INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

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EVALUATION OF THE IMMUNE STATUS OF BROILER CHICKEN DURING OCHRATOXIN, CITRININ AND THEIR COMBINED TOXICITY

[a]Jayaramu G.M., [b]Satyanarayana M.L., [a]Ravikumar, P. & [b]Narayanaswamy H.D.
[a]Department of Veterinary Pathology, Veterinary College, Hassan - 573201, KVAFSU, Karnataka
[b]Department of Veterinary Pathology, Veterinary College, Bangalore – 560024, KVAFSU, Karnataka

ABSTRACT
The study was undertaken to evaluate the effect of feeding ochratoxin A and citrin either alone or in combination in broiler chicken. Two hundred broiler chicks were divided into four groups of 50 chicks each with the following treatment viz. Control diet, (group I), OA 1 ppm, (group II), CTN 12.5 ppm (group III) and combination 1 ppm OA plus 12.5 ppm CTN (group IV) up to 35 days of the trial. The blood samples were collected from all the groups at weekly interval to study the effect of this toxin on HI titre against NDV and ELISA titre against IBD. Reduction in HI titre against NDV and ELISA titre against IBD were observed in all the mycotoxin fed birds. Reduction in the mean skin fold thickness at 24, 48 and 72 hours post application of DNCB were noticed in toxin fed birds. Stray collection of lymphoid cells in skin was observed in mycotoxin fed birds 72 hours after application of DNCB. Findings of the present study pointed out that effect of combined toxicity were more pronounced in target organs than the individual toxicity. Severity of these lesions was found to be enhanced and suggested the additive or synergistic effect of these toxins in the broiler chicken.

KEYWORDS: ochratoxin A, citrinin toxicity, broiler chicken, HI titre, ELISA titre, DNCB.

INTRODUCTION

Mycotoxins comprise a structurally diverse family of naturally occurring fungal toxins which directly or indirectly contaminate the feed of livestock and poultry resulting in toxicities. In poultry, mycotoxicosis causes reduced growth rate, lowered feed conversion, impaired resistance to infectious disease and reduced vaccination efficacy with lesions in many organs (Coulombe, 1993). Ochratoxin and citrinin may occur as co-contaminants of feed and feed ingredients. Considering the effects of these mycotoxins on health and performance of birds as well as huge economic losses involved the present work was taken up to study in detail the effect of individual and combined toxicosis of ochratoxin and citrinin in broilers. The present investigation was undertaken to asses the HI titre against NDV and ELISA titre against IBD, mean skin fold thickness at 24, 48 and 72 hours post application of DNCB.

MATERIAL AND METHODS

Unsexed, day old Vencobb broiler chicks (200 numbers) were obtained from M/S Akash Hatcheries, Bangalore. They were provided with optimum conditions of brooding and management. Poultry mash, both starter and finisher without addition of toxin binder. They were tested for the presence of mycotoxins such as Aflatoxin, ochratoxin and citrinin. After ascertaining the mycotoxin free status of the feed, they were kept in individual labeled bins for further use. On day one of age, the broiler chicks were randomly divided into four different dietary treatment groups of 50 birds each *viz.,* Group I, fed standard mytoxin free basal diet (control), Group II, diet containing 1 ppm OA, Group III, diet containing 12.5 ppm CTN, Group IV, diet containing 1 ppm OA + 12.5 ppm CTN. Six birds from each group were sacrificed on day 7th, 14th, 21st, 28th and 35th day of the experiment.The blood samples were collected in non-heparinized tubes from six birds in each treatment by puncturing the brachial vein. Serum was separated after 8 hours and stored at -20^{o}C until further use. The sera samples were assayed for antibody titers against Newcastle disease virus by ELISA on day 7, 14, 21, 28 and 35 of the experiment. Similarly, the titers against Infectious bursal disease virus were recorded on day 7, 14, 21, 28 and 35. Commercial test kits (ProFLOK® PLUS NDV and IBD ELISA kit, Synbiotics Corporation, San Diego. CA) for detection of antibodies against Newcastle disease and Infectious bursal disease were used for the purpose. The plates were read on ELISA plate reader (Labsystems-Multiscan M.S., USA) at 405 nm. Treatment wise, means of titers were calculated and analyzed. Di Nitro Chloro. Benzene (DNCB) Hypersensitivity Skin Test was carried out as per the method described by Tiwary & Goel (1985) in birds aged five weeks. One per cent (1%) solution of 2, 4 – DNCB (Loba chemicals) in acetone was prepared by dissolving one gram of 2, 4 DNCB in 100 ml acetone and was used to assay cell mediated immune response. DNCB (0.1 ml of 1 percent) was injected to two birds in each group intradermally at interdigital (Plate 10) space between third and fourth digit of right leg using one ml tuberculin syringe. This was allowed to dry immediately by blowing so as to avoid the solution running down the sides. The thickness of the skin at the sight was measured using digital slide caliper (Plate 11) before challenge (0 hour) and 24, 48 and 72 hours after challenge and expressed in millimeters. The tissues were collected in 10 percent neutral buffered formalin. Paraffin embedded tissues were

sectioned to 5 µm thickness and stained by haematoxylin and eosin (H&E) for histopathological examination.

RESULTS & DISCUSSION

In the present investigation, the mycotoxin fed groups (Group II, III and IV) showed significantly lower antibody titre against NDV when compared to Group I. Recording of reduction in HI titre against NDV in OA fed birds in the present study fully agrees with the findings of Thyagarajan *et al*., (1996), Stoev *et al.,* (2000) and Anil Kumar (2002). The decreased antibody titre in OA fed broiler may be attributed to regression of the immunological organs and thus lymphoid cell population leading to reduced immunoglobulin level in the serum (Dwivedi and Burns 1984). In the present study, feeding of OA and CTN either alone or in combination to broiler chicks caused significant reduction in HI titre. This may be due to reduction in complement activity as rightly pointed out by Campbell *et al*. (1981) and Verma *et al*. (1995). Apart from hypoglobulinaemia, histopathological lesions revealed lymphoid depletion in bursa of Fabricius and spleen in CTN group, which might have had the deleterious effect on the humoral immunity of the birds. This particular findings fully agrees with the findings of Anandkumar, (2006). The Group IV showed significant decrease in the overall mean ND-HI titre value when compared to control and other mycotoxin groups which indicated that both OA and CTN played a synergistic role in causing the reduction when compared to OA alone. The observations such as lymphoid depletion and atrophy of lymphoid organs, hymproteinaemia in the mycotoxin fed birds could also contribute to the lower HI titre against ND. The mean ELISA titre against IBD was decreased significantly in all the mycotoxin fed groups when compared to Group I. This might have had the deleterious effect on the humoral immunity of the birds. The decreased antibody titres in OA fed broiler chicks could be attributed to regression of the lymphoid organs and depletion of lymphoid cell population leading to reduced immunoglobulin levels in serum (Dwivedi and Burns, 1984; 1984b). This feature was also consistently seen in the present study. In the present study, feeding of OA, CTN either alone or in combination (OA+CTN) to broiler chicks caused significant ($P \leq 0.05$) reduction in the ELISA titres against ND and IBD vaccines. This may be due to the reduction in the complement activity as pointed out by Campbell *et al*. (1983) and Verma *et al.* (1995). Reduction in the mean skin fold thickness at 24, 48 and 72 hours post application of DNCB was noticed in birds fed with OA (Group II) as compared to the control. The same could be due to the lymphocytolytic activity of OA leading to low CMIR. Similar reduction in the mean skin fold thickness after application of DNCB has been recorded in OA fed birds by Anil Kumar (2002) and Santhosh Kumar (2003), thus lending support the findings of the present study. The microscopic observation of skin from control birds (Group I) at 72 hours post application of DNCB, revealed multifocal areas of lymphoid cell aggregation with mild epidermal necrosis. Section of skin from Group II, III and IV birds revealed stray lymphoid aggregates when compared to control birds at 72 hours post application of DNCB. These findings were in accordance with that of Dwivedi and Burns (1984), Singh *et al*. (1992), Anilkumar (2002) and Santhosh Kumar (2003) who fed 1 ppm OA to broiler chicken for six weeks. The observation made in the present study could be attributed to immuno suppressive effect of mycotoxin on the immune response (CMRI). The present set of observation also draws support from the fact that the lymphoid organs in all the mycotoxin treated groups showed lymphoid depletion and massive lymphocytolytic activity.

TABLE 1 : Mean (± SE) Haemagglutination Inhibition (HI) titers against Newcastle Disease Virus (NDV) in broiler chicken fed with ochratoxin (OA -1 ppm), citrinin (CTN- 12.5 ppm) and their combination during different weeks of observation (n=24)

Groups	1st week	2nd week	3rd week	4th week	5th week	Mean value
I	3.50 ± 0.09	3.30 ± 0.12	4.00 ± 0.15	4.75 ± 0.12	5.00 ± 0.25	4.11^{a} ± 0.85
II	3.00 ± 0.17	3.00 ± 0.17	3.60 ± 0.07	4.00 ± 0.15	3.50 ± 0.09	3.42^{b} ± 0.86
III	2.83 ± 0.26	2.80 ± 0.26	3.50 ± 0.09	3.60 ± 0.07	3.00 ± 0.17	3.15^{bc} ± 0.87
IV	2.50 ± 0.11	2.60 ± 0.07	3.00 ± 0.17	3.20 ± 0.06	2.60 ± 0.07	2.78^{c} ± 0.88

Mean values bearing at least one common superscripts indicates no significant difference ($P \geq 0.05$) with each other

TABLE 2 : Mean (± SE) ELISA titers against Infectious Bursal Disease Virus (IBDV) in broiler chicken fed with ochratoxin (OA -1 ppm), citrinin (CTN- 12.5 ppm) and their combination during different weeks of observation (n=24)

Groups	1st week	2nd week	3rd week	4th week	5th week	Mean value
I	87.50 ± 3.82	236.67 ± 31.82	475.83 ± 35.76	723.33 ± 18.15	826.67 ± 7.92	470.00^{a} ± 53.09
II	85.67 ± 3.32	247.50 ± 39.87	483.33 ± 22.05	670.00 ± 23.94	793.50 ± 15.54	456.00^{b} ± 49.83
III	91.33 ± 5.73	217.5 ± 31.08	423.33 ± 19.94	531.67 ± 15.79	786.17 ± 24.96	410.00^{bc} ± 46.12
IV	82.50 ± 3.82	158.33 ± 21.82	373.33 ± 30.62	553.33 ± 18.56	727.50 ± 20.81	379.00^{c} ± 45.38

Mean values bearing at least one common superscripts indicates no significant difference ($P \geq 0.05$) with each other

TABLE 3: Mean (± SE) skin thickness (mm) to DNCB in broiler chicken fed with ochratoxin (OA -1 ppm), citrinin (CTN- 12.5 ppm) and their combination during different weeks of observation (n=24)

Groups	0 hour	24 hours	48 hours	72 hours	Mean value
I	1.27 ± 0.00	2.16 ± 0.03	1.62 ± 0.01	1.38 ± 0.04	1.61^{a} ± 0.72
II	1.07 ± 0.07	1.87 ± 0.17	1.29 ± 0.10	1.16 ± 0.19	1.35^{a} ± 0.75
III	1.13 ± 0.06	1.64 ± 0.18	1.23 ± 0.04	1.16 ± 0.02	1.29^{a} ± 0.75
IV	1.11 ± 0.09	1.44 ± 0.51	1.19 ± 0.13	1.02 ± 0.04	1.19^{a} ± 0.77

Mean values bearing at least one common superscripts indicates no significant difference (P≥0.05) with each other

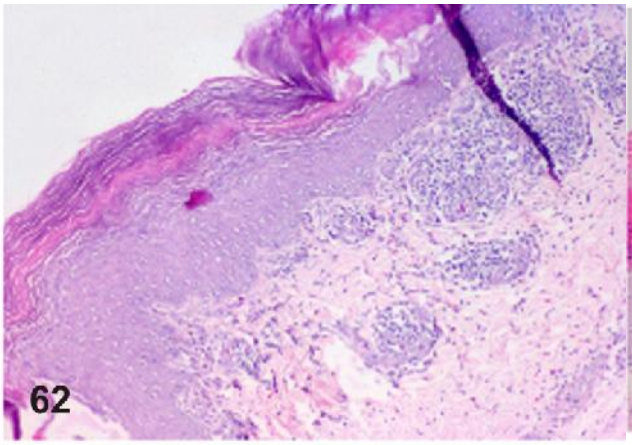

PLATE 1: Section of skin from control bird at 38 days of age showing severe degree of lymphoid aggregation in the dermis with intact epidermis. H&E X 100

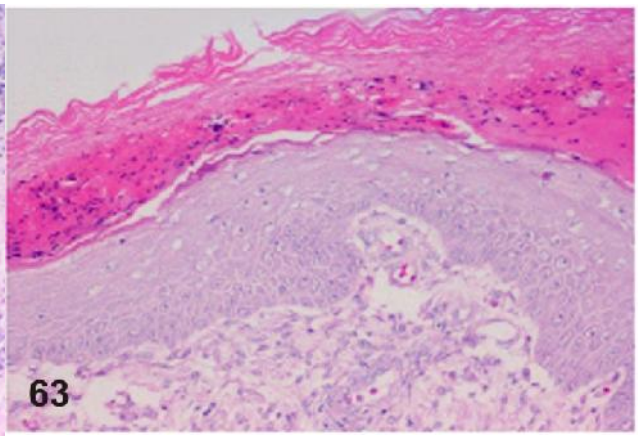

PLATE 2: Section of skin from control bird 38 days of age showing hyperkeratosis of epidermis increased thickness of stratum corneum with layers of keratin and moderate degree of inflammatory cells in the dermis. H&E X 100

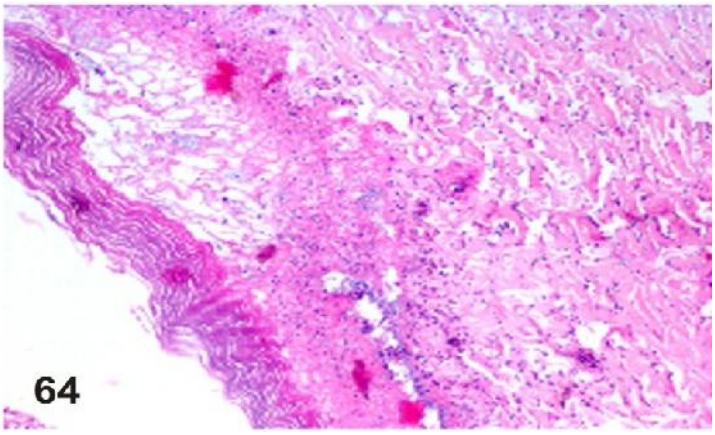

PLATE 3: Section of skin from OA fed bird 38 days of age showing mild epidermal necrosis and stray lymphoid aggregation in the dermis. H&E X 100

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