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Effect of aflatoxin on haematology, gross and histopathology of internal organs in white pekin ducks and its amelioration by dietary incorporation of bentonite clay

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ABSTRACT

The present experiment was carried out to examine the effect of aflatoxin exposure through experimentally contaminated feed (48 or 98 µg/kg of feed) on hematological parameters, and gross and histopathology of liver, kidneys, thymus, spleen and bursa of fabricious in ducks, and to assess the prophylactic efficacy of bentonite clay at three different dose levels (1, 2 or 3g/kg of feed) in protecting the birds from the toxic effects of aflatoxin. The experiment was carried out on 9 weeks old white pekin ducks for a period of six weeks. The experimental ducks (n=72) were randomly divided into six equal groups. Group 1 birds served as healthy controls and were continued with basal diet without addition of toxin or bentonite clay. The birds of Group 2 were provided with a diet containing 48 µg toxin/kg of feed. The diet of the grower ducks of Group 3 to 6 were added with 96 µg of toxin/kg of feed. The birds of Group 4, 5, and 6 were provided with aflatoxin-contaminated feed and the diet was supplemented with bentonite clay @ 1, 2 or 3g/kg of feed, respectively. The hematological parameters such as Hb, PCV, TLC and Differential Leukocyte Count were estimated at 4th and 6th week post exposure. Haemoglobin, PCV and TLC reduced significantly in ducks fed with aflatoxin-contaminated feed. The birds fed with 48 µg aflatoxin/kg of feed had moderate gross lesions in different internal organs. The feed supplementation with bentonite clay in aflatoxin-exposed birds reduced the inflammation of hepatocytes.

Keywords: Aflatoxin, Bentonite clay, Gross pathology, Hematology, Histopathology

Ducks constitute about 10% of the total poultry population in India, and contribute about 6-7% of total eggs produced in the country. Ducks are more preferred for farming over chicken by small and medium scale producers as they require lesser management attention and thrive well in scavenging conditions, thus reducing feeding cost. The White Pekin duck (*Anas domesticus*), the most popular duck breed in the world, was developed in China. It has a large body, orange feet and beak, and creamy white feathers.

Ducks are sturdy against many diseases that affect other poultry birds. Mycotoxicosis is the most important non-infectious disease in ducks, and aflatoxicosis is very common. Aflatoxin imposes toxic effects in animals and birds upon its consumption through contaminated feed, and affects health and productivity. Ducks are most sensitive to aflatoxin among the poultry (Bennett and Klich 2003). The chronic exposure to aflatoxin, even at low doses, can cause growth retardation, severe damage of various organs including liver, spleen, heart, thymus, bursa of fabricious and kidney, and inflicts hemorrhagic syndrome and mortality. Thus, the poultry producers require cost-effective methods of detoxifying potentially contaminated grain (Fowler *et al*. 2015).

Detoxification and inactivation of aflatoxins include use of binders or sequestering agents, added to feed, to reduce toxicity of mycotoxins by reducing reactivity of bound mycotoxins, and intestinal absorption. The use of binders offers an alternate approach over salvaging of feeds with low levels of mycotoxins, and to protect animals and birds from the low levels of mycotoxins that routinely occur and cause chronic toxicity problems, including decreased performance. The present investigation examined the effect of aflatoxin-contaminated feed in grower ducks on hematological parameters and histopathology of liver, kidney and bursa of fabricious and to evaluate the efficacy of dietary supplementation of sodium bentonite clay in protecting the ducks from toxic effects of aflatoxin.

MATERIALS AND METHODS

The present investigation was carried out using 72 day-old White pekin ducks with the approval of Institute Animal Ethical Committee (IAEC) (Letter No. 51/IAEC

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dated 07.01.2017). Ducklings were provided with standard duck feed (Table 1)and *ad lib.* water, and vaccinated against duck cholera and duck plague. The grower ducks (n=72) were randomly divided into six experimental groups with twelve birds in each group. The grower birds of Group 1 (n=12) served as healthy controls and were provided with basal diet without addition of toxin or bentonite clay. The birds of Group 2 were provided with diet containing 48 µg toxin/kg of feed. The ducklings of Group 3 to 6 were fed with diet containing 96 µg of toxin/kg of feed. Toxin containing feed was fed during the grower stage from 9th week onwards for a period of 6 weeks.

Experimental design: Purified toxin for the experiment was procured from commercial source. Toxin was added to the feed with different desired proportions (48 or 96 µg/kg) though premix making. Handling of toxin was done with strict bio-safety measures. The birds of Group 4, 5, and 6 were provided with feed containing 96 µg aflatoxin/kg of feed along with bentonite clay at the dose rate of 1, 2 or 3g of clay/kg of feed, respectively (Table 2).

Table 1. Composition of basal diet fed to the ducks

MERCK feed ingredient	Duck grower
DORB (kg)	96
Soyabean (kg)	
Fish meal (kg)	6
Oysters shell grit (kg)	0
DCP (kg)	1.5
Calcite (kg)	1.5
Trace min. (kg)	1
DL-Metheonine (g)	150
L-Lysine (g)	195
Vit.A, D3, B2 & K (kg)	45
Vit.E & Se (g)	90
B Complex (g)	60
Toxin binder (g)	450
Ch. Chloride (g)	300
Wheat (kg)	159
Total (kg)	300

Feed preparation: Proximate analysis of the feed samples was done before feeding to the ducks (Table 1). Medicated ration was prepared on weekly basis and kept in closed containers for feeding of ducks. The required amount of aflatoxin was dissolved in acetone. This was sprayed over a small amount of feed which was thoroughly mixed with bulk feed to get the required quantity of feed with desired level of aflatoxin.

Clinical sign: The birds were observed daily for any changes in their behavioural pattern throughout the day, especially during noon hours when the sun is at peak.

Collection of samples: The peripheral venous blood samples were collected from the brachial vein of ducks using commercially available EDTA vials for estimation of hematological parameters. Tissue samples from ducks exposed to aflatoxin were collected in 10% buffered neutral formalin solution after completion of feeding experiment of 6 weeks duration, after sacrificing birds for gross and histopathological examinations of liver, spleen, kidney, thymus, and bursa.

Hematological study: The packed cell volume (PCV) estimation was done by Wintrob's haematocrit method. Haemoglobin was estimated by Sahli's haemoglobinometer and differential count (Heterophils, Eosinophils, Basophil, Lymphocytes and Monocyte %) were carried out after staining of blood smears with modified Giemsa stain.

Statistical analysis: The data were analyzed by applying unpaired student 't' test and two way analysis of variance (ANOVA) followed by Bonferroni's post test using the Graph Pad Prism v4.03 software program (San Diego, CA, USA), and the differences between the groups were considered statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

There was significant decrease in body weight in Group 2 and 3 in comparison to control group (2308.14±10.94 g) on completion of 4th week of feeding toxin contaminated feed. However, body weight was significantly high in Group 4, 5 and 6 as compared to Group 2 and 3. The aflatoxin treated groups also showed decreased body weight in comparison to the control group after the end of 6th week of feeding aflatoxin contaminated feed.

Haemoglobin level in Group 2, 3, 4, 5 and 6 did not change significantly on 4th week as compared to control (Table 3). Haemoglobin level was significantly lower in Group 2 and 3 in comparison to Group 4, 5 and 6 on 6th week. However, Group 5 and 6 birds showed significantly higher hemoglobin level from all other groups on completion of 6th week of the experiment. The mean PCV level in all groups did not change significantly ($P<0.05$) in comparison to control group on 4th week. There was significant decrease in the mean PCV level in Group 2 and 3 in comparison to Group 1, 4, 5 and 6. However, Group 6 showed significantly higher PCV level in comparison to only aflatoxin treated group, and there was no significant difference from the control group on 6th

Table 2. Experimental design and distribution of ducks into different groups

Group	No. of ducks	Toxin level (in µg/kg feed)	Bentonite clay	Remarks
1	12	0	0g/kg of feed	Negative/ Healthy Control
2	12	48	0 g/kg of feed	Positive Control lower dose
3	12	96	0 g/kg of feed	Positive Control higher dose
4	12	96	1g/kg of feed	Supplemented group
5	12	96	2g/kg of feed	Supplemented group
6	12	96	4g/kg of feed	Supplemented group

Table 3. Effects of bentonite clay on haematological parameters in ducks exposed to aflatoxin

Parameter	wk	Treatment group 1	2	3	4	5	6
Haemoglobin gm%	0	10.288±0.49[a]	9.858±0.37[a]	10.658±0.42[a]	10.312±0.32[a]	9.794±0.30[a]	10.105±0.32[a]
	4	10.343±0.33[a]	9.954±0.31[a]	9.154±0.35[a]	9.323±0.31[a]	9.811±0.23[a]	10.248±0.32[a]
	6	10.308±0.37[a]	9.481±0.41[ab]	8.296±0.40[b]	9.910±0.41[b]	10.040±0.44[b]	11.011±0.41[b]
PCV (%)	0	30.791±1.10[a]	30.845±1.12[a]	30.323±1.12[a]	30.229±1.00[a]	31.111±0.95[a]	31.025±0.92[a]
	4	31.029±0.92[a]	30.462±0.93[a]	30.104±0.90[a]	29.769±0.93[a]	29.933±0.94[a]	30.744±0.95[a]
	6	31.424±1.11[a]	29.709±1.23[a]	29.456±1.2[a]	30.279±1.23[b]	30.782±1.32[b]	31.403±1.23[b]
Heterophil %	0	19.05±1.16[a]	18.26±1.34[a]	19.33±1.29[a]	20.25±1.04[a]	19.51±0.97[a]	19.06±0.96[a]
	4	19.34±0.88[a]	16.12±0.91[ab]	13.69±1.60[b]	15.88±0.89[b]	16.03±0.94[b]	17.94±0.91[ab]
	6	19.86±1.26a	15.74±1.57[b]	12.38±1.89[b]	16.59±1.03[bc]	18.07±0.92[c]	19.57±0.86[c]
Lymphocyte %	0	70.16±1.051[a]	70.46±1.037[a]	70.64±1.205[a]	69.55±1.101[a]	70.63±0.95[a]	70.94±0.91[a]
	4	70.54±1.508[a]	73.92±0.161[ab]	75.47±2.510[b]	74.41±1.209[a]	75.01±1.174[b]	72.47±0.931[ab]
	6	70.06±1.261[a]	76.04±1.907[b]	79.31±2.067[b]	75.98±1.604[b]	73.15±0.85[bc]	70.61±0.811[c]
Monocyte %	0	2.6±0.34a	2.54±0.61[a]	2.61±0.15[a]	2.46±0.67[a]	2.48±0.33[a]	2.63±0.14[a]
	4	2.61±0.68a	1.31±0.31[b]	1.17±0.84 [b]	1.84±0.46[b]	1.98±0.34[b]	2.09±0.16[ab]
	6	2.67±0.62a	0.91±0.67[b]	0.84±0.98[b]	1.92±054b[c]	2.01±0.55[c]	2.31±0.19[c]
Basophil %	0	1.45±0.081[a]	1.5±0.017[a]	1.42±0.046[a]	1.64±0.016[a]	1.64±0.016[a]	1.48±.047[a]
	4	1.40±0.34a	1.151±0.086[a]	1.03±0.094[a]	1.08±0.084[a]	1.24±.074[ab]	1.41±.062[a]
	6	1.49±0.42[a]	1.09±0.047[ab]	1.00±0.115[ab]	1.27±0.051[b]	1.38±0.043[b]	1.54±.018[b]
Eosinophil %	0	5.01±0.08[a]	6.14±0.02[a]	6.21±0.05[a]	6.04±0.02[a]	6.17±0.04[b]	5.98±0.03[a]
	4	5.56±0.04[a]	5.51±0.05[a]	5.45±0.08[a]	5.84±0.02[a]	5.89±0.05[a]	5.95±0.02[a]
	6	5.61±0.04[a]	5.32±0.06[a]	5.22±0.06[a]	5.98±0.06[a]	6.01±0.04[a]	6.45±0.06[b]
TLC ($\times 10^3$)	0	16.3±1.1[a]	15.4±1.0[a]	16.6±1.2[a]	16.4±1.1[a]	16.0±1.0[a]	16.0±0.9[a]
	4	16.1±1.5[a]	14.4±1.6[ab]	12.4±2.5[b]	13.4±1.2[ab]	14.7±1.2[ab]	16.5±0.9[a]
	6	16.8±1.3[a]	13.0±1.9[ab]	10.3±2.1[b]	15.0±1.6[a]	15.7±0.9[a]	17.6±0.8[a]

Data were expressed as mean±SE; n=12; mean data bearing different superscript in a row differ significantly ($p<0.05$) within a parameter between the group.

week of the treatment. There was a significant decrease in total leukocyte count (TLC) on 4th week in Group 2 and 3 as compared to control group. Significant increase in total leukocyte count (TLC) was observed in Group 4, 5 and 6 as compared to only aflatoxin treated groups. On 6th week post exposure, the total leukocyte count (TLC) decreased significantly in both Group 2 and 3. However Group 4, 5 and 6 showed significantly higher TLC as comparison to Group 2 and 3 and no significant difference was observed in TLC in Group 1and 6 on 6th week of the experiment.

The hematological examination revealed significantly higher lymphocyte % in aflatoxin exposed group at the end of 4th and 6th week in comparison to other groups (Table 3). The mean lymphocyte % decreased significantly in bentonite supplemented groups. However, hemoglobin, PCV, heterophil, monocyte, eosinophil, and basophil values were significantly less in aflatoxin-exposed groups. However, these values significantly increased in bentonite supplemented groups. Heterophil level on the 4th week showed a significant decrease in Group 2 and 3 as compared to control group. There was significant increase in heterophil level in Group 4, 5 and 6. On 6th week, the heterophil % decreased significantly in both Group 2 and 3. However, Group 4, 5 and 6 showed significantly higher heterophil level as compared to Group 2 and 3, and no significant difference was recorded between Group 6 and control group on 6th week of the experiment. There was a significant increase in Lymphocyte percentage on 4th week in Group 2 and 3 as compared to control group. Significant decrease in Lymphocyte percentage was observed in Group 4, 5 and 6 as compared to only aflatoxin treated groups. On 6th week post exposure, the lymphocyte % increased significantly in both Group 2 and 3. However, Group 4, 5 and 6 showed significantly lower lymphocyte level in comparison to Group 2 and 3.

A significant reduction in monocyte percentage was seen on 4th week in Group 2 and 3 as compared to control group (Table 3). Significant elevation in monocyte % was observed in Group 4, 5 and 6 compared to Group 2 and 3. On 6th week post exposure, the monocyte % decreased significantly in both Group 2 and 3. However, Group 4, 5 and 6 showed significantly higher monocyte % as compared to Group 2 and 3. On completion of 4th week, there was no significant difference in basophil % in Group 4, 5, 6 in comparison to Group 2 and 3. The aflatoxin treated groups showed very little reduction in basophil % in comparison with the control group. All aflatoxin exposed and bentonite clay supplemented group showed slight increase in basophil %, as compared to only aflatoxin treated group. At the end of 4th week, there was no significant change in eosinophil % in Group 4, 5, 6 in comparison to Group 2 and 3. The aflatoxin treated groups showed very little

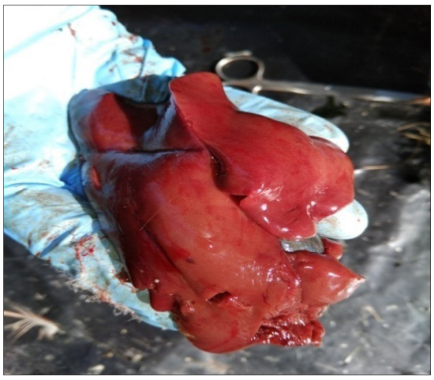

Fig. 1. Liver from birds of Group II fed with aflatoxin @ 48 µgm/kg of feed showing haemorrhagic areas.

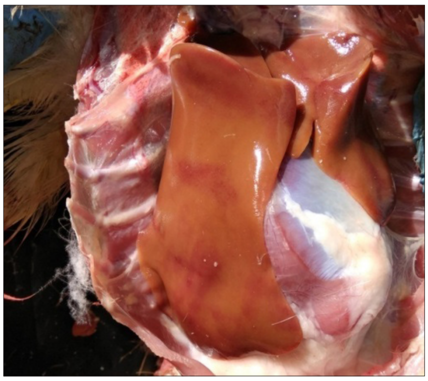

Fig. 2. Liver of birds of Group III fed with aflatoxin @ 96 µgm/kg of feed showing congested and pale areas.

Fig. 3. Heart from birds of Group III fed with aflatoxin @ 96 µgm/Kg of feed, showing petechial hemorrhage.

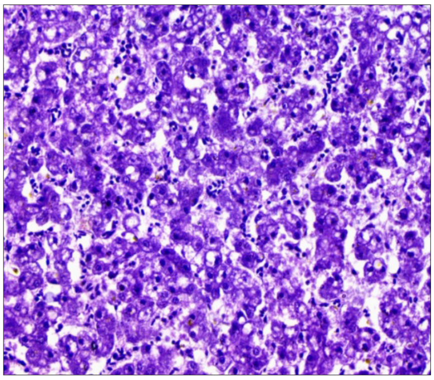

Fig. 4. Group II Liver showing vacuolar degeneration of hepatocytes and diffused congestion (40×) (H&E).

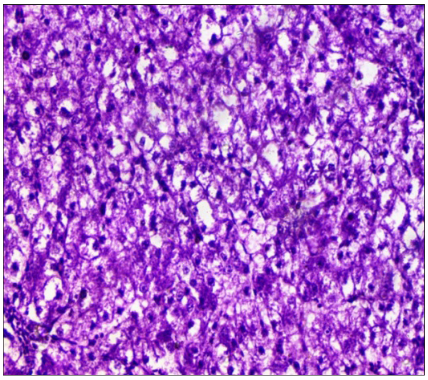

Fig. 5. Group II Liver showing vacuolar degeneration and individualization of hepatocytes (40×) (H&E).

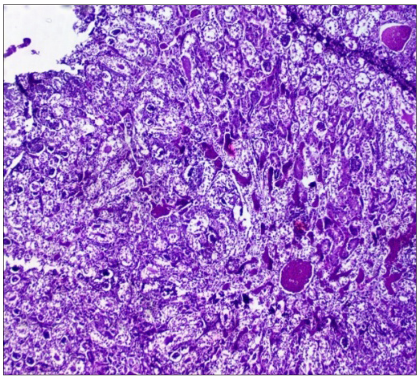

Fig. 6. Group II Kidney showing degeneration of tubular epithelium with marked congestion (10×) (H & E).

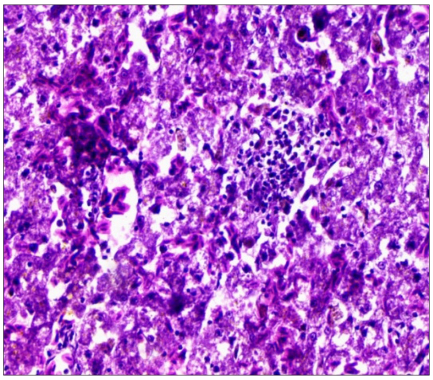

Fig. 7. Group III Liver- Necrotic hepatocytes with focal infiltration of inflammatory cells (40×) (H&E).

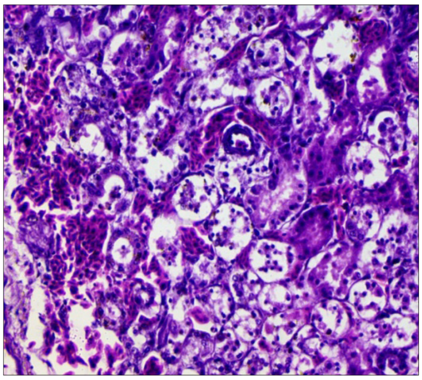

Fig. 8. Group III Kidney showing degeneration and necrosis of tubular epithelium with interstitial congestion and hemorrhage (40×) (H&E).

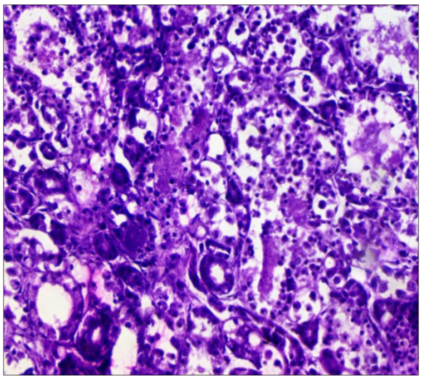

Fig. 9. Group III Kidney- Necrotic tubular epithelium with interstitial infiltration of inflammatory cells (40×) (H&E).

reduction in eosinophil % in comparison with the control group. All aflatoxin exposed and bentonite clay supplemented group showed slight increase in eosinophil %, as compared to only aflatoxin treated group.

Gross and histopathological changes: The birds fed with 48 µg aflatoxin/kg of feed showed moderate gross lesions in different organs including liver (Fig. 1), heart, and spleen. The liver showed pale and congested areas (Figs 4, 5). Heart showed moderate hypertrophy with petechial hemorrhage in the pericardium. Kidney was congested in most of the birds (Fig. 6). Thymus and bursa of fabricious showed no significant lesions on completion of 6th week of experiment. On post mortem examination of birds fed with 96µg of aflatoxin/kg of feed for 6 weeks, there were severe lesions in different organs revealing the toxic effect of aflatoxin (Figs 2, 3, 7, 8 and 9). The liver showed varying areas of congestion and hemorrhagic areas. Liver of some birds were dark reddish in colour as a result of severe congestion, whereas the liver of some birds were completely pale showing severe anemic appearance. The liver from most of the birds of this group showed rounded edges instead of normal sharp edge with varying degree of inflammation and hypertrophy. Kidneys of the birds were soft and friable and easily broken when palpated. Spleen showed severe mottling with dark red to brownish appearance. The hearts showed petechial hemorrhagic areas on both pericardium and endocardium. Thymus also showed areas of petechial hemorrhage. The bursa of fabricious showed varying degrees of inflammation. There was significant increase in most of the organs weight, as compared to control group. On microscopic examination, the liver showed centrilobular and perilobular hypertrophy of hepatocytes in Group II (Figs 4 and 5) and III (Fig. 7). The hepatocyte also revealed hydropic and vacuolar degeneration with some areas showing coagulative necrosis, mostly in the periportal areas. Varying degree of hemorrhages was seen with presence of degenerated red blood cells. There was infiltration of mononuclear cells in the hepatocytes with severe hypertrophy. The degenerated hepatocytes showed presence of large vacuoles at places. The droplets showed positive for Sudan III showing fatty infiltration and fatty degeneration. Microscopic study of spleen revealed necrosis of lymphoid follicles with degeneration and hyperplasia of lymphoid cells. There was severe rarefaction of lymphoid cells in spleen of birds receiving 96 µg aflatoxin/kg of feed for 6 weeks. The kidneys showed varying degree of inflammation of nephrons, mostly glomerulonephritis (Figs 8 and 9). The bursa of fabricious showed vacuolar degeneration with infiltration of mononuclear cells in the trabeculae. Heart showed hypertrophy of cells with infiltration of degenerated red blood cells. The groups fed with bentonite clay along with aflatoxin in the diet showed improvement in the lesions with moderate inflammation of hepatocytes. Grossly, the congestion was also found to be less severe. Spleen revealed very few areas of necrosis and degeneration in lymphoid follicles. Reduced discolouration of various organs was found as compared to organs from birds maintained with aflatoxin contaminated and bentonite non-supplemented feed.

Aflatoxin is mainly hepatotoxic. However, it has varied other toxic effects on body systems like immunotoxicity, carcinogenicity, blood clotting, protein utilization and many more. Bio-activation of AFB1 predominantly occurs in hepatocytes after absorption in the small intestine, especially the duodenum. Cytochrome P450 isoenzyme system metabolises aflatoxin B1 into reactive and electrophilic exo-AFB18,9-epoxide. This AFBO forms adducts with DNA, RNA and protein mediating the toxic effects of aflatoxin. AFB1-DNA and AFB1-RNA adducts can inhibit transcription and translation, induce DNA mutations during DNA repair and replication, and even initiate apoptosis or carcinogenesis.

Experimental contamination of diet with aflatoxin caused a significant reduction in body weight gain of the grower ducks by completion of 4^{th} and 6^{th} week of feeding in a dose dependant manner. Diets containing 50 micrograms of aflatoxin B1 equivalent or more/kg of feed have been reported to cause significant reduction in body weight gain in growing Alabio ducks (Ostrowski-Meissner 1984). A dose-dependent reduction in weight gain and feed consumption was also observed when broiler chicks were fed with a diet contaminated with increasing level of purified AFB1 (Dalvi and Mcgowan 1984). Significant reduction in body weight gain and feed intake was recorded in cherry valley ducks maintained on aflatoxin contaminated diet (Han *et al.* 2008). Negative impact of aflatoxin on body weight gain in ducks has also been reported. The reduced body weight gain was effectively reversed by addition of bentonite clay to the diet containing aflatoxin (Wan *et al*. 2012). This may be due to the adsorption of aflatoxin by bentonite clay in gut which reduces the availability of aflatoxin (Ostrowski-Meissner 1984). However, no significant difference in the body weight gain was observed between control and aflatoxin plus sodium bentonite fed group (Rosa *et al*. 2001). In the present study, aflatoxin in the feed of ducks adversely affected most of the haematological parameters. Most of the blood constituents like haemoglobin, PCV, eosinophil, basophil, heterophil and monocyte were significantly lower but lymphocyte count increased significantly. There was no significant change in haemoglobin % and PCV in aflatoxin exposed ducks at the end of 4^{th} week. However, haemoglobin % and PCV decreased significantly on 6^{th} week post exposure to aflatoxin. This confirms the earlier observations of significant decrease in haematocrit in chickens given aflatoxin alone as compared to the control chickens (Kececi *et al.*1997). Significantly decreased hemoglobin percentage has been reported following exposure to 3 ppm aflatoxin (Gholami and Zia-Jahromi 2012). The haemoglobin % and PCV value increased to a significant level in groups fed with bentonite clay supplemented feed by the end of 6^{th} week of experimental feed.

Broiler chickens fed with aflatoxin treated feed had a lower heterophil count as compared to the control (Bianchi *et al*. 2005). But, there are contradictory observations of significant increase in heterophil count in broiler chickens fed with different mycotoxins including aflatoxin (Kececi *et al*. 1997). No significant change in heterophil % was observed in chicks fed with aflatoxin (Gholami and Zia-Jahromi 2012). In the present study, the heterophil percentage decreased significantly in groups fed with diets containing only aflatoxin on both 4^{th} and 6^{th} week post exposure. However, the ducks fed with diets treated with both aflatoxin and bentonite clay showed a significant increase in the heterophil percentage as compared to the birds fed with only aflatoxin in diet on both 4^{th} and 6^{th} week of experiment.

The lymphocyte count showed a significant increase in ducks fed with increasing level of aflatoxin at the end of 4^{th} and 6^{th} week. This is in agreement with the results of Bianchi *et al*. 2005. This is in contradiction to the earlier findings in broiler chickens (Kececi *et al*. 1997, Gholami and Zia-Jahromi 2012). This adverse effect was reversed by addition of bentonite clay to the aflatoxin intoxicated feed.

The percentage of monocyte was significantly decreased in groups fed with diets containing aflatoxin on 4th and 6th week. This supports the earlier findings of reduced monocyte % in aflatoxin treated broilers (Kececi *et al*. 1997) and contradicts the findings of no significant alteration in monocyte count in broiler chickens fed with aflatoxin (Gholami and Zia-Jahromi 2012). However, the birds fed with feed containing aflatoxin plus bentonite clay showed a marked increase in the monocyte count. The eosinophil and basophil counts showed very slight to no difference in groups taking diets containing aflatoxin on 4th and 6th week post exposure as reported earlier (Kececi *et al*. 1997). Bentonite clay had a very little to no effect on the levels of eosinophil and basophil counts. The total leukocyte count (TLC) reduced to a great extend in groups fed with only aflatoxin in diet on completion of 4th and 6th week, confirming earlier findings of decreased TLC in aflatoxin intoxicated chicks (Gholami and Zia-Jahromi 2012). However, this contradicts the observation of increased TLC in chicks fed with diet containing aflatoxin (Kececi *et al*. 1997). At the end of 4th and 6th week, the birds provided with feed containing aflatoxin plus bentonite clay showed a marked increase in the total leukocyte count.

The birds fed with 48 µg of aflatoxin/kg of feed showed moderate gross lesions in different organs including liver, heart, and spleen. There was significant increase in the organ weights as compared to control group. Aflatoxin treated ducklings had increased relative weights of the liver, kidneys, and spleen but decreased relative weight of bursa of fabricius (Liu *et al*. 2017). The liver of birds fed with 48 µg of aflatoxin/kg of feed showed varying areas of congestion and hemorrhagic patches indicating liver damage. Severe liver damage in ducklings fed with aflatoxin have been reported earlier (Ostrowski-Meissner 1984). Liver of some birds were dark reddish in colour as a result of severe congestion, whereas the liver of some birds were completely pale showing severe anemic appearance, similar to earlier findings in broilers (Miazzo *et al*. 2005). The liver of the birds of this group showed rounded edges instead of normal sharp edge showing varying degree of inflammation and hypertrophy. Microscopically, the liver showed centrilobular and perilobular hypertrophy of hepatocytes with hydropic and vacuolar degeneration, and coagulative necrosis, mostly in the periportal area. Perilobular degeneration of hepatocytes and cytoplasmic vacuolations with infiltration of mononuclear cells in the hepatocytes and fatty degeneration of liver has been recorded earlier in aflatoxin intoxicated broilers (Miazzo *et al*. 2005, Kumar *et al*. 2015). Microscopically, spleen revealed necrosis of lymphoid follicles with degeneration and hyperplasia of lymphoid cells. Increased vacuoles in the lymphoid follicles of the bursa and the white pulp of the spleen were observed in AFB1-exposed chicks (Chen *et al*. 2014). Kidney of aflatoxin treated ducks showed varying degree of inflammation of nephrons and glomerulonephritis. Heart showed hypertrophy of cells with infiltration of degenerated red blood cells. The bursa of fabricious showed vacuolar degeneration with infiltration of mononuclear cells in the trabeculae. This substantiates findings of more vacuoles and inflammatory debris in the bursal lymphoid follicle (Chen *et al*. 2014).

Clay-based binders in animal feeds have been evaluated to adsorb aflatoxin with reported success. However, no adsorbent has been approved for the prevention or treatment of aflatoxicosis (Kubena *et al.* 1990, Kubena *et al*. 1993). Bentonite is chemical absorbent, aluminum phyllosilicate clay, consisting of mostly montmorillonite, originated from weathering of volcanic ash. Its adsorption properties are attributed to the lamellar crystalline micro-structures that accounts for its marked swelling abilities when added to water. It has also been introduced into poultry sector as a binding and lubricating agent in pellet food preparation. Its supplementation increased the rate of egg lying, feed efficiency, egg size, shell quality, feed efficiency and decreased mortality in layers and pullets. *In vivo* use of 1% Sodium bentonite and 0.5% calcium bentonite separately against aflatoxicosis have been reported to restore performance partially, and liver function without influencing mineral metabolism (Schell *et al*. 1993).The irreversible binding of the clay with the toxin in the gut and thereby, preventing the absorption of the toxin across the intestinal wall, might have provided a prophylactic effect in reducing the toxic effects (Schell *et al*. 1993). It is concluded that bentonite clay supplementation in the diet @ 3g/kg of feed protected the white pekin grower ducks from deleterious health effects of consuming aflatoxin-contaminated feed.

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