International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2018; 3(1): 10-14

www.veterinarypaper.com
Received: 03-11-2017
Accepted: 04-12-2017

VP Anand
Veterinary Officer, Veterinary Hospital, Mangalagatti, Tahsil-Dharwad, District- Dharwad, 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-Dharwad, District- Dharwad, Karnataka, India

Study on efficacy of diatomaceous earth on growth parameters in experimental ochratoxicosis in broiler chickens

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 the feed conversion ratio in comparison to the control groups. Supplementation of DAE to the OTA mixed feed could significantly ameliorate the adverse effect of ochratoxin on the growth and feed conversion in 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, growth and performance, 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* (Frisvad *et al.*, 1991) [5]. The primary toxin was identified as ochratoxin A (OA); its less toxic dechloro analog is ochratoxin B (OB) (Vander Merwe *et al.*, 1965 a, b) [16, 17]. 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 (Huff *et al.*, 1988) [8]. Ochratoxin ingestion also impairs the defence mechanism of the birds resulting in increased susceptibility to various infections (Kubena *et al.*, 1988) [11]. 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 (Kamigasa and Kato, 2000) [10]. These clay-like, chalky remains are referred as diatomite (Eraslan *et al.*, 2006) [4]. 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.

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 based on the growth and performance parameters.

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.* (1971) [15] with some minor modifications. Whole wheat (100g) 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 on day 7, 14, 21, 28 and 35 of the experiment using electronic balance. Feed consumption was recorded at weekly intervals and feed conversion ratio was calculated using the following formula.

$$FCR = \frac{\text{Average feed consumption per bird during the week (g)}}{\text{Average weight gain per bird during the week (g)}}$$

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 $P<0.05$.

Growth rate study

Body weight

The weekly mean body weights of different groups have been graphically depicted in Fig. 1.

The mean body weights among the birds of Group I, II, III were similar throughout the experimental period, however insignificant numerical increase in the body weight was observed in group II and III on day 35 of the experiment. However, significant ($P<0.05$) reduction in the body weight was observed in toxin fed birds (Group IV) from day 7, till the end of the experiment and in Group V significant ($P<0.05$) reduction was seen from day 28 to the end of the experiment.

The birds supplemented with DAE along with toxin (Group VI and VII) showed numerical increase in the bodyweight throughout the period of experiment, but significant ($P<0.05$) increase in the weight gain was seen from day 28 and continued till the end of experiment as compared to only toxin fed groups (Group IV). The birds group VIII and IX showed numerical increase in their body weight gain from day 28 onwards, but significant ($P<0.05$) increase in the body weight was seen on day 35 of experiment as compared to toxin fed Group V. There was 51.81 and 39.18 per cent reduction in body weights of birds fed with only toxin (Group IV and V) was noticed at the end of the fifth week as compared to Control (Group I) birds. The mean body weights recorded was 1516 ± 19.55, 1372.33 ± 46.13, 1359.5 ± 19.09, 730.5 ± 28.09, 922.0 ± 25.17, 953.33 ± 15.78, 1119.5 ± 62.47, 1176.5 ± 41.50 and 1133.83 ± 33.72 in Group I to IX, respectively at the end of the experiment. There was 23.37 and 34.77 per cent increase in body weight gain was observed in birds of Group VI and VII on day 35 of age as compared to only toxin fed birds (Group IV) and 21.66 and 18.69 percent body weight gain was observed in birds of Group VII and IX at 35 days in comparison to the toxin fed birds (Group V).

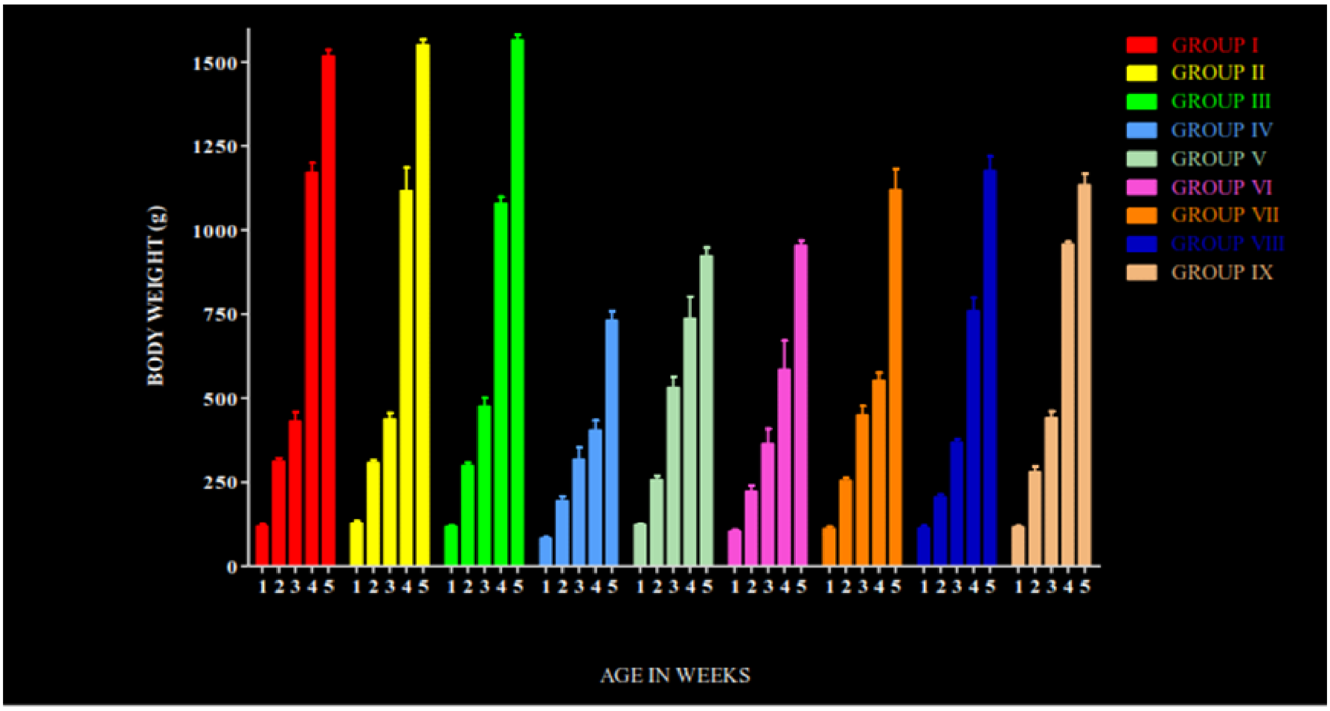

Fig 1: mean (+SE) weekly body weight (g0of broiler chicks fed with ochratoxin (OA)dlatomaccous earth (DAE) and their combination in feed

Feed conversion ratio (FCR)

The mean FCR with different dietary treatments have been indicated in Table 2 and graphically represented in Fig. 2. In comparison to control groups, birds supplemented with diatomaceous earth (Group II and III) showed insignificant decreased FCR values in on day 35 of experimentation. The FCR was significantly (P<0.05) increased in toxin fed birds (Group IV and V) throughout the experimental period as compared to control groups (Group I, II and III). The toxin fed birds had FCR value of 3.44 and 2.93 (Group IV and V). However, the birds of co-treatment groups (Group VI, VII, VIII and IX) recorded the FCR of 2.82, 2.44, 2.30 and 2.22, respectively at the end of day 35 of experiment. The FCR was highest in Group IV (3.44) and it was least in Group I (1.85) on the day 35 of the experiment.

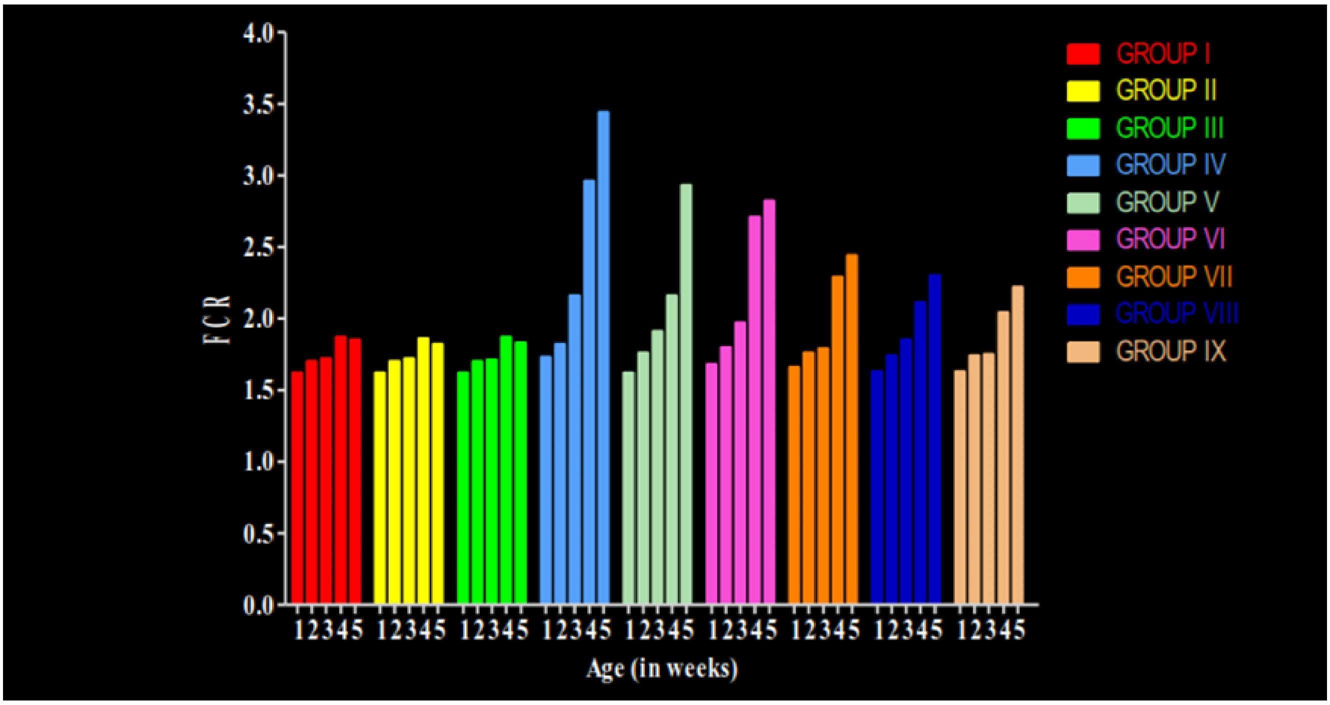

Fig 2: Feed conversion ratio of broiler chicks feed with ochratoxin (OA), diatomaceous earth (DAE) and their eombination

Discussion

Body weight

In the present study, there was a significant reduction in the body weight in ochratoxin fed group (Group IV and V) as compared to control birds. The reduction in the body weight due to Ochratoxin feeding in the present study is in agreement with findings of previous workers (Gentles *et al.*, 1999; Rajeev, 2001) [6]. The reduction in body weight gain observed in OA fed birds may be attributed either to decreased feed intake or impaired protein metabolism (Gibson *et al.*, 1989) [7], Huff *et al.* (1988) [9] have reported that OA competes with phenylalanine for binding sites of phenylalanine t-RNA synthetase enzyme, thus inhibiting the protein synthesis leading to decreased body weight gain. The birds of Group VI and VII which were fed with toxin and supplemented with diatomaceous earth (DAE) showed better weight gain as compared to only toxin fed birds (Group IV). This could be attributed to protective role of DAE and this was well supported by histological lesions of reduced severity and intensity in organs, thus it can be construed that DAE is partially effective in ameliorating the growth depressing effect of OA. Similarly birds fed with toxin and treated with DAE (Group VIII and IX) showed a marginal increase in weight gain as against to only toxin fed birds (Group V). This could be attributed to the incomplete binding of ochratoxin by the DAE. The findings of the present study were in agreement with Manafi *et al*., (2009) [12], who reported that binding of mycotoxins could be attributed to the toxin binding ability of DAE in feed since diatomaceous is considered as a powerful natural adsorbent and might adsorb the toxin effectively through the polar ends.

Feed conversion ratio (FCR)

In the present study, the FCR significantly increased in birds of Group IV and V in comparison to control birds. Increased FCR due to feeding of OA in the feed has been reported earlier by several workers (Gibson *et al*., 1989; Anil kumar, 2002) [7, 2]. Increased FCR could be attributed to competition of OA with phenyl alanine t RNA synthetase enzyme, which inhibits the protein synthesis as reported by Creepy *et al*. (1980). In addition, reduced feed intake due to toxin contaminated feed may also be a contributing factor for increased FCR in birds fed with OA. The present study indicated that in Group VI, VII, VIII and IX showed FCR of 2.82, 2.44, 2.30 and 2.22 as compared to only toxin fed birds (Group IV and V), which showed 3.44 and 2.93 respectively on day 35 of observation. These observations clearly shows supplementation of DAE along with toxin in the diet partially helped in increasing the feed efficiency of broilers.

The dietary inclusion of DAE improved the FCR of OA fed birds indicating the possible role of DAE in amelioration of mycotoxin as reported earlier Silambarasan *et al*., 2013; Manafi and Khosravinia, 2013) [13, 14]. Literature revealed paucity of information on the role of DAE in ameliorating the toxic effect of ochratoxin in poultry. However, study conducted on supplementation of AflaDetox (a natural product obtained from diatomaceous earth extracted from a quarry) to AFB1 contaminated feed, significantly improved the feed intake, BW gain, and feed conversion rate in AFB1 fed broilers. The improvement in the performance of the broilers is suggestive of ameliorating effect of DAE on toxic effects of AFB_1 in the broilers. The presence of highly porous surface of diatomaceous earth with high cationic interchange capacity is attributed for high in vitro adsorption capacity against AFB_1. In the light of the above, it can be construed that the ochratoxin might also have adsorbed to DAE in the gastro-intestinal tract in the similar manner to aflatoxin.

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 and could improve the growth and performance in broiler birds. 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.

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.

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