



OCHRATOXIN A AND CITRININ INDUCED PATHOMORPHOLOGICAL CHANGES IN BROILER CHICKEN

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ABSTRACT

The study was undertaken to evaluate the effect of feeding ochratoxin A and citrinin 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 experimental and the control birds were sequentially sacrificed and examined at 7, 14, 21, 28 and 35th day of the experiment. On post-mortem examination grossly, the toxin fed birds showed congestion, enlargement, pallor or yellowish discoloration of liver with distended gall bladder, swollen and congested kidneys. In addition, congestion of heart with prominent vasculature, pale, dehydrated and shrunken skeletal muscles, presence of small quantity of semisolid ingesta with slight mucous in crop and proventriculus, dry and shrunken gizzard, congested appearance of intestine with small quantity of mucous and congested pancreas was observed in all the toxin fed groups throughout the period of experimentation. Microscopically degenerative changes in hepatocytes, periportal fibrosis, periductular mononuclear cell infiltration, fatty degeneration, focal necrosis in the liver, degeneration and necrotic changes in the tubular epithelial cells in kidneys, myocardial degeneration, hyaline degeneration of muscle, mucosal hyperplasia of crop, proventriculitis, ventriculitis, catarrhal enteritis, pancreatitis, lymphoid depletion in the spleen, bursa of Fabricius and thymus were the prominent lesions observed when both the toxins were fed to birds from second to fifth week of age. 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, Pathology.

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). Exposure to low concentration of Ochratoxin A through diet is known to cause structural and functional changes in different organ systems, especially the kidneys and liver of several domestic and experimental animals^{5,9}. Citrinin is known to be nephrotoxic besides affecting the growth and productivity of birds^{3,18,19}. 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.

MATERIAL AND METHODS

Pure cultures of *Aspergillus ochraceus* (NRRL-3174) and *Penicillium citrinum* (NRRL 1841), maintained at Department of Poultry Science and Animal Nutrition, Veterinary College, KVAFSU, Hebbal, Bangalore respectively were used in the study for the production of OA and CTN. The concentrations and purity of OA and CTN were estimated using thin layer chromatography at the Animal Feed Analytical and Quality Control Laboratory, Veterinary College and Research Institute,

Nammakal – 637 001. 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 mycotoxin 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 plus 12.5 ppm CTN. Six birds from each group were sacrificed on day 7th, 14th, 21st, 28th and 35th day of the experiment. The broiler chicken sacrificed during the course of experiment, were systematically necropsied and gross lesions were recorded. After thorough examination, representative tissue pieces from the liver, kidneys, heart, skeletal muscle, crop, proventriculus, gizzard, intestine, pancreas, spleen, bursa of Fabricius and thymus were collected and preserved in 10% buffered formalin for routine histopathological examination. Tissues were processed by routine paraffin embedding technique and 5 micron sections were cut and stained with H&E as per the procedure (Luna, 1969). Histological lesions observed in various tissues of treated and control groups were systematically recorded and photo micrograph of interest was taken.

RESULTS & DISCUSSION

The carcasses of toxin fed birds were congested, dehydrated, emaciated and parboiled in appearance. These changes could be attributed to poor feed consumption, decreased protein synthesis and effect of toxin on various organs and tissues. The gross lesions observed in birds of Group II and III included enlarged, pale or congested liver with distended gall bladder containing thick bile. Histologically, the liver of ochratoxin fed birds showed congestion of vessels and sinusoids, occasional area of haemorrhages, mild degree of vacuolar degeneration and necrosis of hepatocytes. In addition, mild degree of biliary hyperplasia with heterophilic and lymphocytic infiltration in portal areas and perivascular and periductular fibrosis were also observed. Similar observation were also made in broiler chicks^{5,9,12,15} fed with ochratoxin. The increase in serum concentration of enzymes in the toxin fed birds and histological lesions observed in the toxin fed groups in the present study was an indication of hepatic damage occurred during ochratoxicosis. Histologically the liver from birds of Group III (CTN fed) revealed congestion of vessels and sinusoidal space and vacuolar degeneration of hepatocytes and fatty changes in the second week (Plate 1). Formation of lymphoid aggregate, mononuclear cell infiltration and acinar (glandular) transformation of hepatocytes in the fifth week. These observations were akin to the findings of earlier workers^{1,3,10,16,18,19} fed with citrinin. The hepatic lesions observed in Group IV birds were similar to those observed in the individual mycotoxin fed groups but they were of severe intensity and could be attributed to the synergistic or combined effect of both the toxin. The gross lesions observed in kidneys of toxin fed birds included tumescence, haemorrhages and paleness. Section of kidneys from Group II birds revealed congestion and haemorrhages in the interstitial and glomerular area, vacuolated appearance of the tubules, distended, misshapened PCT with loss of brush border and presence of eosinophilic proteinaceous material in their lumen (Plate 2). Such observations were also recorded in broiler chicks^{7,11,12,14} fed with ochratoxin.

In ochratoxicosis, the proximal tubular epithelium was primarily affected which could be related to inhibition of mitochondrial function by acting as a competitive inhibitor of carrier protein of inner membranes^{6,10}. Increased size of kidneys in toxin fed birds observed grossly could be due to enormous dilatation of the tubules and infiltration of inflammatory cells seen histologically. Tubular damage along with thickened glomerular basement membrane indicated the impaired renal function, which in turn reflected the cause of increased serum creatinine levels observed in this study. Similar findings were also recorded in broiler chicks^{4,5,3,18} fed with ochratoxin and citrinin individually. The lesions observed in the Group IV were of severe nature and could be attributed to combined effect / synergistic effect of both the toxins. The heart appeared congested with prominent vasculature. In few cases mild hydropericardium was also evident. These changes could be attributed to effect of toxins on various tissues including the heart. Section of heart in Group II birds showed mild degree of congestion and haemorrhages in the epicardium. Loss of cross striation, separation and hyalinization of cardiac fibers was also evident (Plate 3). Edema, congestion, haemorrhages, loss of cross striation,

enucleation, sarcolysis and hyalinization of the cardiac fibers were the lesions observed in the heart of CTN fed and combined toxicity groups. Similar findings were also recorded in broiler chicks^{1,19} fed with citrinin. The degenerative lesions in the heart might be due to venous congestion in the systemic circulation leading to anoxia and poor supply of nutrients to the vital organs like liver and kidneys including heart. The skeletal musculature appeared pale, shrunken and dehydrated in all the toxin fed groups. Histologically, mild degree of congestion, haemorrhages, loss of cross striation and hyalinization were observed from second to fifth week of age in all the toxin fed groups. Similar findings were also recorded in broiler chicks¹⁶ fed with ochratoxin. The histological lesions observed in the skeletal musculature in all the toxin fed birds could be attributed to action of toxins on the vasculature of the skeletal muscle leading to degeneration and necrosis in addition to hypoproteinaemia and malabsorption. The crop contained small quantity of mucous.

The sections of crop mucosa showed hyperplastic epithelium projected into the lumen, superficial ulceration, desquamation and edema of submucosa in all the toxin fed groups from third week onwards. Similar findings were also reported in broiler chicks³ fed with citrinin. The lesions observed in the crop of toxin fed birds in the present study could be attributed to irritant action of toxins on the crop mucosa. The proventriculus contained small quantity of mucous. The sections of proventriculus in the toxin fed birds revealed slight denudation and disruption of villous structure, infiltration of mononuclear cells in lamina propria, oedema of submucosa, during first and second week of the experiment. In addition to the above changes crypt elongation, increased disruption of crypt, increased goblet cell activity and presence of lymphoid aggregation in the submucosa were evident during third to fifth week of experiment in all the toxin groups. These findings were also recorded in broiler chicks fed with ochratoxin by earlier workers^{5,3,9,18}.

The gizzard appeared dry congested and reduced in size. Microscopically, gizzard revealed degeneration and desquamation of epithelial cells in mucosa, increased mucus secretion, hyperplasia of villous epithelium, edema of submucosa, disruption and separation of kiolin layer, cystic dilatation of glands and periglandular fibrosis in all the toxin fed groups. The changes noticed in the crop, proventriculus and gizzard in the present study could be attributed not only to the poor feed consumption and hypoproteinaemia but also to the direct action of toxins on these organs. Thus, the lesions in the gizzard could interfere with the grinding of feed and affect digestion and absorption in the intestine leading to decreased serum electrolyte concentration and hypoproteinaemia. Similar findings were also recorded in broiler chicks fed with ochratoxin^{8,5} and citrinin^{18,3} in the diet. The intestines appeared small, shrunken congested with small quantity of mucus. In few cases, catarrhal and haemorrhagic changes were observed. The catarrhal¹⁹ and haemorrhagic¹⁶ changes reported were in accordance with the findings of the present study in citrinin fed broiler chicks.

The histological lesion comprised of disruption of villous structure, increased goblet cell activity and partial necrosis of mucosal epithelium from second to fifth week of age in

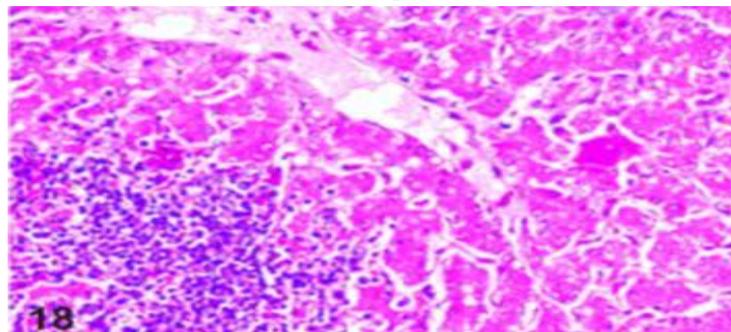
all the mycotoxin fed groups. The lesion observed in the present study could be attributed to direct irritant effect of toxin on the mucosa of GIT. In Group IV, besides increased goblet cell activity, fusion of villi and severe necrosis were also observed. These alimentary tract lesions affected the digestion and absorption of nutrients as was evident from the reduced weight gain, poor feed conversion observed in this study. Section of pancreas in Group II birds revealed congestion, haemorrhages and focal necrosis of acini during second to fifth week of age. Whereas, in Group III birds mild vacuolar degeneration of acinar cells and haemorrhages were observed.

In addition to the above lesions, in Group IV birds reduced zymogen granules and interstitial mononuclear cell infiltration were also observed from second to fifth week of age. The lesions observed in the present study could be attributed to action of toxins on the glandular and endothelial cells of pancreas. Similar findings were also reported in broiler birds^{1,3,9} fed with citrinin either alone or in combination with aflatoxin. The spleen appeared enlarged with few petechiae on them in all the toxin fed groups. This could be attributed to vascular and hyperplastic changes noticed during toxicosis. The histopathological lesions observed in the spleen of birds fed with OA revealed congestion and haemorrhages along with lymphocytolytic activity with increase in number of histiocytes in splenic corpuscles. Similar features were also reported in broiler birds^{5,12} fed with ochratoxin.

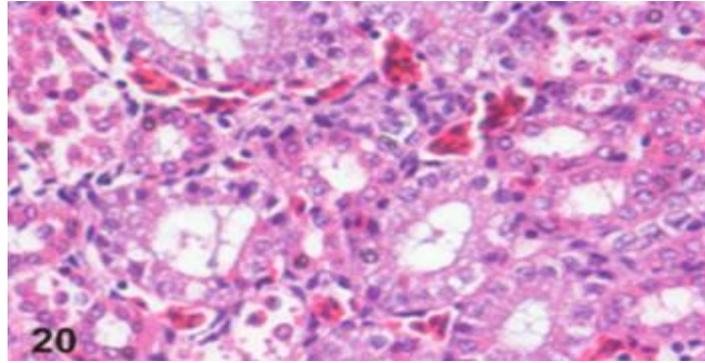
In Group IV birds, in addition to severe degree of above lesion presence of secondary follicles containing blast type of cells were also observed. Similar lesions were also reported in broiler birds^{1,3,18} fed with citrinin in combination with ochratoxin or aflatoxin. The findings of the present study indicated that these two mycotoxins affected the lymphoid system and compensatory lymphoid hyperplasia occurred in these organs. The birds fed with ochratoxin, grossly showed atrophic changes in bursa of Fabricius and thymus. These findings draw support from the fact that the relative weights of these organs were also marginally decreased consequent to lymphocytolytic activity observed in these organs. Similar observations were also reported in broiler chicks fed with ochratoxin either alone² or in combination with aflatoxin⁴/T-2¹³ toxin. Lymphocytolysis coupled with cellular sparcity with infiltration of histiocyte, presence of micro cyst in mucosa along with proliferation of connective tissue were the lesions observed in bursa of Fabricius of birds fed with ochratoxin in the diet (Group

II). Similar lesions were also reported in broiler birds⁷ fed with ochratoxin.

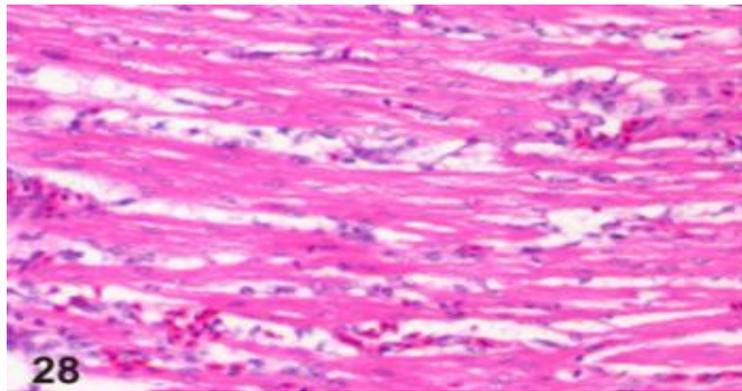
The immunotoxic property of OA is primarily responsible for lymphocytolytic activity resulting in lymphoid cell depletion and immunosuppression as observed by feeding ochratoxin in broiler chicken.^{5,15,17} Atrophy of bursa of Fabricius observed in this study during citrinin toxicity. The bursa of Fabricius in Group III revealed congestion, mild generalised lymphoid depletion in the follicles and lymphocytolysis with starry sky appearance in the third week (plate 4). During fifth week, atrophic changes with severe lymphoid depletion and interfollicular fibrosis were observed. Similar lesions were also reported in broiler birds¹ fed with citrinin. Similar observation were also recorded in broiler chicks¹⁶ fed with *P. citrinum*. In the present study, prolonged exposure of CTN for five weeks leading to increased lymphoid depletion might have caused the reduction in size. Slight congestion of bursa also observed during this study. Similar observation were also recorded in broiler chicks¹⁹ fed with 150 ppm CTN from 1 to 28 days of age. The gross lesions observed in birds of Group IV were similar to those recorded for individual toxin fed groups but they were of increased intensity. The Group IV birds showed lesions similar to those recorded in Group II and III birds but were of severe intensity. Similar lesions were also reported in broiler birds^{3,10} fed with citrinin along with ochratoxin or aflatoxin. The reason for the same could be inappetence, hypoproteinaemia and combined or synergistic effect of both toxin on various organs and tissues. Similar observation were also made in broiler chicks^{1,3} fed with CTN and AF in the diet. Rapidly proliferating cells of the immune system are reported to be more prone for the peroxidation damage by free radicals. This could be the possible reason for extensive damage noticed in the lymphoid organ of toxin treated birds. The microscopic changes recorded in thymus of ochratoxin and citrinin treated birds included moderate degree of congestion, haemorrhages, lymphocytolytic activity and reduction in the thickness of the cortex in the toxin fed birds (Plate 5). The Group IV (OA+CTN fed) birds revealed severe degree of above lesions during the entire period of experimentation. Similar lesions were also reported in broiler birds^{1,3} fed with citrinin along with ochratoxin or aflatoxin. Recording of lymphoid depletion in the thymus of mycotoxin fed groups in the present study further strengthen and the hypothesis that these toxin have immunotoxic potentials in rapidly dividing cells such as lymphoid organs.



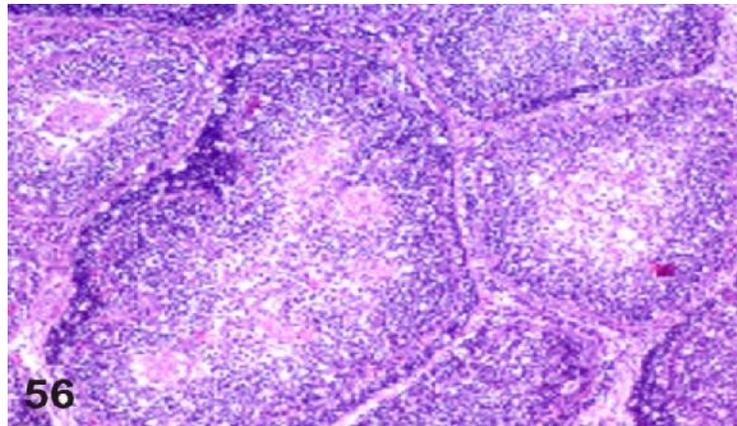
1. Section of Liver from OA and CTN fed bird at 28 days of age showing focal areas of hydrophic degeneration, fatty change and necrosis with infiltration of lymphoid cells.



2. Section of Kidney from OA fed bird at 28 days showing congestion, haemorrhages, swollen and vacuolated tubular epithelium, loss of brush border, desquamation of epithelial cells into the tubular lumen and presence of proteinaceous casts in the lumen.



3. Section of heart from OA fed bird at 28 days of age showing edema, haemorrhage, separation and disruption of cardiac fibres with loss of cross striation.



4. Section of bursa of Fabricius from CTN fed bird at 28 days of age showing severe lymphocytolysis with histiocytosis giving starry sky appearance.

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