EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 4
411-419
Year: 2017

BIOCHEMICAL ALTERATIONS IN AFLATOXICOSIS AND ITS AMELIORATION USING DIATOMACIOUS EARTH AS TOXIN BINDER IN BROILERS

A.W. Lakkawar[1]*, H. D. Narayanaswamy[2] and M.L. Satyanarayana[2]

[1]Department of Pathology, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Kurumbapet, Pondicherry- 605009, India.
[2]Department of Pathology, Veterinary College, Bangalore- 560024, Karnataka India.

***Corresponding Author: Dr. A.W. Lakkawar**
Department of Pathology, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Kurumbapet, Pondicherry- 605009, India.

Article Received on 31/01/2017 **Article Revised on 20/02/2017** **Article Accepted on 13/03/2017**

ABSTRACT
The ability of Diatomaceous earth (DAE) in reducing the toxic effects of aflatoxin (AF) in broiler diet was evaluated. DAE was supplemented @ 2000 mg kg-1 of feed along with 0.5 and 1 ppm of AF kg-1 of feed for a period of 35 days of age. Healthy unsexed day old broiler chicks (n=240) were assigned to 6 groups comprising of control and treatment groups. Feeding of AF resulted in significantly lower feed intake as well as body weight gain and increase in feed conversion ratio in comparison to the control groups. Supplementation of DAE in aflatoxin mixed diet significantly reduced the deleterious effects of AF on growth parameters. Feeding of AF alone caused significant decrease in serum levels of total proteins, albumin and cholesterol. However, significant increase was found in serum levels of AST, ALT, ALP, urea, triglycerides and creatinine in AF fed broilers. Supplementation of DAE to the AF mixed feed ameliorated the adverse effect of AF on the serum biochemical values by causing increase in serum total proteins, albumin and cholesterol levels. In addition, a significant decrease was recorded in AST, ALT, ALP, BUN, triglycerides and creatinine levels in DAE supplemented broilers. The study emphasised that diatomaceous earth can be used as an effective adsorbent to decrease the toxic effects of aflatoxin in broiler chicken.

KEYWORDS: Aflatoxin, amelioration, diatomaceous earth, serum biochemistry, broilers.

1. INTRODUCTION
Aflatoxins (AF) are secondary metabolites produced predominantly by *Aspergillus flavus* and *Aspergillus parasiticus*.[1] These toxins are worldwide in occurrence in feed and feed ingredients resulting in severe economic losses to poultry and livestock industries.[2]

Many studies have shown the negative effects of aflatoxin in broiler chickens in relation to body weight gain, efficiency of feed utilization, poor performance and immune responses.[3]

These toxins also cause pathologic alteration in important organs such as liver, kidneys and lymphoid tissues.[4] The pathological lesions in broilers include hepatic lesions such as enlargement, paleness, hydropic degeneration and necrosis, bile duct hyperplasia and periportal fibrosis.[5] Furthermore, the transmission of AF and its metabolites from feed to animal edible tissues and products, such as liver and eggs have become particularly important as a potential hazard for human health.[6]

Decontamination of feed from AF is a major task in the poultry industry. Producers and researchers desire to develop an effective de-contamination technology to deal with the feed- borne toxin.[7] Approaches for decontamination include the physical, chemical and biological methods. A successful detoxification process must be economical and capable of eliminating all traces of toxin without leaving harmful residues and also should not impair the nutritional quality of the commodity.[8] Adsorbents are preferred over most of the decontaminating agents. The major advantages of adsorbents include cost effectiveness, safety and easy administration through animal and poultry feeds.

Lately, approaches to decontaminate or remediate the feed and feedstuffs have been proposed with more emphasis on use of naturally occurring compounds/substances.[9] Diatomite or diatomaceous earth (DAE) is a kind of clay that consists of 90 per cent silicon dioxide. It is fine-grained, biogenic siliceous sediment and is available in large quantities at low cost.[10] DAE consists essentially of amorphous silica derived from opalescent frustules of diatoms resulting in an inert dust, lightweight, highly porous, super-absorbent material and has a fine porous structure with low

density.[11] Denli and Okan[12] concluded that diatomite is not effective in reducing the detrimental effects of aflatoxin in broiler diets. However, Modirsanei *et al.*[13] reported diatomaceous earth significantly increased body weight gain, feed intake, and feed conversion ratio as well as productive efficiency index, serum albumin, and the activity of serum LDH in the birds that were fed AFB_1.

Considering the above beneficial properties of DAE, the present study was undertaken to evaluate the efficacy of DAE @ level of 2000 mg per kilogram of feed in ameliorating the toxic effects of aflatoxin in broiler birds.

2. MATERIALS AND METHODS

The present study was carried out in the Department of Pathology, Veterinary College, Hebbal, Bangalore, Karnataka Veterinary, Animal and Fisheries Sciences University Bangalore, India.

2.1. Production and quantification of aflatoxin

*Aspergilus parasiticus (*MTCC-2796) culture was procured from Institute of Microbial Technology (IMTECH) Chandigarh, India and sub-cultured on potato dextrose agar. Aflatoxin was produced on the rice using *Aspergillus parasiticus* as per the method described by Shotwell *et al.*[14] with some modifications. Briefly, polished rice (50 gm) was soaked overnight in water measuring approximately 40 per cent of the weight and was autoclaved (15 psi at 121°C) for 20 minutes and then cooled. It was then inoculated with spores of 7 days old culture of *Aspergillus parasiticus*, sub-cultured on potato dextrose agar (PDA), using inoculation loop. The inoculated rice flasks were incubated at the room temperature in dark place for 14 days with vigorous shaking three times a day, to break the mycelial mass. On 15th day, the inoculated rice flasks were autoclaved (15 psi at 121°C for 20 minutes) to stop fungal growth. The fermented rice was dried in hot air oven (70^{0}C overnight), powdered and stored in dark place until further use. Aflatoxin content in dried rice sample was confirmed and quantified from Government Analytical Laboratory (AFAQCL), Namakkal, Tamil Nadu, India.

2.2. Experimental birds

Two hundred and fourty unsexed day-old healthy broiler chicks were procured from a reputed commercial hatchery and reared in battery cage system in experimental sheds with average temperature ranging from 27 to 31^{0}C and relative humidity of 59% to 62% with 16:8±I h L:D cycle of intensity of 10 to 20 lux. All chicks were vaccinated on days 7 and 11 of age with the Lasota strain of Newcastle disease virus and Infectious bursal disease (intermediate strain) respectively.

Optimum conditions of brooding and management was provided to the birds throughout the period of experiment. Toxin free and diatomaceous earth (DAE) free Starter and finisher broiler feed was procured from Department of Poultry Science, Veterinary College, Hebbal, Bangalore, India as recommended by the National Research Council. Required quantity of dried rice powder containing aflatoxin was added to make the final concentration of aflatoxin in feed to be 0.5 ppm and 1ppm.

The birds were randomly divided into 6 groups, each comprising of 40 chicks. The different experimental group were as per the Table 1.

GROUPS	TREATMENT	NO. OF BIRDS
I	CONTROL (Toxin free & DAE free feed)	40
II	AGRIPOWER DAE @ 2000 mg/Kg of feed	40
III	AFLATOXIN (1 ppm)	40
IV	AFLATOXIN (0.5 ppm)	40
V	AFLATOXIN (1 ppm) + DAE @ 2000 mg/Kg of feed	40
VI	AFLATOXIN (0.5 ppm)+ DAE 2000 mg/Kg of feed	40

All the birds were checked daily for the health and husbandry conditions. All the sanitary and hygienic precautions were strictly followed throughout the experiment. Prior permission of the Institute Animal Ethical Committee (IAEC) was obtained before the conduct of experiment. The birds were observed daily for clinical signs and mortality (if any). Six birds selected randomly from each group were weighed individually and blood was collected in non heparinized vials on day 7, 14, 21, 28 and 35th day post treatment and serum was separated by standard procedures and stored under -20^{0}C until further use.

The individual serum samples were analysed for Alanine aminotransferase (ALT) concentration, Aspartate aminotransferase (AST) concentration, Alkaline phosphatase (ALP), Creatinine, Blood Urea Nitrogen (BUN), Total proteins, Albumin, Total cholesterol and Triglycerides by the Semi-automatic analyser (Semi-Automatic Biochemistry Analyser, STATFAX 2000+, CPC Diagnostics Pvt. Ltd., India). The methodology and the set of reagents used in respect of each parameter were as per the recommendations of the manufacturer of the analyser system.

2.3. Statistical Analysis

The experimental data collectedwas analysed using the General Linear models (GLM) procedure of software

SPSS 16 of 2010 version. If appropriate, post-hoc analyses were carried out using the Duncan's test for multiple comparisons.[15] Statements of statistical significance are based on $P < 0.05$.

RESULTS AND DISCUSSION

The birds of Group I (Control) and Group II (DAE mixed in control feed) were healthy and did not show any clinical signs throughout the period of experiment.

Serum Aspartate Aminotransferase (AST)

The weekly mean serum aspartate aminotransferase (AST) of different groups have been represented in Fig 1. The mean serum ALT values of Group I to VI birds were 250.94±13.21, 262.97±12.23, 441.77±12.22, 371.37±11.03, 362.94±7.72 and 288±5.55, respectively at the end of 35th day. The mean serum AST values of Group I and II did not alter significantly throughout the experimental period. A significant increase was seen in first four weeks of the experiment in birds fed with toxin (Group III and IV). The mean serum AST values of birds supplemented with toxin binder along with toxin (Group V and VI) showed significant decrease in mean serum AST values as compared to toxin fed birds (Group III and IV) on day 35 of experiment.

Serum Alanine Aminotransferase (ALT)

The results of mean serum alanine aminotransferase (ALT) value in birds with different dietary treatments have been graphically represented in Fig 2. The mean serum ALT values of Group I to VI birds 11.73±0.66, 13.06±1.59, 18.82±0.46, 15.26±0.69, 14.26±0.28 and 14.59±0.22, respectively at the end of day 35.

The mean serum ALT values of the control Groups I and II were similar throughout the experimental period, however there was significant increase in serum ALT values seen in toxin fed birds (Groups III and IV) in comparison to control birds (Group I) from second week onwards till the end of experiment, while numerical decrease in serum ALT values was seen in birds supplemented with toxin binder (Group V and VI) as compared to toxin fed birds (Group III and IV) from second week onwards.

Serum Alkaline Phosphatase (ALP)

The results of mean serum alkaline phosphatase (ALP) value in birds with different dietary treatments are graphically represented in Fig.

3. The mean serum ALT values of Group I to VI birds were 1072.9±38.1, 1047.07±29.51, 1957.3±24.35, 1637.14±6.82, 1219.34±48.23 and 1375.4±40.85, respectively at the end of day 35th of experiment.

The mean serum ALP values of toxin fed birds (Group III and IV) showed a significant increase in comparison to control birds (Group I and II) on second and fifth week, but numerical increase was seen on 14th day of observation. However, the birds supplemented with toxin binder (Group V to VI) showed significant decrease with that of only toxin fed birds (Group III and IV) on 35th day.

Serum Creatinine

The weekly mean serum creatinine values of birds fed with different dietary treatments have been graphically represented in Fig 4. The mean serum creatinine levels for Group I to VI were 1.07±0.09, 0.97±0.09, 1.77±0.09, 1.24±0.04, 1.14±0.13 and 0.97±0.04 mg/dl respectively, at 35 days of age.

The mean serum creatinine values of birds of Group I and II did not alter significantly on all the days of observation. There was numerical increase in the mean serum creatinine of toxin fed group (Group III and IV) was seen throughout the experimental period however it increased significantly ($P \leq 0.05$) as compared with control birds (Group I) from day 28 of observation. Supplementation with toxin binder in (Group V to VI) showed significant decrease in serum creatinine values as compared to toxin fed birds (Group IV) on day 28th and 35th of experiment.

Total Serum Protein

The weekly mean serum total protein levels in different groups have been presented in Fig 5. The mean Total serum proteins values of Group I to VI birds were 3.82±0.02, 3.74±0.09, 2.17±0.14, 2.97±0.13, 3±0.06 and 3.3±0.06, respectively at the end of 35th day of experiment.

It was observed the mean serum total protein of Group I and II did not alter significantly throughout the period of experiment. The mean serum total protein of toxin fed group (Group III and IV) altered significantly with untreated control (Group I and II) from Day 7 till the end of experiment. However, with addition of toxin binder, birds of Group V and VI showed significant increase in total protein as compared to Group III and Group IV (toxin fed group) on day 7th, 28th and 35th day of observation, and numerical increase was observed on day 14th and 21st of the observation. It was also observed that there was a significant increase in total protein in DAE supplemented birds of Group V and VI as compared to Group III and IV (Toxin fed) on day 35th of observation.

Serum Albumin

The weekly mean serum albumin values in the birds of Group I to VI have been depicted in Fig 6. The mean serum albumin value of Group I to VI at the end of fifth week were1.6±0.06, 1.5±0.16, 1.04±0.09, 1.27±0.04, 1.3±0.06 and 1.47±0.04, respectively.

The serum albumin values did not show a significant change in birds of Group I and II during the entire period of experiment. The mean serum value of Group III and IV (toxin fed group) decreased significantly in comparison to the control group throughout the period of study. However, birds of Group V to VI showed increase

in serum albumin in comparison to the toxin fed group (III and IV) at 35 days of experiment.

Serum Triglycerides

The data pertaining to the mean serum triglycerides in birds of Group I to VI have been presented in Fig. 7. The mean serum triglycerides for Group I to VI were 75.7±1.39, 73.97±1.51, 158.07±4.91, 115.2±3.22, 118.04±2.67 and 97.47±0.62 mg/dl respectively at 35 days of age.

There was no significant difference seen in mean serum triglyceride level in control groups (Group I and II) throughout the period of experiment, however a significant decrease was seen in toxin fed groups (Group III and IV) on day 7 and 14 of observation but numerical decrease was seen on day 21, 28 and 35 of observation in comparison to the control group (Group I and II).

There was significant decrease in mean serum triglycerides in birds supplemented with DAE (Group V and VI) in comparison to the toxin fed birds (Group III and IV) throughout the period of experiment.

Serum Urea

The weekly mean serum urea levels in different groups have been graphically depicted in Fig 8. The mean serum urea values of Group I to VI birds were 17.04±0.3, 18.94±1.6, 29.8±1.16, 23.04±1.21, 20.84±0.58 and 19.74±0.64, respectively at the end of 35th day.

The mean serum urea levels of birds in Group I and II did not alter significantly during entire period of the experiment. A significant ($P \leq 0.05$) increase in the mean serum urea levels in Group III and IV toxin fed birds was observed as compared with control (Group I and II) birds from day 28 till end of the experiment The birds in Group V and VI (DAE supplemented) showed significant decrease as compared to control group at the end of 35th day.

Serum Cholesterol

The weekly mean serum cholesterol values of birds fed with different dietary treatments have been graphically represented in Fig 9. The mean serum cholesterol values of Group I to VI birds were 123.1±1.46, 120.4±2.91, 58.27±2.95, 73.24±3.79, 80.1±5.22 and 90.4±1.13, respectively at the end of day 35 of the experiment**.**

The mean serum cholesterol values of birds of Group I to II did not alter significantly on all the days of observation. The mean serum cholesterol of toxin fed groups (Group III and IV decreased significantly ($P \leq 0.05$) as compared with control (Group I and II) birds, from second week observation. Supplementation with toxin binder in (Group V to VI) showed significant increase as compared to toxin fed birds (Group III and IV) at the end of the experiment.

In the present study, the performance of the chicks was not affected by the addition of DAE alone in the diet (Group II) indicating the inert and non-toxic property of diatomaceous earth. Feeding of AF alone (Group III and IV) in the diet caused significant ($P \leq 0.05$) decrease in serum total protein, albumin and cholesterol levels. However, significant ($P \leq 0.05$) increase was observed in serum levels AST, ALT, ALP, urea, triglycerides and creatinine.

The incorporation of DAE in toxin mixed diet could improve the adverse effects of AF on the serum biochemical values as evident by increase in the total protein, albumin and cholesterol levels. However, there was decrease in the serum levels of AST, ALT, ALP, urea triglycerides and creatinine in the co-treatment groups of V and VI.

The derangement in serum biochemical parameters recorded in AF fed birds in the present study corroborate with the previous studies on aflatoxicosis in broiler birds.[9,16,17]

Liver is considered as target organ for aflatoxicosis, where most aflatoxins are bioactivated to the reactive 8, 9-epoxide form, which is known to bind DNA and proteins causing damage to the hepatocytes followed by inflammatory changes leading to increase in liver weight.[18] Aflatoxin inhibits DNA dependent RNA polymerase and cause impairment of nuclear DNA template function resulting in inhibition of protein synthesis.[19] Serum levels of AST, ALT and ALP in aflatoxin fed birds were higher throughout the study, which could be attributed to seepage of enzymes due to hepatocyte membrane damage. Reduction in serum cholesterol and triglycerides levels in aflatoxicosis reflects impaired liver metabolism. Marked increase in serum levels of BUN and creatinine during induced aflatoxicosis in the present study reflects impaired renal function resultant to tubular damage.[20] The significantly higher BUN and creatinine levels indicate disturbed transportation function of epithelial cells in collecting tubules and diffuse impairment of proximal tubules function.[21] Further, the changes in blood urea nitrogen and creatinine could be secondary to necrotic changes in renal parenchyma.[22]

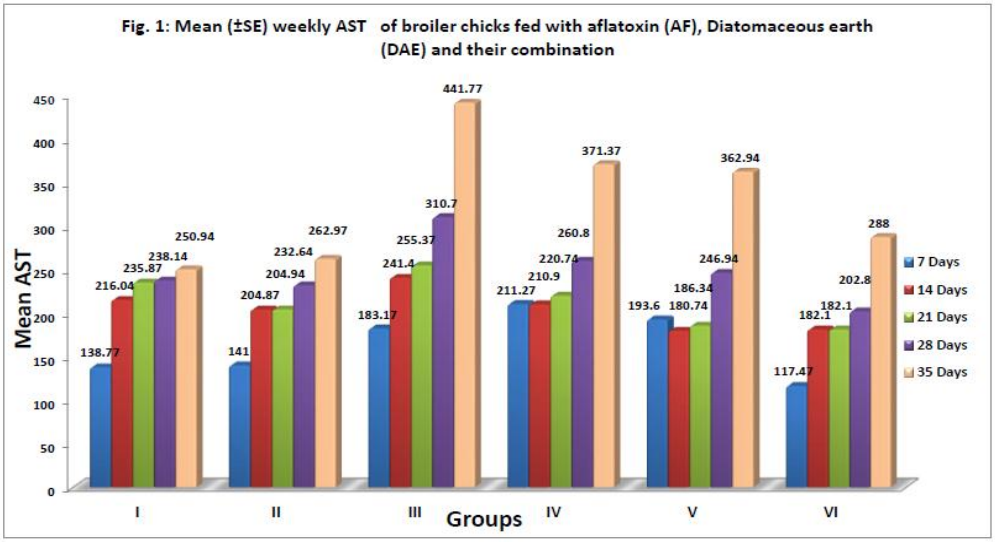
In the combined treatment groups (V and VI) fed with combination of DAE and aflatoxin in feed showed marked improvement in serum biochemical parameters in comparison to aflatoxin alone fed birds. These findings of serum biochemical changes associated with AF contamination could be ameliorated by the supplementation of DAE to the aflatoxin fed birds.[23] Similar study conducted earlier reported that reduced growth rate and serum biochemical changes associated with AFB1 contamination (0.1 mg/kg) could be ameliorated by the supplementation of a modified

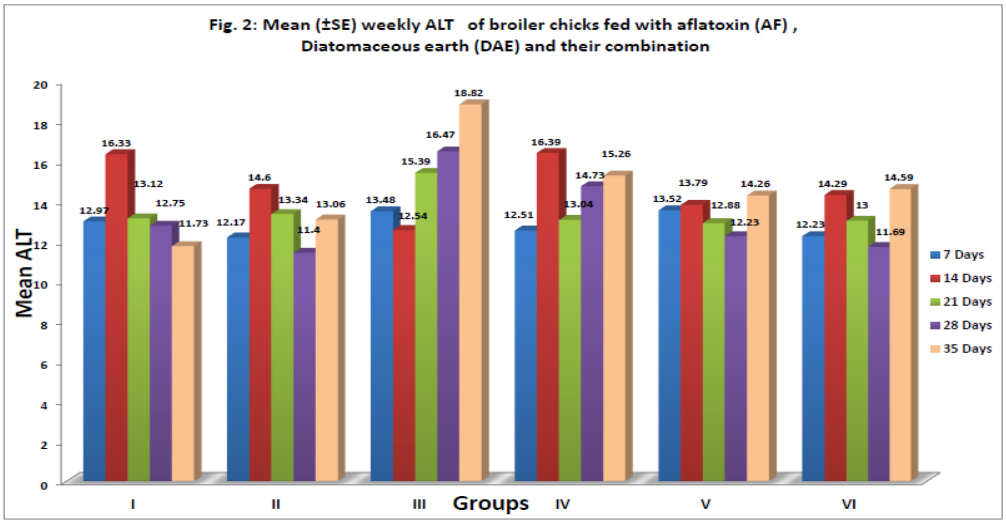
montmorillonitenano composite (a compound comprising of DAE) at doses of 3 g/kg.[20] Similarly, Bailey *et al*[24] reported that montmorillonite clay in broiler diets provided protection on growth performance, serum biochemistry, and the relative organ weights from over 4 mg of AFB1/kg diets.

Contrary to our findings, limited or no protective effect of DAE on the growth performance and biochemical parameter in AF fed birds has also been reported earlier.[12,13,16] These conflicting results are possibly due to the differences in the cationic compounds or chemical content of the adsorbents tested. Differences among studies could also be explained by different levels of adsorbents or the aflatoxin exposure dose tested.

Diatomaceous earth has a small mass (0.5-0.8 g/cm^3), high porosity and high content of silicon, which are responsible for the high adsorption capacity.[25] Natour and Yousef[26] reported significantly higher in- vitro adsorption ability of DAE to aflatoxins, which is directly proportional to the number of diatom valves. In- vitro study showed that DAE has high (94.71%) ability to adsorb AF from the feed at pH 6.5.[27] The normal pH of the chicken intestinal tract contents is 5.7–6.0 in the duodenum/jejunum, 6.3–6.4 in the ileum/rectum and finally up to pH 7.0 or higher in the caecum.[28] Considering the correlation between the pH and ability of mycotoxin binder in *in-vitro* studies, a higher binding ability of DAE to the aflatoxins can be expected at the P^H of 6-7 in the intestinal tract of chicken to reduce the absorption and systemic availability of this mycotoxin.

In the present study, the incorporation of DAE in the diet during the period of exposure to AF could prevent the toxic effects of aflatoxin. The protective effects of DAE might plausibly be due to its capability of specific chemisorptions of AF in gastrointestinal tract, which reduces AF bioavailability for absorption and systemic circulation in broiler birds.[29] Extrapolation of the data from the earlier reports *in-vitro* experiments as well as *in- vivo* studies and the present study warrants further studies employing the broader perspectives to determine whether lower level of DAE in broiler feed will be effective in controlling or preventing the occurrence aflatoxicosis in poultry.





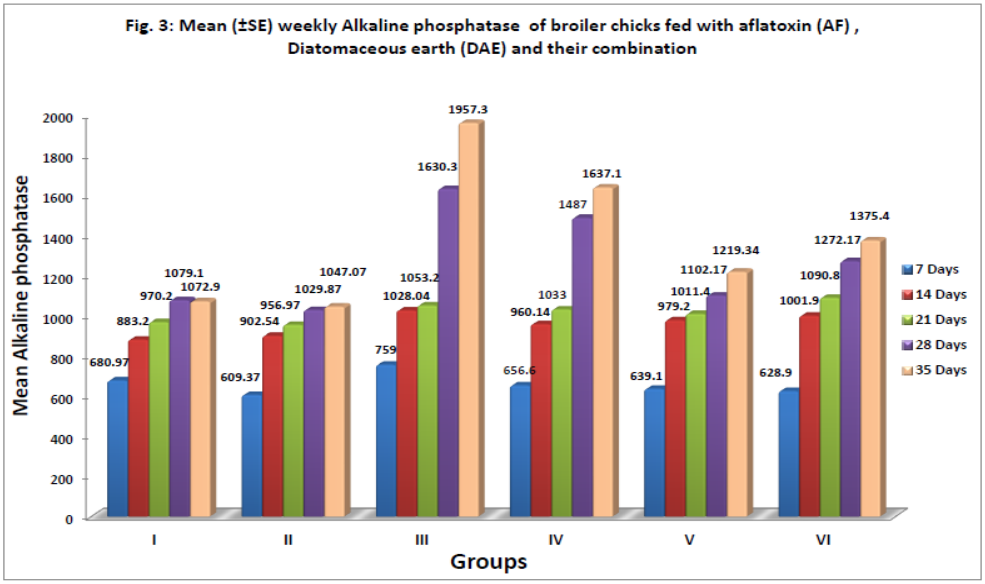


Fig. 3: Mean (±SE) weekly Alkaline phosphatase of broiler chicks fed with aflatoxin (AF) , Diatomaceous earth (DAE) and their combination
Mean Alkaline phosphatase
Groups
I
II
III
IV
V
VI
7 Days
14 Days
21 Days
28 Days
35 Days
680.97
883.2
970.2
1079.1
1072.9
609.37
902.54
956.97
1029.87
1047.07
759
1028.04
1053.2
1630.3
1957.3
656.6
960.14
1033
1487
1637.1
639.1
979.2
1011.4
1102.17
1219.34
628.9
1001.9
1090.8
1272.17
1375.4

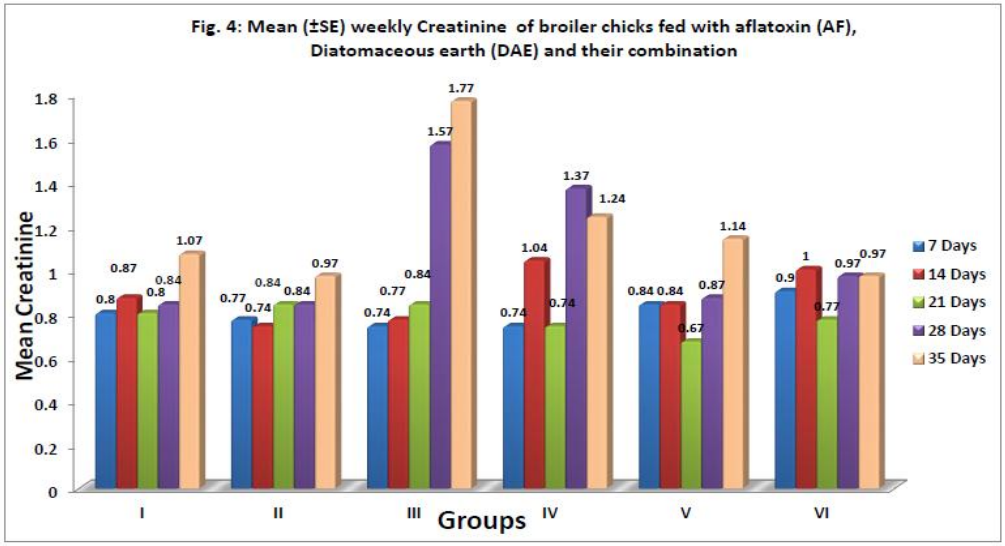


Fig. 4: Mean (±SE) weekly Creatinine of broiler chicks fed with aflatoxin (AF), Diatomaceous earth (DAE) and their combination
Mean Creatinine
Groups
I
II
III
IV
V
VI
7 Days
14 Days
21 Days
28 Days
35 Days
0.8
0.87
0.8
0.84
1.07
0.77
0.74
0.84
0.84
0.97
0.74
0.77
0.84
1.57
1.77
0.74
1.04
0.74
1.37
1.24
0.84
0.84
0.67
0.87
1.14
0.9
1
0.77
0.97
0.97

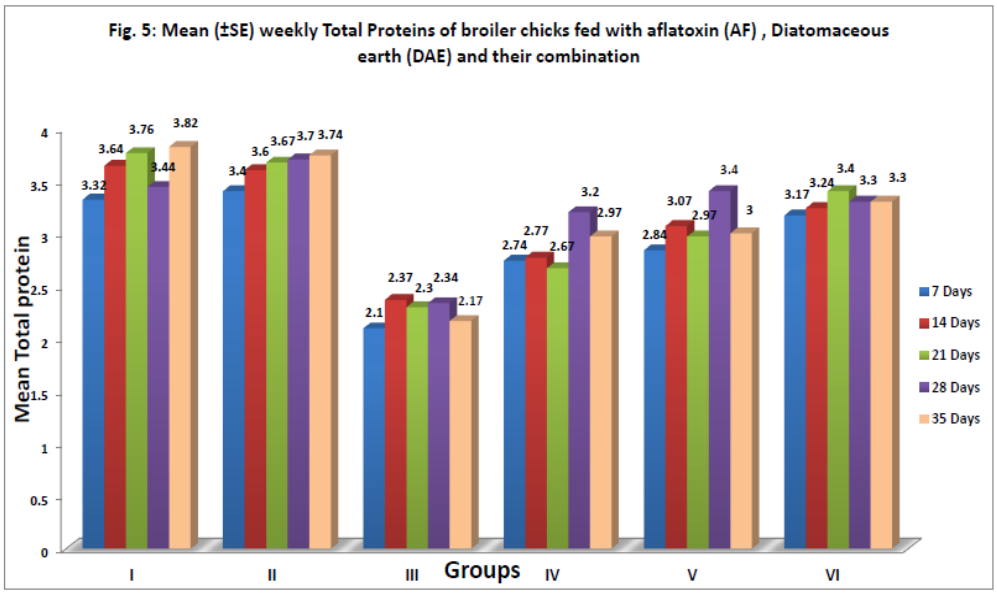


Fig. 5: Mean (±SE) weekly Total Proteins of broiler chicks fed with aflatoxin (AF) , Diatomaceous earth (DAE) and their combination
Mean Total protein
Groups
I
II
III
IV
V
VI
7 Days
14 Days
21 Days
28 Days
35 Days
3.32
3.64
3.76
3.44
3.82
3.4
3.6
3.67
3.7
3.74
2.1
2.37
2.3
2.34
2.17
2.74
2.77
2.67
3.2
2.97
2.84
3.07
2.97
3.4
3
3.17
3.24
3.4
3.3
3.3

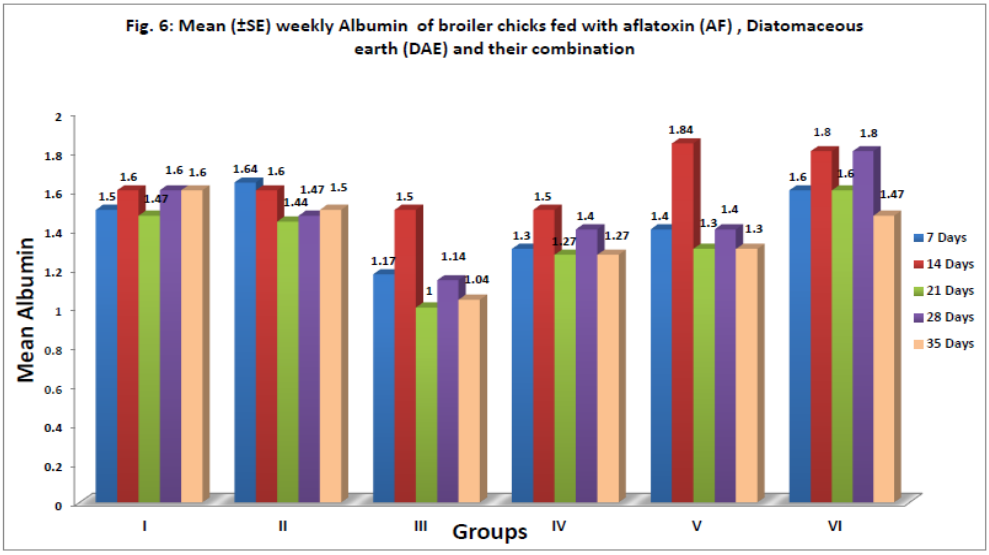


Fig. 6: Mean (±SE) weekly Albumin of broiler chicks fed with aflatoxin (AF) , Diatomaceous earth (DAE) and their combination
Mean Albumin
Groups
I
II
III
IV
V
VI
7 Days
14 Days
21 Days
28 Days
35 Days

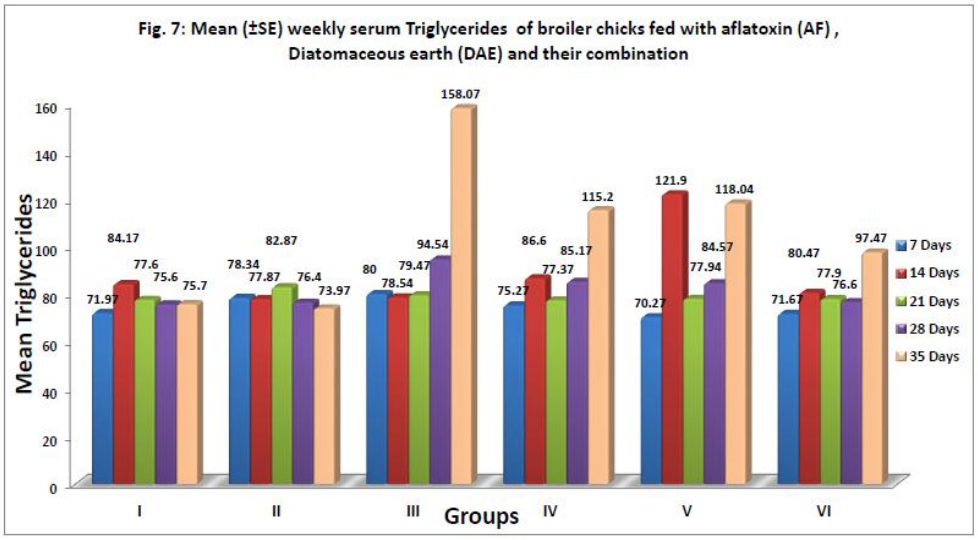


Fig. 7: Mean (±SE) weekly serum Triglycerides of broiler chicks fed with aflatoxin (AF) , Diatomaceous earth (DAE) and their combination
Mean Triglycerides
Groups
I
II
III
IV
V
VI
7 Days
14 Days
21 Days
28 Days
35 Days

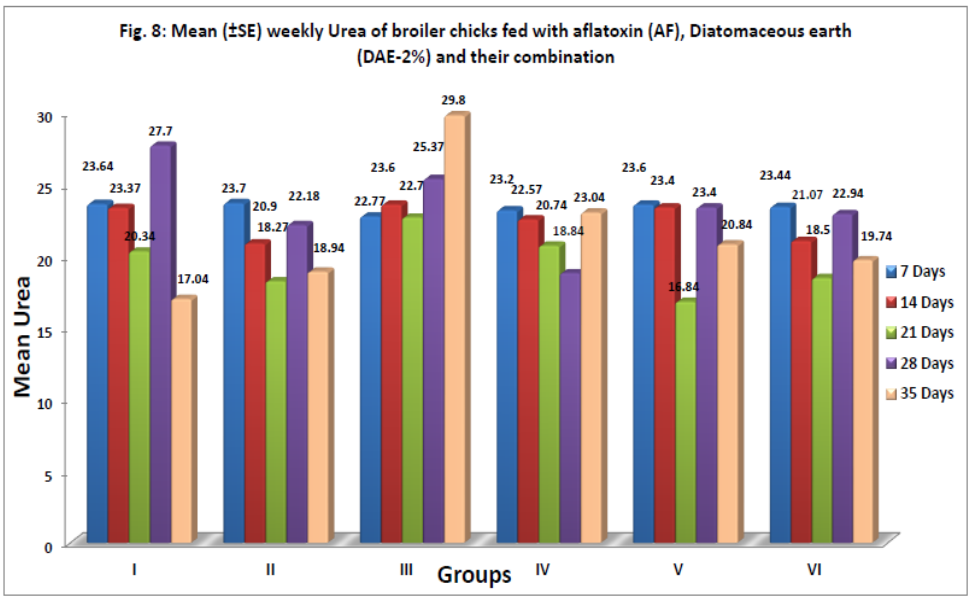
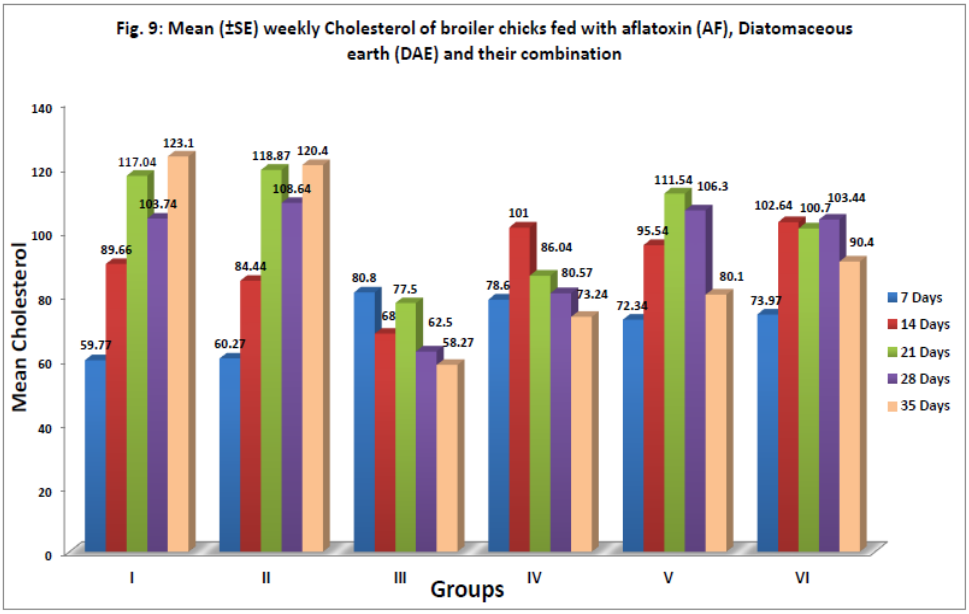


Fig. 8: Mean (±SE) weekly Urea of broiler chicks fed with aflatoxin (AF), Diatomaceous earth (DAE-2%) and their combination
Mean Urea
Groups
I
II
III
IV
V
VI
7 Days
14 Days
21 Days
28 Days
35 Days



CONCLUSION

Addition of diatomaceous earth to aflatoxin containing feed showed marked improvement in the biochemical parameters suggesting the protective effect of 2000 mg of DAE Kg^{-1} feed against the toxic effects of AF; and these improvements could be due to counter action of aflatoxicosis problem on the basis of higher binding ability of diatomite.

ACKNOWLEDGEMENT

Authors are the thankful to the Vice Chancellor, KVAFSU, Bidar; Dean, Veterinary College, Bangalore and ICAR-NAE Project for providing the necessary facilities. Thanks are due to Agripower, Australia for providing DAE for study purpose and financial support to carry out the research work.

DISCLOSURE OF INTERESTS

The authors have no conflicts of interest to declare. All authors participated and approved the manucsript for publication.

REFERENCES

1. Huwig A, Freimund S., Kappeli O and Dutler H. Mycotoxin detoxication of animal feed by different adsorbents. Toxicol Lett, 2001; 122: 179-188.
2. Miller JD. Fungi and mycotoxins in grain: implications for stored product research. J Stored Prod Res, 1995; 31: 1-16.
3. Hamilton PB. Determination safe levels of mycotoxins. J Food Protect, 1984; 47: 575.
4. Dafalla R, Yagi A and Adam SE. Experimental aflatoxicosis in hybro-type chicks; sequential changes in growth and serum constituents and histopathological changes. Vet Hum Toxicol, 1987; 29: 222-226.
5. Ledoux DR, Rottinghaus GE, Bermudez AJ and Alonso-Debolt M. Efficacy of hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. Poult Sc, 1999; 78: 204-210.
6. Bintvihok A, Thiengnin S, Doi K, Kumagai S. Residues of aflatoxins in the liver, muscle and eggs of domestic fowls. J Vet Med Sci, 2002; 64: 1037–1039.
7. Leeson S, Diaz GJ and Summers JD. Poultry metabolic disorders and mycotoxins, University Books, Ontario, Canada, 1995.
8. Parlat SS, Yildiz AO, Oguz H. Effect of clinoptilolite on performance of Japanese quail (*conturnix japonica*) during experimental aflatoxicosis. Br Poult Sci, 1999; 40: 495-500.
9. Ledoux DR, Rottinghaus GE, Bermudez AJ and Alonso-Debolt M. Efficacy of hydrated sodium calcium aluminosilicate to ameliorate the toxic effects of aflatoxin in broiler chicks. Poult Sci, 1999; 78: 204-210.
10. Kamigasa S and Kato H. Recent conditions and prospects of diatomite resources. Energy Resources, 2000; 21: 166-172.
11. Wajima T, Haga M, Kuzawa K, Ishimoto H, Tamada O, Ito K, Nishiyama T, Downs RT and Rakovan JF. Zeolite synthesis from paper sludge ash at low temperature (90^0C) with addition of diatomite. J Hazard Mat, 2006; 132(2-3): 244-252.
12. Denli M and Okan F. Efficacy of different adsorbents in reducing the toxic effects of aflatoxin B1 in broiler diets. South African J Anim Sci, 2006; 36(4): 222-228.
13. Modirsanei M, Mansoori B, Khosravi AR, Kiaei M M, Khazraeinia P, Farkhoy M, Masoumi Z. Effect of diatomaceous earth on the performance and blood variables of broiler chicks during experimental aflatoxicosi. J Sci Food Agric, 2008; 88(4): 626-632.
14. Shotwell OL, Hesseltine CW, Stubblefeild RD and Sorenson WG. Production of aflatoxin on rice.

Appl. Microbiol, 1966; 52: 425-428.
15. Duncan, DB. Multiple Range and Multiple F tests. Biometrics, 1955; 1: 1-42.
16. Rosa CAR, Miazo R, Oli C, Salvano M, Chiacchiera SM, Ferrero S, Saenz Carvalho ECQ and Dalcero A. Evaluation of the dietary effect of bentonite from the south of Argentina in ameliorating the toxic effects of aflatoxin in broilers. Poult Sci, 2001; 80: 139-144.
17. Dhanapal, SK, Rao S, Govindaraju PKP, Hukker R and Mathesh K. Ameliorative Efficacy of Citrus Fruit Oil in Aflatoxicosis in Broilers: A Growth and Biochemical Study. Turk J Vet Anim Sci, 2014; 38: 207-211.
18. Shankaran R, Raj HG and Venkatasubramanian TA. Effect of aflatoxin on carbohydrate metabolism in chick liver. Enzymlogia, 1970; 39: 371-378.
19. Verma, RJ. Aflatoxin cause DNA damage. Int. J Human Genetics, 2004; 4: 231- 236.
20. Shi YH, Xu ZR, Feng ZR and Wang CZ. Efficacy of modified montomorillonitenano composite to reduce the toxicity of aflatoxin in broiler chickens. Anim Feed Sci Tech, 2006; 129: 138.
21. Umar S, Arshad A, Ahmad B and Arshad M. Clinico biochemical and haematological changes in broilers induced by concurrent exposure to aflatoxin B1 and ochratoxin A. J Public Health Bio Sci, 2012; 1: 79–85.
22. Valchev D, Kanakov TS, Hristov L, Lazarov R, Binev N, Grozeva Y and Nikolov Y. Effects of experimental aflatoxicosis on renal function in broiler chickens. Bulg J Vet Med, 2014; 17(4): 314-324.
23. Denli M, Blandon JC, Guynot ME, Salado S and Perez JF. Effects of dietary Afla Detox on performance, serum biochemistry histopathological changes and aflatoxin residues in broilers exposed to aflatoxin B1. Poult. Sci, 2009; 88: 1444- 1451.
24. Bailey CA, Latimer GW, Barr AC, Wigle WL, Haq AU, Balthrop JE and Kubena LF. Efficacy of montmorillonite clay (NovaSil PLUS) for protecting full-term broilers from aflatoxicosis. J Appl Poult Res, 2006; 15: 198–206.
25. Whitlow LW. Evaluation of mycotoxin binders, 4th Mid-Atlantic Nutrition Conference (Zimmerman, N.G. ed.), Proceedings, 2006; 132-143.
26. Natour RM and Yousef SM. Adsorption efficiency of diatomaceous earth for mycotoxin. Arab Gulf J Scientific Res, 1998; 16: 113–127.
27. Soleimani R, Faradonbeh OP and Bagheri H. Mycotoxin detoxification of commercial broiler feed by a mycotoxin binder. Res Opin Anim Vet Sci, 2011; 1(12): 778-780.
28. Denbow D. Gastrointestinal anatomy and physiology. In: Whittow, G.C., ed. Sturkie's Avian Physiology. 5th edn. Academic Press, San Diego, CA, USA, 2000; 299-324.
29. Abdel-Wahhab MA, Nada SA and Amra HA. Effect of aluminosilicates and bentonite on aflatoxin-induced developmental toxicity in rat. J Appl Toxicol, 1999; 19: 199–204.