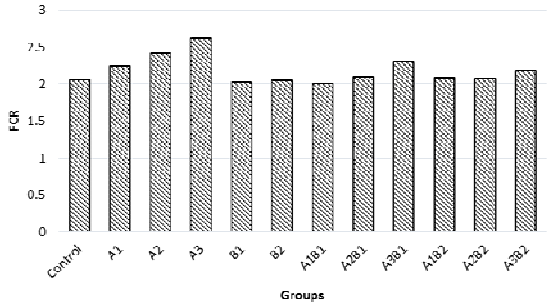

Fig. 2: Feed conversion ratio of broiler chicks given different dietary levels of AFB1 and Bentonite Clay.

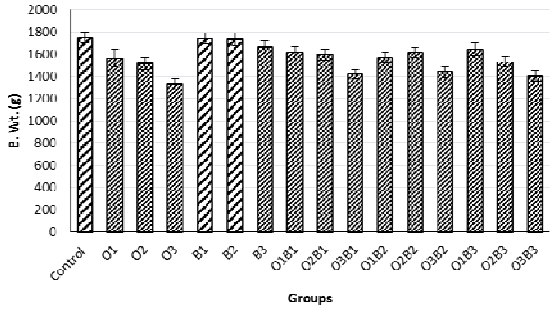

Fig. 3: Body weight (g) of broiler chicks given different dietary levels of OTA and Bentonite Clay (Mean±SD). Bar column bearing different filling pattern are significantly different (P<0.05).

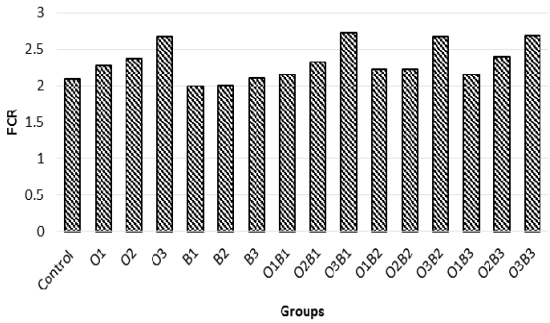

Fig. 4: Feed conversion ratio of broiler chicks given different dietary levels of OTA and Bentonite Clay.

Table 3: The mean total antioxidant capacity (Mean±SD) (TAC, mmol Vit. Eequiv. L^{-1}), of broiler chicks given different dietary levels of AFB1 and Bentonite Clay

Group	Plasma	Liver	Kidney	Muscle
Control	3.44±0.05a	2.99±0.08a	2.75±0.08a	0.98±0.03a
A1	3.29±0.03c	2.83±0.09c	2.60±0.06cd	0.84±0.04cd
A2	3.20±0.02d	2.73±0.11d	2.51±0.04e	0.74±0.02e
A3	2.97±0.05g	2.10±009f	2.31±0.05g	0.54±0.03h
B1	3.43±0.03a	2.96±0.11ab	2.74±0.04a	0.97±0.03a
B2	3.41±0.02a	2.95±0.07ab	2.72±0.03ab	0.96±0.02a
A1B1	3.35±0.02b	2.87±0.10bc	2.65±0.02bc	0.89±0.03b
A2B1	3.28±0.02c	2.82±0.04c	2.58±0.04d	0.81±0.03d
A3B1	3.09±0.05f	2.63±0.10e	2.39±0.03f	0.62±0.05g
A1B2	3.36±0.04b	2.88±0.04bc	2.66±0.05bc	0.89±0.03b
A2B2	3.33±0.05b	2.84±0.04c	2.63±0.06cd	0.87±0.04bc
A3B2	3.14±0.04e	2.66±0.05de	2.44±0.06f	0.66±0.03f

Values in each column followed by different letters are significantly (P<0.05) different.

Serum biochemical parameters of broiler chicks kept on different dietary levels of OTA and BN have been presented in Table 4. Serum ALT concentration of the control group had non-significant difference from groups B1, B2, B3, O1B1, O2B1 and O1B2.The serum urea concentration of the groups B1, B2, O1B1, O2B1, O1B2, O2B2 and O1B3 had non-significant differences from control. Serum total protein and albumin concentrations of all the experimental groups had significantly lower values than the control group. The concentration of serum globulin of the control group had non-significant difference from groups B1 and B2.

The total antioxidant capacity (Table 5) in plasma and muscle of the control group had a non-significant difference from groups B1 and B2. The total antioxidant capability in the liver and kidney of the control group differed non-significantly from groups B1, B2 and B3.

DISCUSSION

AFB1 and OTA are of great concern due to the deleterious health and production effects in livestock and poultry birds and therefore, the majority of the research is focused on these mycotoxins (Battacone *et al*., 2010; Zahoor-ul-Hassan *et al*., 2010). The injurious effects reported earlier have also been reproduced in the experimental feeding of AFB1 and OTA in the present study.

Table 4: Serum biochemical parameters of broiler chicks given different dietary levels of OTA and Bentonite Clay (Mean±SD)

Group	ALT (IU/µL)	Urea (mg/100 ml)	Creatinine (mg/100 ml)	Total protein (g/100 ml)	Albumin (g/100 ml)	Globulin (g/100 ml)
Control	21.46±3.54h	15.09±2.27f	0.34±0.02	5.56±0.04a	3.91±0.07a	1.64±0.09a
O1	32.76±3.72d	19.10±1.50de	0.38±0.01	3.98±0.06g	3.33±0.03g	0.66±0.06g
O2	36.81±3.37bc	20.75±3.07cd	0.42±0.01	3.62±0.19i	3.17±0.05h	0.44±0.23h
O3	44.89±2.56a	30.89±2.42a	0.53±0.01	2.69±0.05k	2.50±0.05k	0.19±0.08i
B1	23.13±3.43fgh	15.58±2.58f	0.34±0.01	5.37±0.06b	3.73±0.04bc	1.64±0.05a
B2	4.92±3.12efgh	15.85±1.79f	0.34±0.01	5.18±0.05c	3.66±0.07cd	1.52±0.09a
B3	4.10±2.51efgh	22.78±3.11bc	0.34±0.01	4.66±0.06d	3.55±0.14ef	1.11±0.14b
O1B1	23.18±2.49gh	15.05±2.27f	0.34±0.01	4.47±0.08e	3.70±0.04cd	0.77±0.11efg
O2B1	5.39±1.84efgh	16.52±1.91ef	0.35±0.01	4.59±0.08d	3.46±0.22f	1.13±0.22b
O3B1	34.43±4.31cd	21.21±2.55cd	0.41±0.01	3.19±0.05j	2.92±0.05j	0.27±0.09i
O1B2	23.61±2.92fgh	15.28±2.25f	0.33±0.01	4.59±0.07d	3.68±0.05cd	0.91±0.08cde
O2B2	27.17±2.43ef	16.12±1.85f	0.34±0.01	4.47±0.04e	3.48±0.08f	0.99±0.11bcd
O3B2	36.84±3.20c	20.69±2.41cd	0.39±0.01	3.89±0.06h	3.03±0.05i	0.86±0.10de
O1B3	26.31±2.57efg	16.79±1.83ef	0.35±0.01	4.47±0.05e	3.80±0.03b	0.67±0.06fg
O2B3	28.43±2.32e	20.67±1.99cd	0.36±0.01	4.41±0.05e	3.61±0.04de	0.81±0.08ef
O3B3	40.42±3.46b	24.27±2.87b	0.45±0.01	4.19±0.05f	3.18±0.05h	1.01±0.06b

Values in each column followed by different letters are significantly (P<0.05) different.

Table 5: The total antioxidant capacity (TAC, mmol Vit. Eequiv. L-1), of broiler chicks given different dietary levels of OTA and Bentonite Clay (Mean±SD)

Group	Plasma	Liver	Kidney	Muscle
Control	3.15±0.04a	2.86±0.08a	2.66±0.10a	0.94±0.04a
O1	2.80±0.04e	2.52±0.10d	2.30±0.09c	0.59±0.04e
O2	2.30±0.04h	2.02±0.09g	1.80±0.09f	0.35±0.03g
O3	1.32±0.05l	1.05±0.11j	0.83±0.10i	0.17±0.04h
B1	3.11±0.04a	2.84±0.08a	2.63±0.09a	0.90±0.04a
B2	3.13±0.04a	2.85±0.06a	2.64±0.09a	0.92±0.04a
B3	3.04±0.04b	2.77±0.09ab	2.56±0.09ab	0.83±0.04b
O1B1	2.92±0.04d	2.64±0.06c	2.45±0.10b	0.71±0.04d
O2B1	2.43±0.06g	2.14±0.09f	1.92±0.08e	0.43±0.04f
O3B1	1.72±0.05k	1.44±0.09i	1.22±0.11h	0.30±0.11g
O1B2	2.99±0.06bc	2.72±0.05bc	2.50±0.10b	0.78±0.06bc
O2B2	2.67±0.05f	2.39±0.08e	2.19±0.09d	0.46±0.05f
O3B2	1.89±0.04i	1.62±0.07h	1.41±0.07g	0.32±0.04g
O1B3	2.97±0.05cd	2.67±0.10bc	2.48±0.10b	0.76±0.05cd
O2B3	2.64±0.06f	2.36±0.09e	2.16±0.09d	0.43±0.06f
O3B3	1.81±0.04j	1.54±0.10h	1.32±0.09gh	0.23±0.04h

Values in each column followed by different letters are significantly (P<0.05) different.

In experiment 1, the dietary addition of BN at graded levels (3.7 and 7.5 g/kg) to the control group did not affect the overall performance of the broiler chicks. In the groups fed BN concurrently with first two dietary levels of AFB1 (0.1 and 0.2 mg/kg) the body weight and FCR were comparable to that of control group suggesting a complete amelioration. However, in the groups fed 0.6 mg/kg AFB1 concurrently with different dietary levels of BN the significantly lower body weight and higher FCR value suggesting a partial or no amelioration at higher AFB1 levels. The improvement might be due to the irreversible binding of AFB1 in the gut and therefore, prevented its systemic availability. The decrease in body weight and increased FCR may be due to liver damage and inhibitory effect on protein synthesis (Johri and Majmudar. 1990). These results were in agreement with previous studies of Indresh *et al*. (2013) and Pasha *et al.* (2007) who reported the protective effect 1.0 and 0.5 % bentonite against the 500 ppb and 100 ppb of total aflatoxin, respectively. However, in the present study attempt was made to observe the ameliorative ability of graded doses of BN against three graded AFB1 levels.

Serum biochemical changes in ALT concentration were non-significant in the groups fed 0.1 and 0.2 mg/kg AFB1, while serum urea concentration was non-significant at level 0.2 mg/kg AFB1 fed concurrently with BN. Serum creatinine, total protein, albumin and globulin concentrations were mostly significantly lower in combination groups suggesting a partial or no amelioration by BN in these parameters. Rosa *et al*. (2001) and Santurio *et al*. (1999) did not observe the protective effect of sodium bentonite (2.5, 3 and 5 g/kg) on biochemical parameters against 3 and 5 mg/kg, dietary AFB1. However, Che *et al*. (2011) reported the re-establishment of serum biochemical parameters in broiler birds fed mold contaminated diet (450.6 ppb AFB1) concurrently with 0.2% hydrated sodium calcium alimunosilicate (HSCAS).

The protective response of BN was absent in terms of oxidative stress as accessed by the total antioxidant capacity of the broiler birds fed AFB1 contaminated diets. Similar to the present study Che *et al*. (2011) reported partial amelioration of HSCAS (0.2%) on the hepatic oxidative status of broiler birds fed mold-contaminated diet. However, no author has so far, reported aflatoxin induced oxidative stress studies in different organ or tissues including plasma, kidneys and muscles and their amelioration by BN as reported in the present work.

In experiment 2, broiler chicks fed 0.37 and 0.75 g/kg dietary BN levels had no deleterious effects on different parameters studied. Different authors have reported no harmful effects of different clays upon the body weight gains and serum biochemical parameters in poultry birds (Safaeikatouli *et al*., 2010; Indresh *et al*., 2013). However, the deleterious effects observed at higher level of dietary BN (15 g/kg) used in the present study might be due to un-specific and broad binding spectra (Ramos *et al*., 1996). At higher dietary level (1.5%) Khanedar *et al*. (2012) has also reported the deleterious effects of bentonite.

The increased FCR and decreased body weight observed in groups fed OTA and BN concurrently were in agreement with the previous findings of Santin *et al*. (2002) and Watts *et al*. (2003) who fed 2 and 0.5 ppm of dietary OTA, respectively. However, the non-significant difference in average daily weight gain and feed intake from control reported by Che *et al*. (2011) by dietary addition of 0.2% HSCAS in broiler feed might be due to the lower dietary contamination of OTA (68.4 µg/kg) compared to the levels used in the present study.

Alterations in different serum biochemical parameters induced by OTA were not completely ameliorated by concurrent feeding of BN. Garcia *et al*.

(2003) and Watts *et al*. (2003) reported that the dietary addition of aluminosilicates based binder did not ameliorate the OTA induced toxic effects on serum biochemical parameters in the broiler diet. The possible reason might be the fairly non-polar nature of the OTA and until now, no single adsorbent has been revealed effective against most types of mycotoxins (Denli and Perez, 2010; Sirhan *et al.*, 2012).

No amelioration was observed in combination groups in terms of total antioxidant capacity of the birds. However, previously no work has been reported on the oxidative stress of the broiler birds fed OTA concurrently with BN.

Conclusions: The results collectively suggested that dietary incorporation of BN (3.7 and 7.5 g/kg) ameliorated the adverse effects of 0.1 and 0.2 mg/kg of AFB1 on various parameters examined in this study. At higher dietary level of AFB1 (0.6 mg/kg) a partial amelioration was observed. The OTA induced injurious effects on production and health parameters in broiler chicks could not be alleviated by dietary incorporation of BN at all levels used.

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