International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(1): 673-679

Received: 20-11-2017
Accepted: 25-12-2017

VP Anand
Veterinary Officer, Veterinary Hospital, Mangalagatti, Tahsil-Dharawad, Dharawad, Karnataka, India

AW Lakkawar
Department of Pathology, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Kurumbapet, Pondicherry, Tamil Nadu, India

HD Narayanaswamy
Department of Pathology, Veterinary College, KVAFSU, Bangalore, Karnataka, India

ML Sathyanarayana
Department of Pathology, Veterinary College, KVAFSU, Bangalore, Karnataka, India

Correspondence
VP Anand
Veterinary Officer, Veterinary Hospital, Mangalagatti, Tahsil- Dharawad, Dharawad, Karnataka, India

Serum biochemical changes in ochratoxin induced toxicity and its amelioration using diatomacious earth in broiler chicken

VP Anand, AW Lakkawar, HD Narayanaswamy and ML Sathyanarayana

Abstract
The ability of Diatomaceous earth (DAE) in reducing the toxic effects of ochratoxin (OTA) in broiler diet was evaluated. DAE was supplemented @ 400 and 800 mg kg^{-1} of feed along with 0.5 and 1 ppm of OTA/kg of feed. Healthy unsexed day-old broiler chicks (n=360) were assigned to 9 groups comprising of control and treatment groups. Feeding of OTA 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 ochratoxin mixed diet significantly reduced the deleterious effects of OTA on growth parameters. Feeding of OTA alone caused significant decrease in serum levels of total proteins, albumin, triglycerides and cholesterol. However, significant increase was found in serum levels of AST, ALT, ALP, BUN and creatinine in OTA fed broilers.
Supplementation of DAE to the OTA mixed feed ameliorated the adverse effect of ochratoxin on the serum biochemical values by causing increase in serum total proteins, albumin, triglycerides and cholesterol levels. In addition, a significant increase was recorded in AST, ALT, ALP, BUN and creatinine levels in DAE supplemented broilers. The study concluded that diatomaceous earth is an effective adsorbent to decrease the toxic adverse effects of ochratoxin in broiler chicken.

Keywords: ochratoxin toxicity, amelioration, diatomaceous earth, serum biochemistry, broiler chicken

Introduction
Poultry industry is one among the most rapidly growing business in India. In intensive poultry farming, birds are exposed to several stress factors, which increase their susceptibility to various disease conditions that are already existing or emerging. Concerted efforts have been made to control disease through intensive immunization, by use of chemotherapeutics and better management practices. In spite of these practices, there has been re-emergence of diseases possibly due to immune-suppression caused by various infectious and non-infectious agents.
In nature, there are thousands of toxins exists that have potential adverse effects on human and livestock health including poultry. Among these natural toxins, mycotoxins are common contaminants of food and feed ingredients. Many mycotoxins affect various organs specifically and are responsible for hampering poultry production. In addition, mycotoxins impair both humoral and cell mediated immunity resulting in increased susceptibility to various infectious diseases. Ochratoxins are toxic metabolites, commonly produced by two species of fungi, *Penicillium verrucosum* and *Aspergillus ochraceus* [1]. The primary toxin was identified as ochratoxin A (OA); its less toxic dechloro analog is ochratoxin B (OB) [2, 3]. In addition, several other forms of ochratoxins also occur. Ochratoxins in recent years has received considerable attention not only because of its effect on animal performance and well-being, but it may also have deleterious effects on humans. In human beings, it is implicated in a fatal kidney disease known as Balkan endemic nephropathy and also it is considered as potent carcinogen.
Ochratoxins are nephrotoxic, hepatotoxic, carcinogenic, immunotoxic and teratogenic mycotoxins. Exposure to low concentration of ochratoxins causes structural and functional changes in different organ systems; especially the kidney and the liver of several domestic and experimental animals are commonly affected. In poultry, the ochratoxin contamination of feed and feed stuffs reduces growth efficiency, lowers feed conversion and reproductive rates, impairs resistance to diseases, reduces vaccination efficacy and induces damage to the liver, kidney and other organs. Further it affects the lymphoid organs and is particularly responsible

for immunosuppresion. Ochratoxin ingestion also impairs the defence mechanism of the birds resulting in increased susceptibility to various infections. Considering the adverse effects of the ochratoxin on health and performance of birds, it becomes essential to develop some large scale, cost effective methods to neutralize or eliminate the preformed toxin in the diet. A possible economical solution could be development of nontoxic dietary additives that would makes birds resistant to the toxic effects. Diatomite or diatomaceous earth (DAE) is a kind of clay that consists of 90% silicon dioxide. It is fine-grained, biogenic siliceous sediment, and is abundantly available at low cost [4]. These clay-like, chalky remains are referred as diatomite [5]. Diatomaceous earth, a clay mineral of the smectite group is formed of highly colloidal material composed of mainly montmorillonite and is produced by *in-situ* diversification of volcanic ash. It has the unique characteristic of swelling to several times its original volume when placed in water and of forming thixotropic gels with water even though the amount of clay is relatively less. DAE may possess higher adsorptive capacity due to its structural composition, which can be responsible for the binding to mycotoxins in the feed materials [6].

Therefore in the present study an attempt has been made to assess the efficacy of diatomaceous earth (DAE) a toxin binder to ameliorate the toxic effects of ochratoxin.

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

Production and quantification of ochratoxin

The *Aspergillus ochraceus* (MTCC No 6037) culture was procured from Microbial Type Culture collection, Institute of Microbial Technology, Sector-39-A Chandigarh- 160036, India. The culture of *Aspergillus ochraceus* (MTCC No 6037) was periodically sub-cultured on potato dextrose agar for 15 days to maintain its viability. Ochratoxin was produced on whole grains of wheat with this culture as outlined by Trenk *et al.* [7] with some minor modifications. Whole wheat (100 g) was soaked in 50 ml water overnight and then autoclaved at 15 Psi for 20 min. Eight day old fungal spore suspension was inoculated to the soaked grains of wheat and the inoculums were incubated for 22 days at room temperature in a dark place with vigorous shaking twice a day. The fermented wheat was autoclaved to kill the spores and dried at 80^{o} C in hot air oven overnight. The dried material was later powdered and stored in a dark place for further use.

Experimental birds:

Two hundred and forty 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± 1h (Light : Dark) 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 management were provided to the birds throughout the period of experiment. Toxin free and DAE free Starter and Finisher broiler feed was procured from Department of Poultry Science, Veterinary College, Bangalore, India as recommended by the National Research Council. Required quantities of cultured ochratoxin material were added to make the final concentration of ochratoxin in feed to be 0.5 ppm and 1 ppm.

The approval of the Institute Animal Ethics Committee (IAEC) was obtained prior to the conduct of the experiment. The birds were randomly divided into 9 groups, each comprising of 40 chicks (Table 1).

Table 1: Experimental design for various treatment groups

Groups	Ochratoxin (ppm)	DAE/Kg feed	No. of Birds
I	0	0	40
II	0	400	40
III	0	800	40
IV	1	0	40
V	0.5	0	40
VI	1	400	40
VII	1	800	40
VIII	0.5	400	40
IX	0.5	800	40

All the birds were checked daily for the health status and husbandry conditions. All the sanitary and hygienic precautions were strictly followed throughout the experiment. The birds were observed daily for clinical signs and mortality (if any). Six birds selected randomly from each group were weighed individually and euthanized by cervical dislocation on day 7, 14, 21, 28 and 35 of the experiment.

Blood was collected from the euthanized birds and sera 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, 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.

Statistical Analysis

The experimental data collected was analysed using the General Linear models (GLM) procedure using Statistical Package for the Social Sciences software 16 (SPSS) of 2010 version. Statements of statistical significance were based on $P<0.05$.

Results and Discussion

The birds of control groups (I, II, III) were healthy and did not show any clinical signs throughout the period of experiment. Birds fed with diet mixed with ochratoxin (Group IV and V) exhibited clinical signs such as dullness, ruffled feathers, poor growth, in appetence and diarrhoea from day 7 of the experiment, which persisted till the end of the experimentation. The birds in co-treatment groups (VI to IX) fed with diet containing ochratoxin and DAE showed similar clinical signs of those recorded for the toxin alone fed birds (Group IV and V). However, the clinical manifestations were of reduced severity and were observed after day10-12 of the experiment.

Serum total protein and albumin: In the present study, the mean serum total protein, albumin and globulin values were significantly ($P<0.05$) decreased in toxin fed birds (Group IV and V) as compared to control birds (vide Fig. 1, 2). Similar observations were also reported in earlier studies [8, 9, 10].

Decrease in the serum total protein and albumin values in these birds could be due to inhibition of hepatic protein synthesis, which occurred at the post transcription level by competitive inhibition of phenylalanine-t RNA synthetase, so that amino-acylation and peptide elongation were affected [11]. Further, Kamagani [12] reported that one of the primary effects of albumin binding on OA was to retard its elimination by limiting the transfer of OA from the blood stream to the hepatic and renal cells resulting in its long half-life.

Supplementation of DAE to the birds fed with of toxins showed improvement in the serum protein concentration indicating the protective effect of DAE against ochratoxin. The results of present study can be compared with that of Shi *et al.* [13], who reported that a reduced growth rate and serum biochemical changes associated with AFB_1 contamination could be ameliorated by the supplementation of a modified montmorillonite nanocomposite (a compound comprising of DAE) at doses of 3 g/kg feed.

Similarly, Bailey *et al.* [14] reported that montmorillonite clay (5 g/kg) in broiler diets provided protection on growth performance, serum biochemistry, and the relative organ weights from over 4 mg of AFB1/kg diets. In the light of the above it can be concluded that the reduction of OA toxicity might be similar to that of AFB

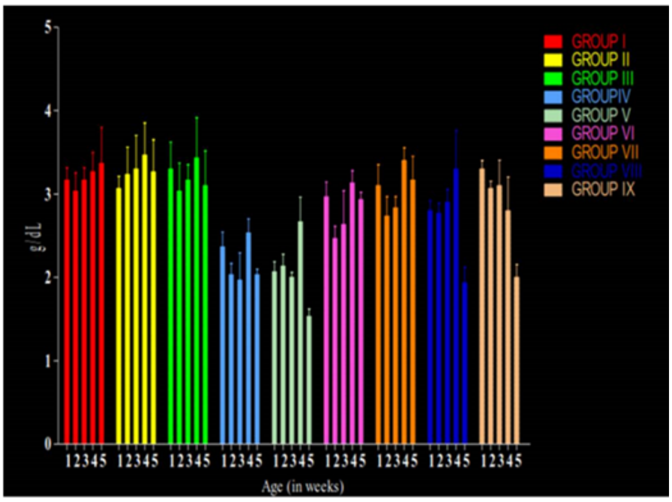

Fig 1: mean (-+SE) weekly total proteins of broiler chicks fed with Ochratoxin, diatomaceous earth and their combination

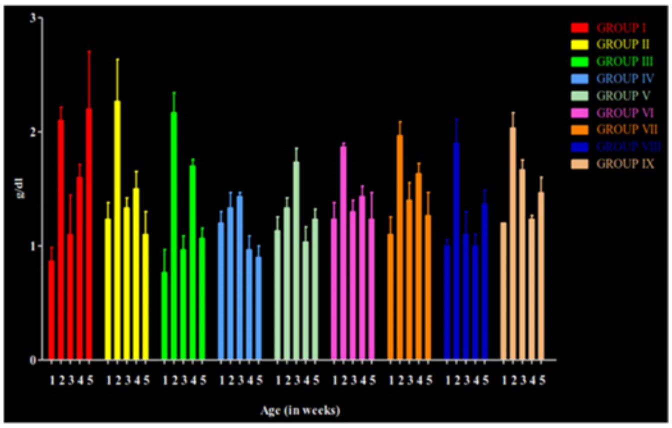

Fig 2: mean (-+SE) weekly albumin of broiler chiks fed with Ochratoxin, diatomaceous earth and their combination

Serum triglycerides

The present study revealed that decrease in serum triglycerides level in birds fed with toxin (Group IV, V) as compared to control groups throughout experimental period (vide Fig. 3). These findings were in accordance with that of earlier reports [15, 16, 17]. Reduction in triglycerides reflects impaired liver metabolism leading to reduced synthesis of triglycerides. However, birds supplemented with DAE (Group VI, VII, VIII and IX) showed marginal increase in the levels this enzyme in comparison to toxin fed birds (Group IV and V), which indicates the protective role of DAE in binding of ochratoxin.

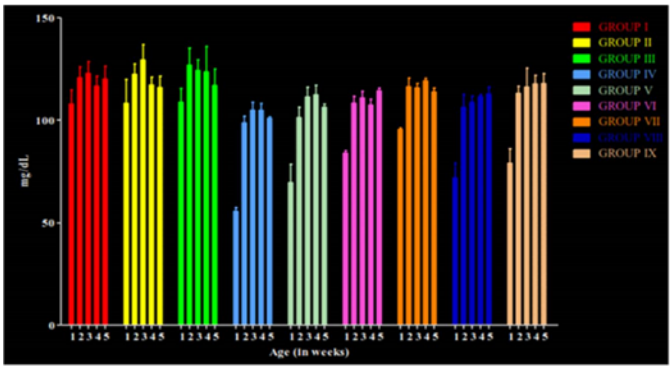

Fig 3: mean (-+SE) weekly triglycerides of broiler chicks fed with ochraftoxin, diatomaceous earth and their combination

Serum cholesterol

Significant decrease in serum cholesterol levels of toxin fed birds (Group IV and V) from second week onwards as compared to control birds (vide Fig. 4) corresponded with the earlier findings [18, 16, 17]. The reduction in serum cholesterol levels during ochratoxicosis reflects impaired liver metabolism, leading to reduced synthesis of cholesterol. However, birds supplemented with DAE along with toxin (Group VI, VII, VIII and IX) showed increase in serum cholesterol levels as compared to toxin fed birds (Group IV and V). The significant improvement in serum cholesterol levels is indicative of protective role of DAE against ochratoxicosis.

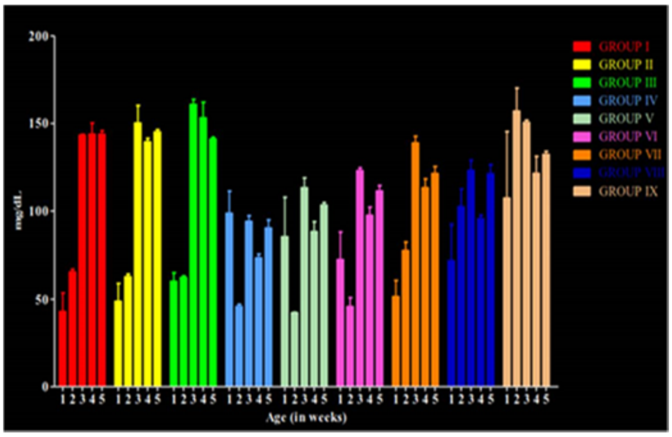

Fig 4: mean (-+SE) weekly cholesterol of broiler chicks fed with Ochratoxin, diatomaceous earth and their combination

Serum aspartate aminotransferase (AST)

The serum levels of AST showed a significant increase in the toxin fed birds (Group IV, V) against control birds (vide Fig. 5). The increase of AST level in the present study could be attributed to leakage of enzyme as a result of liver damage, due to degenerative changes as reported by Kumar *et al.* [19] which can be correlated to degenerative changes for increase in AST levels in the liver.

However, supplementation of DAE along with toxin in Groups VI, VII VIII and IX showed only a marginal decrease with that of toxin fed birds (Group IV, V) on all days of observation indicating the protective role of DAE in binding of ochratoxin from the feed.

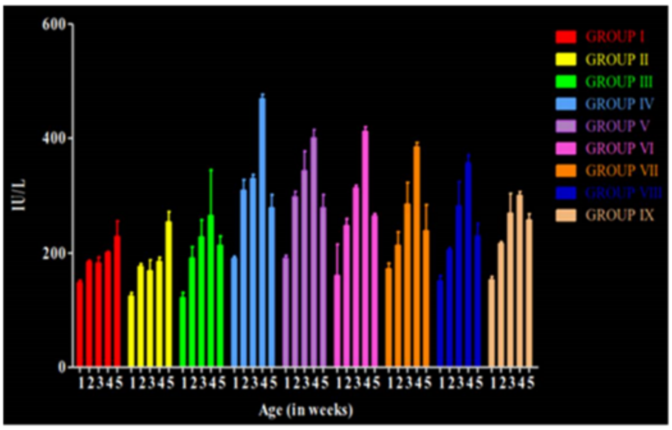

Fig 5: mean (-+SE) weekly AST of broiler chicks fed with Ochratoxin, Diatomaceous earth and their combination

Serum alanine aminotransferase (ALT)

In the present investigation, birds showed increased level of ALT in toxin fed birds (Group IV and V) as compared to control birds (vide Fig. 6). Increase in ALT values in the toxin fed birds could be attributed to the hepatic damage caused by ochratoxin. Similar observations were also reported by [20, 21]. Supplementation of DAE in co-treatment groups of VI to IX showed a marginal decrease in serum ALT levels as compared to toxin fed birds (Group IV and V) indicating their beneficial effect in ameliorating the effects of ochratoxin

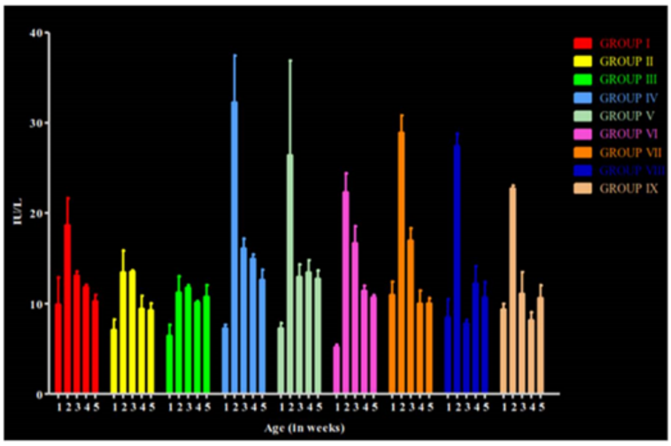

Fig 6: mean (±SE) weekly ALT of broiler chicks fed with Ochratoxin, Diatomaceous earth and their combination

Serum alkaline phosphatase (ALP)

The present study revealed that toxin fed birds (Group IV and V) showed a significant increase in serum ALP level as compared to control birds (Group I) on second and fifth week, and whereas birds supplemented with DAE (Group VI, VII VIII and IX) showed a marginal decrease in ALP levels in their serum respectively as against toxin fed birds (vide Fig. 7). The increased level of this enzyme may be correlated to the degeneration changes noticed in the liver leading to seepage of enzyme into the serum and this was supported by similar findings of Raina *et al.* [22] and Manafi and Bagher [23].

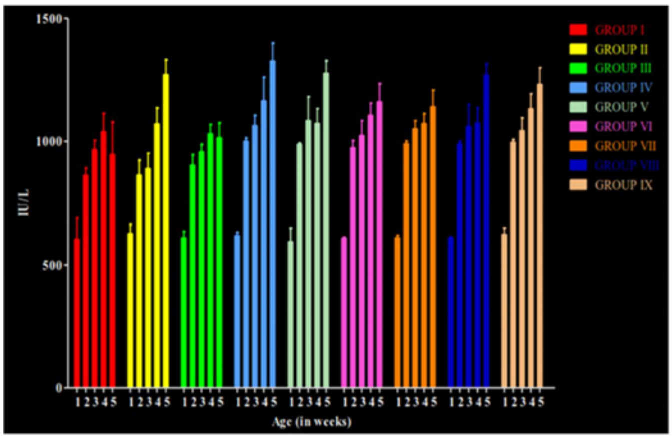

Fig 7: mean (±SE) weekly ALP of broiler chicks fed with Ochratoxin, diatomaceous earth and thieir combination

Serum Creatinine

The levels of serum creatinine were increased in birds fed with ochratoxin (Group IV and V) as compared to control groups (vide Fig. 8). Similar findings have also been reported earlier [24, 25, 26]. The increase in serum creatinine concentration in these birds could be due to damage of glomeruli and proximal convulted tubules by ochratoxin. Further, significant increase in kidney relative weights were indicative of renal damage in the present study and serum creatinine level may be considered as a sensitive indicator of ochratoxin induced renal damage.

Supplementations of DAE decreased the creatinine level in the serum of birds in Group VI to IX, respectively and decrease in relative kidney weight of Group VI to IX as against Group IV and V indicating the ameliorative effect. Supplementation of DAE prevented the rise in these values in toxin fed groups indicating its protective effect on kidney during ochratoxicosis.

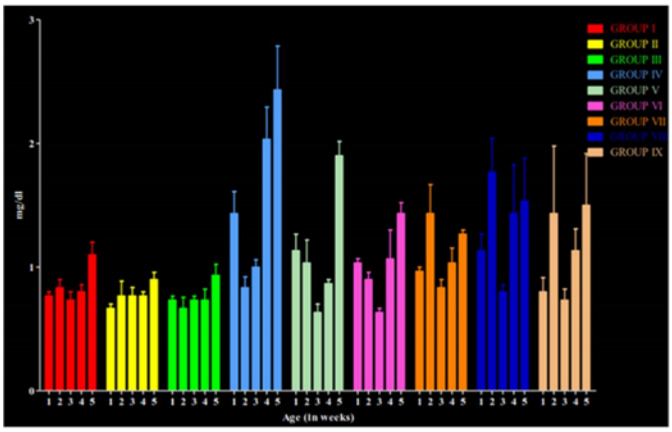

Fig 8: mean (-+SE) weekly creatinine of broiler chicks fed with Ochratoxin, diatomaceous earth and their combination

Serum Urea

The findings of significant increase in serum levels of urea of toxin fed birds (Group IV and V) as compared to control birds (Group I, II, and III) (vide Fig. 9) was in agreements with the earlier reports [27-31]. The increased levels of urea can be attributed to inflammatory or degenerative changes in the kidney. Nephrotoxicity is assumed to be due to interference with transport function in collecting tubular cells together with diffused impairment of proximal tubular function. However, decrease in serum urea levels was seen in birds supplemented with DAE along with toxin (Group VI, VII, VIII and IX) as compared to toxin fed birds (Group IV and V) indicates that supplementation of DAE could restore the increased levels of serum urea by its protective effect in case of ochratoxicosis.

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 triglycerides and cholesterol levels. However, there was decrease in the serum levels of AST, ALT, ALP, creatinine and urea in the co-treatment groups of VII to IX.

Perusal through the available literature indicated paucity of information on studies conducted on amelioration of ochratoxicosis using DAE as binder in poultry feed. Hence, the extrapolation of data on studies conducted on efficacy of DAE to bind aflatoxin could explain the findings of improvement in serum biochemical changes in birds of co-treatment group [32].

Similar study conducted earlier reported that reduced growth rate and serum biochemical changes associated with AFB_1 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 [13]. Similarly, Bailey *et al.* [14] reported that montmorillonite clay in broiler diets provided protection on growth performance, serum biochemistry, and the relative organ weights from over 4 mg of AFB_1/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 [33, 34, 35]. 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 [36]. Natour and Yousef [37] 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 [38]. 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 [39]. 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.

Manafi *et al.* [40] observed at pH 4.5 the highest percentage binding of mycotoxins was noticed for AF (90.68%) whereas the lowest binding ability was recorded for OA (61.73%) and at pH 6.5 highest binding percentage of mycotoxins was recorded for AF (94.71%) while on contrary with OA it was (63.13%) in in-vitro studies.

Soleimani *et al.* [38] observed that at pH 6.5, highest binding percentage of mycotoxins was recorded for AF (94.71%) and lowest binding percentage was noticed for OA (63.13%) with diet containing 0.2 percent binder, and in combination treatments the highest binding ability was noticed for AF+OA (65.80) and lowest binding ability was recorded for AF+OA+T-2 (6.26) for the pH of 6.5 in in-vitro studies.

In the present study, the incorporation of DAE in the diet during the period of exposure to AF could prevent the toxic effects of ochratoxin. The protective effects of DAE might plausibly be due to its capability of specific chemisorptions of ochratoxin in gastrointestinal tract, which reduces ochratoxin bioavailability for absorption and systemic circulation in broiler birds [41]. 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 ochratoxicosis in poultry.

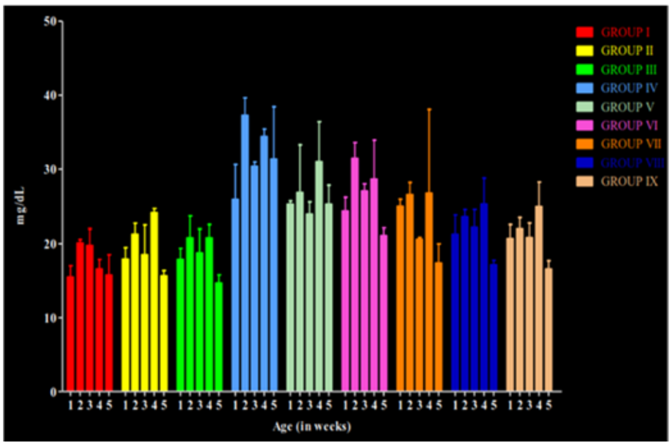

Fig 9: mean (±SE) weekly urea of broiler chicks fed with Ochratoxin, diatomaceous earth and their combination

Conclusions

The results collectively suggested that dietary incorporation of DAE significantly ameliorated the adverse effects of 0.5 and 1 ppm of ochratoxin on various serum biochemical parameters. The OTA induced injurious effects on production and health parameters in broiler chicks could not be alleviated by dietary incorporation of DAE at all levels used. Therefore, the use of DAE in ochratoxin- contaminated feed is an alternative method to reduce the adverse effects of ochratoxicosis in broilers. Further studies are suggested to investigate and compare the adsorption efficiencies of adsorbents against various mycotoxins using these methods.

Acknowledgement

Authors are the thankful to AGRIPOWER, Australia for providing DAE and financial support from ICAR-NAE project and the Dean, Veterinary College, Bangalore, India for providing the necessary facilities to carry out the research work.

References

1. Frisvad JC, Samson RA. Mycotoxins produced in species of *Penicillium* and *Aspergillus* occurring in cereals. In: Chelkowski (Ed) Cereal Grain Mycotoxins, Fungi and Quality in Drying and Storage. Elsevier, Amsterdam. 1991, 441-476.
2. Vander Merwe KJ, Steyn PS, Fourie L. Mycotoxins. Part II. The constitution of ochratoxin A, B and C, metabolites of Aspergillus ochraceus. J Chem. Soc. 1965a; 7083.
3. Vander Merwe KJ, Steyn PS, Fourie L, Scott De B, Theron JJ. Ochratoxin A, a toxic metabolite produced by *Aspergillus ochraceus.* Nature. 1965b; 205:1112-1113.

4. Kamigasa S, Kato H. Recent conditions and prospects of diatomite resources. Energy Resources. 2000; 21:166-172.
5. Eraslan G, Essiz D, Akdogan M, Karaoz E, Oncu M, Ozyildiz Z. Efficacy of dietary sodium bentonite against sub-chronic exposure to dietary aflatoxin in Broilers. Bull. Vet. Inst. Pulawy. 2006; 50:107-112.
6. Parker SP. Encyclopedia of the geological sciences, 2nd edn. New York, McGraw-Hill. 1988; 32-33:69-72:400-401.
7. Trenk HL, Butz ME, Chu FS. Production of ochratoxin in different cereal products by *Aspergillus ochraceus*. Appl. Microbiol. 1971; 21:1032-1035.
8. Singh GSP, Chauhan HVS, Jha G, Singh KK. Immunosuppression due to chronic ochratoxicosis in broiler chicks. J Comp. Path. 1990; 103:399-410.
9. Prakash GC. Pathology of combined effects of aflatoxin and ochratoxin in broiler chickens. M.V. Sc. Thesis, University Agricultural Science, Bangalore, India, 2001.
10. Rajeev K. Pathology of ochratoxin and T-2 toxin and their combined toxicity in broiler chickens. M.V.Sc. Thesis, University Agricultural Science, Bangalore, India, 2001.
11. Creepy EE, Lugnier AAJ, Fasiolo F, Heller K, Roschethaler R, Dirheimer G. *In-vitro* inhibition of yeast phenylalanyl- t- mRNA synthetase by ochratoxin A. A Chem. Biol. Interact. 1979; 24:257-261.
12. Kamagani S. Ochratoxin A. Plasma concentration and excretion into bile and urine in albumin- deficient rats. Food Chem. Toxicol. 1985; 23:941-943.
13. Shi YH, Xu ZR, Feng ZR, Wang CZ. Efficacy of modified montmorillonite nanocomposite composite to reduce the toxicity of aflatoxin in broiler chickens. Anim. Feed Sci. Tech. 2006; 129:138
14. Bailey CA, Latimer GW, Barr AC, Wigle WL, Haq AU, Balthrop JE, *et al*. Efficacy of montmorillonite clay (NovaSil PLUS) for protecting full-term broilers from aflatoxicosis. J Applied Poult. Res. 2006; 15:198-206.
15. Srikanth MK, Jeevana Latha M, Gopala Reddy A. Polyherbal compound Immuplus® ameliorates oxidative stress induced by ochratoxins in broilers. Int J Recent Scientific Res. 2013; 4(6):883-886.
16. Sawarkar AR, Sonkusale PM, Kurkure NV, Jangade CR, Maini S, Ravikanth K. Experimental Afla and Ochratoxin induced mixed mycotoxicosis in broilers and its amelioration with herbal toxin binder Toxiroak gold®. Int. J Poult. Sci. 2011; 10(7):560-566.
17. Sakhare PS, Harne SD, Kalorey DR, Warke SR, Bhandarkar AG, Kurkure NV. Effect of Toxiroak polyherbal feed supplement during induced aflatoxicosis, ochratoxicosis and combined mycotoxicoses in broilers. Vet. Arhiv. 2007; 77(2):129-146.
18. Kalorey DR, Sakhare PS, Kurkure NV. Effect of toxiroak on immunological response during induced mycotoxicosis in broilers. Indian Vet. J. 2005; 82:486-489.
19. Kumar A, Jinda N, Shukla CL, Yash Pal, Ledoux DR, Rottinghaus GE. Effect of ochratoxin A on *Escherichia coli* - challenged broiler chickens. Avian Dis. 2003; 47:415-424.
20. Mohiuddin SM, Vikram Reddy M, Ahmed SR. Studies on ochratoxicosis in broiler chicks. Indian Vet. J. 1992; 69:1011-1014
21. Umar S, Arshad A, Ahmad B, Arshad M. Clinico biochemical and haematological changes in broilers induced by concurrent exposure to aflatoxin B1 and ochratoxin A. J Pub. Health. Bio. Sci. 2012; 1(3):79-85
22. Raina JS, Roy KS, Balwant Singh. Biochemical and histochemical studies in experimental mycotoxicosis in chicks. Indian J Anim. Sci. 1991; 62:1276-1281
23. Manafi M, Bagheri H. Effect of combination of *Aspergillus ochraceus* (NRRL-3174) and *Penicillium citrinum* (NRRL-1841) on blood parameters of commercial chicks. Res. Opin Anim. Vet. Sci. 2011; 1(S):23-29.
24. Huff WE, Kubena LF, Harvey RB. Progression of ochratoxicosis in broiler chickens. Poult. Sci. 1988; 67:1139-1146.
25. Kubena LF, Huff WE, Harvey RB, Corrier DE. Influence of ochratoxin A and deoxynivalenol on growing broiler chicks. Poult. Sci. 1988; 67:253-260.
26. Bailey CA, Gibson RM, Kubena LF, Huff WE, Harvey RB. Ochratoxin A and dietary protein: 2. effects on hematology and various clinical chemistry measurements. Poult. Sci. 1989; 68:1664-1671.
27. Indresh HC, Umakantha B. Effects of ochratoxin and T-2 toxin combination on performance, biochemical and immune status of commercial broilers. Vet. World. 2013; 6(11):945-949.
28. Jayasri A, Raghavendra Srikanth N. Combined effect of aflatoxin and ochratoxin on liver enzymes of broilers and amelioration using adsorbents. J Livestock Sci. 2016; 7:26-29.
29. Bhatti SA, Khan MZ, Saleemi MK, Saqib M. Aflatoxicosis and ochratoxicosis in broiler chicks and their amelioration with locally available bentonite clay. Pak. Vet. J. 2016; 36(1):68-72.
30. Jayasri A, Sampath Kumar B, Narasa Reddy GV, Ravinder Reddy V. Studies on nephrotoxicity due to combined effect of Aflatoxin and Ochratoxin in broilers and their amelioration with adsorbents. J Livestock Sci. 2017; 8:172-175.
31. Qu D, Huang X, Han J, Man N. Efficacy of mixed adsorbent in ameliorating ochratoxicosis in broilers fed ochratoxin A contaminated diets. Italian J Animal Sci. 2017, 1-7.
32. Denli M, Blandon JC, Guynot ME, Salado S, Perez JF. Effects of dietary AflaDetox on performance, serum biochemistry histopathological changes, and aflatoxin residues in broilers exposed to aflatoxin B1. Poult. Sci. 2009; 88:1444-1451.
33. Rosa CAR, Miazo R, Oli C, Salvano M, Chiacchiera SM, Ferrero S, Saenz Carvalho ECQ, 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.
34. Denli M, Okan F. Efficacy of different adsorbents in reducing the toxic effects of aflatoxin B_1 in broiler diets. South African J Anim Sci. 2006; 36(4):222-228.
35. Modirsanei M, Mansoori B, Khosravi AR, Kiaei MM, Khazraeinia P, Farkhoy M, *et al*. 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.
36. Whitlow LW. Evaluation of mycotoxin binders, 4th Mid-Atlantic Nutrition Conference (Zimmerman, N.G. ed.), Proceedings. 2006; 132-143.
37. Natour RM, Yousef SM. Adsorption efficiency of diatomaceous earth for mycotoxin. Arab Gulf J Scientific Res. 1998; 16:113-127.

38. Soleimani R, Faradonbeh OP, Bagheri H. Mycotoxin detoxification of commercial broiler feed by a mycotoxin binder. Res Opin Anim Vet Sci. 2011; 1(12):778-780.
39. Denbow D. Gastrointestinal anatomy and physiology. In: Whittow, G.C., ed. Sturkie's Avian Physiology. 5thedn. Academic Press, San Diego, CA, USA. 2000, 299-324.
40. Manafi M, Narayanaswamy HD, Pirany N. Invitro binding ability of mycotoxins binder in commercial broiler feed. Afr. J Agri. Res. 2009; 4(2):141-143.
41. Abdel-Wahhab MA, Nada SA, Amra HA. Effect of aluminosilicates and bentonite on aflatoxin-induced developmental toxicity in rat. J Appl Toxicol. 1999; 19:199-204.