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PATHOLOGICAL EFFECT OF CITRININ AND AFLATOXIN IN BROILER CHICKEN

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ABSTRACT

The individual and combined pathological effects of citrinin (CTN) at 5 ppm and aflatoxin (AF) at 0.5 ppm were studied in broiler chicken by feeding the mycotoxins from 0 to 6 weeks of age. In the entire toxin fed groups, inappetance and brownish diarrhoea were observed from first week onwards. The AF and CTN+AF fed groups showed ruffled feathers, lethargy, and stunted growth from the third week. There was no mortality in the control and mycotoxin fed groups. There was a significant ($P<0.05$) increase in the relative weight of the liver and spleen and decrease in the bursa of Fabricius in the entire mycotoxin treated groups when compared to the control group. In birds fed with CTN, the liver showed congestion, enlargement, pallor or yellowish discolouration and distended gall bladder. Kidneys revealed swelling, congestion and a few petechiae. Splenomegaly, atrophy of the bursa of Fabricius and catarrhal enteritis was also observed. In the AF group, the lesions were severe, affecting all birds. The gross lesions were pronounced in the sixth week. Microscopically, glomerular basement membrane thickening, degeneration and necrotic changes in the tubular epithelial cells in kidneys, degenerative changes in hepatocytes, microgranuloma, periportal fibrosis, periductular mononuclear cell infiltration, fatty degeneration, focal necrosis and fibrosis in the liver, mucosal hyperplasia of crop, proventriculitis, ventriculitis, catarrhal enteritis, pancreatitis, myocardial degeneration, mycocarditis, hyaline degeneration of muscle, lymphoid depletion and atrophic changes in the bursa of Fabricius, lymphoid depletion and reticulum cell hyperplasia in spleen, lymphoid depletion in caecal tonsils and plasma cell depletion in the Harderian gland were observed in the mycotoxin fed birds. Combined toxicity was more severe when compared to the individual mycotoxin fed groups. However, the effect was less than additive.

Key words: Citrinin, aflatoxin, broiler chicken and pathology

INTRODUCTION

The hepato-nephrotoxic mycotoxins citrinin (CTN) and aflatoxin (AF) are secondary metabolites of fungi *Penicillium citrinum* and *Aspergillus parasiticus* respectively which become dangerous, when the bird tries to detoxify them by conjugation. Co-occurrence of these two mycotoxins affect the productivity of broiler chicken by producing lesion in many organs, lowering the growth rate, feed conversion and resistance to infectious diseases by impairing both the cellular and humoral immunity of chicken which also leads to vaccination failures (Coulombe, 1993). Natural occurrence of these mycotoxins in poultry feeds causes major economic loss to poultry sector. The natural occurrence of CTN and AF in the feed ranged from 40 and 4800 ppb (Ahamad and Vairamuthu, 2000) and 1 ppb to 12 ppm (Natrajan *et al.,* 1999; Anandkumar *et al.,* 2005), and their cooccurrence in the feed was 9.3% and in combination with AF, the CTN concentration ranged from 40-800 ppb (Ahamad and Vairamuthu,

2000). Considering the recent reports on the increased frequency of co-occurrence of CTN and AF at sublethal doses in feed, the individual and combined pathological effects of CTN at 5 ppm and AF at 0.5 ppm level were studied in broiler chicken.

MATERIALS AND METHODS

Citrinin was produced on maize (Nelson *et al.,* 1980) and rice (Carlton *et al.,* 1974) and the AF was produced on rice (Shotwell *et al.,* 1966). The CTN and AF from the ground solid substrate samples were quantified by using thin layer chromatography (Tapia, 1985) at the Animal Feed Analytical and Quality Control Laboratory, Veterinary College and Research Institute, Namakkal-637 001and at the Central Animal Feed and Food Residue Laboratory, Centre for Animal Health Studies, TANUVAS, Chennai. Two experimental trials were conducted using 96 broiler chicks. In each trial, a total of 48 newly hatched broiler chicks were randomly allotted to 4 groups of 12 birds each and fed with control, CTN (5 ppm), AF (0.5 ppm) and CTN (5 ppm) + AF (0.5 ppm) diets from 0 to 6 weeks of age. Six birds from each group were sacrificed at 3rd and 6th week of age. After recording the gross lesions, liver, spleen and bursa of Fabricius were weighed to calculate their relative weights. Representative pieces of tissues from kidneys, liver, crop, proventriculus, gizzard, duodenum, pancreas, heart, pectoral muscle, bursa of Fabricius, spleen, thymus, caecal tonsils and Harderian gland, were collected in 10 percent formal saline to study the histopathological changes. Paraffin embedded tissues were sectioned to 5 µM thickness and stained by haematoxylin and eosin (H&E) for histopathological examination (Bancroft *et al.,* 1996). For ultrastructural studies of these two hepatonephrotoxic mycotoxins, liver and kidney samples were prefixed in 3 percent glutaraldehyde and stored at 4°C. The tissues were then dehydrated in ascending grades of cold alcohol (50, 70, 80, 90, 95 percent and absolute ethyl alcohol) and propylene oxide and then embedded in epon-araldite mixture. The ultra thin sections prepared on Leica ultracut microtome were stained with uranyl acetate and lead citrate and examined under Philips (Teknai-10) computer augmented transmission electron microscope operated at 60-kilowatt ampere (KVA). For statistical analysis the data generated from the experimental trials were subjected to one or two way analysis of variance using SPSS version 9.0 software for windows.

RESULTS

In the entire mycotoxin fed groups, inappetance and brownish diarrhoea were observed from first week onwards. The AF and CTN+AF fed groups showed a reduced appetite, ruffled feathers, lethargy and stunted growth from the third week. In CTN+AF group, the clinical signs were more pronounced. No mortality was observed in the control and toxin fed groups.

Relative organ weights

Mean ± SE relative weights of liver, spleen and bursa of Fabricius of broiler chicks fed with control, CTN, AF and CTN+AF are shown in Tables 1 to 3. There was a significant ($P<0.05$) increase in the relative weight of the liver and spleen and significantly ($P<0.05$) decrease in the bursa of Fabricius in the entire mycotoxin treated groups when compared to the control group.

Liver

The relative weights of liver were significantly increased in the mycotoxin fed group, when compared to the control. The increase that was significant among the mycotoxin fed groups was in the ascending order of CTN, AF and CTN+AF groups.

Spleen

Significant increase in the relative weight of the spleen during sixth week was observed in all the mycotoxin fed groups when compared to the control.

Bursa of Fabricius

There was a significant decrease in the relative weight of bursa of Fabricius in the CTN and CTN+AF fed groups when compared to control. The weights of bursa of Fabricius in the third and sixth weeks were not significantly different and the size was increased in the sixth week when compared to the third week. The weight of bursa of Fabricius in the AF group was equal to that of control and this was due to the increased weight in the third week,

when compared to all other groups, which had compensated the reduced weight during sixth week. The bursa of Fabricius in CTN group was significantly lower than the control. In the AF group, the bursa of Fabricius weight, though numerically lower in the sixth week, was compensated by the increased size during the third week and therefore the overall mean weight was not significantly different from that of control weight.

Gross pathology

In birds fed with CTN, kidneys showed swelling, congestion and a few petechiae when compared to the control. The liver showed enlargement, congestion, pallor or yellowish discolouration with distended gall bladder. Splenomegaly, atrophy of the bursa of Fabricius and catarrhal enteritis was also observed. In four out of 12 CTN fed birds, the lesions were mild. In the birds fed with AF, the lesions observed were similar to the CTN group but were severe and encountered in all the birds. Gall bladder was distended with thin or thick greenish bile. Catarrhal enteritis was observed. In the CTN+AF group, the lesions in the kidneys (Fig.1), liver (Fig.2) and lymphoid organs were more pronounced in all birds when compared to CTN and AF fed groups. Gall bladder was distended with thick greenish bile. In all mycotoxin fed groups, the gross lesions were pronounced in the sixth week.

Histopathology - Kidneys

The CTN treated birds showed thickening of glomerular basement membrane (Fig.3), degeneration of tubular epithelium and necrosis. Occasionally stasis of baso-eosinophilic fluid in the lumen of tubules was observed (Fig.4). The changes were similar in both third and sixth weeks. In the third week, AF treated kidneys showed mild thickening of glomerular basement membrane, vacuolar degeneration and necrosis of tubular epithelial cells were observed. Two birds showed atrophic glomeruli and calcification in the sixth week besides the above changes. In combined group, kidneys revealed glomerular basement membrane thickening, atrophy of glomeruli with calcification (Fig.5) and tubular epithelial cell degeneration and necrosis (Fig. 6), during the third week. Besides the above changes, mononuclear cell infiltration in the interstitium was observed in the sixth week (Fig.7).

Liver

The liver from birds of CTN group revealed congestion of veins and focal to extensive vacuolar degeneration of hepatocytes during the third week. Ballooning degeneration, microvesicular to macrovesicular fatty degeneration (Fig.8), focal haemorrhage, mononuclear cell infiltration and acinar transformation of hepatocytes (Fig.9) were also encountered. Microgranulomas consisting of mononuclear cells (Fig.9) were found in the parenchyma. Kupffer cell hypertrophy was seen in the third week. During sixth week, bile duct hyperplasia, periductular mononuclear cell infiltration and fibrosis were observed. Liver from AF treated birds showed congestion, venous dilatation and extensive vacuolar degeneration of hepatocytes. Ballooning degeneration and fatty cyst formation were also observed. The regenerating hepatocytes were arranged in acinar pattern and also revealed fatty degeneration. Microgranulomas, bile duct hyperplasia with villi projecting into lumen, periductular mononuclear cell infiltration and in a few cases periportal fibrosis were also observed in the third week. In sixth week, focal necrosis and fibrosis were observed, in addition. In CTN+AF fed birds, the above hepatic changes were more pronounced (Fig.10,11).

Crop

The mucosa showed hyperplasia in all mycotoxin fed birds. In CTN, apart from hyperplasia, two birds showed colonies of bacilli organisms embedded in the mucosal surface (Plate 9d). The hyperplastic mucosa in the AF fed birds showed projections into the lumen as in other mycotoxin fed birds. In the CTN+AF group, the hyperplastic changes were more pronounced. Muscular layer showed degenerative changes (Fig.12).

Proventiculus

The proventricular muscosa of the CTN fed birds showed crypt elongation and mononuclear cell infiltration at the base of the villi during the third and sixth weeks. The AF fed birds showed infiltration of mononuclear cells in the proventricular mucosa and crypt elongation during third week. In the sixth week, the lamina propria showed oedema and mononuclear cell infiltration. The CTN+AF fed birds showed shortened villi (Fig.13), oedema in the

lamina propria (Fig.14) and mononuclear cell infiltration in the mucosa (Fig.14). Partial necrosis of mucosa, elongation of crypts and necrosis of proventricular glands were also observed.

Gizzard

The birds of CTN group showed dilatation of glands, lack of secretion, elongation of crypts and mild interglandular fibrosis in a few cases. In the sixth week, mononuclear cell infiltration in the interglandular area was observed. The AF fed birds showed dilatation of glands with eosinophilic secretion in the third week (Fig.15) and interglandular fibrosis in the sixth week. The CTN+AF combined group showed dilatation of glands, eosinophilic secretion, severe interglandular fibrosis in a few cases and vacuolar degeneration of the muscle (Fig.16) in the third week. During sixth week, extensive fibrosis and glandular atrophy (Fig.17) were observed.

Intestine

The CTN fed birds showed increased goblet cell activity and fusion of villi in the third and sixth weeks. The AF fed birds showed increased goblet cell activity and partial necrosis of mucosal epithelium in the third and sixth weeks. In the CTN+AF fed birds, besides increased goblet cell activity, fusion of villi and severe necrosis were observed. During sixth week, shortened villi and hyperplastic changes were observed.

Pancreas

In the CTN fed birds, no changes were observed. In the AF fed birds reduced zymogen granules were observed in the third and sixth weeks. In the CTN+AF fed birds, reduced zymogen granules and interstitial mononuclear cell infiltration (Fig.18) were observed in the third and sixth weeks.

Heart

In the CTN fed birds, no changes were noticed. In the AF fed birds, myocardial degeneration was noticed in the sixth week. In the CTN+AF fed birds, myocardial degeneration was noticed in the third and sixth weeks with mononuclear cell infiltration (Fig.19).

Skeletal muscle

In the CTN fed birds, no changes were observed. The birds of AF fed group showed hyaline degeneration of the pectoral muscle in both third and sixth weeks. The CTN+AF fed birds showed hyaline degeneration of the pectoral muscle (Fig.20) in the third and sixth weeks.

Lymphoid organ-Bursa of Fabricius

The CTN fed group revealed congestion, mild generalised lymphoid depletion both in the cortex and medulla and lymphocytolysis with starry sky appearance in the third week. During sixth week, atrophic changes with severe lymphoid depletion and corrugation of plical epithelium were observed. In the AF fed birds, the bursa of Fabricius showed medullary lymphoid depletion and lymphocytolysis with starry sky appearance and mild oedema during third week and in the sixth week, six birds showed atrophic changes with increase in interfollicular fibrous tissue, variable sized follicles and lymphocytolysis. In the CTN+AF treated birds, medullary lymphoid depletion, medullary cyst formation, follicular haemorrhages, epithelial hyperplasia, atrophy and lymphocytolysis were observed in the third week. Lymphocytolysis (Fig.21), severe atrophy of follicles with corrugation of plical epithelium, interfollicular fibrosis and multiple cysts (Fig.22) were observed during sixth week.

Lymphoid organ-Spleen

In the CTN fed birds, spleen revealed heterophilic infiltration, apoptotic bodies (Fig.23), lymphoid depletion, lymphocytolysis and reticulum cell hyperplasia, during third week. In the sixth week, severe lymphoid depletion with reticulum cell hyperplasia was observed. The spleen of AF treated birds showed moderate lymphoid depletion and reticulum cell hyperplasia in the third week. During sixth week, necrosis of lymphoid cells were observed. Severe lymphoid depletion, lymphocytolysis and reticulum cell hyperplasia were observed in the spleen of CTN+AF group during the third week. During sixth week, increase in germinal centres was observed.

Lymphoid organ-Caecal tonsils

In the third week, mild lymphoid depletion and lymphocytolysis in the nodular and diffuse lymphoid tissue were observed in the caecal tonsils of CTN fed birds. During sixth week, moderate to severe lymphoid depletion was observed. In the third week, AF fed birds showed moderate to severe lymphoid depletion both in the nodular and diffuse lymphoid tissues. During sixth week, the AF fed birds revealed severe lymphoid depletion. Lymphoid depletion (Fig.24) was seen in the third week itself in the CTN+AF birds, which continued in the sixth week also (Fig.25).

Lymphoid organ-Thymus

The CTN group revealed mild lymphoid depletion in thymus in the third and sixth weeks. The AF group revealed mild lymphoid depletion in thymus in the third and sixth weeks. The CTN+AF group birds showed mild lymphoid depletion in the third week and lymphocytolysis in the sixth week.

Lymphoid organs-Harderian gland

The CTN group showed mild plasma cell depletion with cystic changes in the epithelium. Plasma cells with Russel bodies (Fig.26) were observed in the third and sixth weeks.The AF treated group showed moderate plasma cell depletion in the Harderian glands during third and sixth weeks. The CTN+AF group birds showed severe plasmacytic depletion in the Harderian gland during third (Fig.27) and sixth weeks.

Ultrastructural Pathology

The CTN fed birds showed vacuoles in the tubular epithelial cells of kidneys and swollen mitochondria with disruption of cristae (Fig.28). A few tubular epithelial cells had large areas of cytoplasm filled with smooth endoplasmic reticulum. Liver showed vacuoles in the cytoplasm and swollen mitochondria. In the kidneys of AF treated birds, thickening of glomerular basement membrane was observed between the epithelial and endothelial surface, although most prominent towards endothelial surface. Some tubules showed swelling with proliferation of mitochondria and generalized disorganization of cell architecture. Hepatocytes showed lipid droplets in the cytoplasm masking the other subcellular organelles except mitochondria. In many hepatocytes, cytoplasmic disorganisation was noticed. A few necrotic hepatocytes and dilatation of bile canaliculi were observed. The changes in CTN+AF mycotoxicoses were comparable to the individual toxicities for kidney and liver (Fig. 29,30).

Table 1

Mean (±SE) relative weight of liver in broiler chicken fed with control, CTN, AF and CTN+AF diets (n=12)

Groups	Relative weight of liver (g)		
	3rd week	6th week	Over all mean
Control	2.66 ± 0.06	2.68 ± 0.20	2.67^{d} ± 0.10
CTN(5 ppm)	4.16 ± 0.12	3.08 ± 0.17	3.61^{c} ± 0.15
AF (0.5 ppm)	4.69 ± 0.30	3.44 ± 0.16	4.06^{b} ± 0.21
CTN+ AF	5.26 ± 0.31	3.95 ± 0.18	4.61^{a} ± 0.22

Means with different superscripts differ significantly (P<0.05)

Table 2

Mean (±SE) relative weight of spleen in broiler chicken fed with control, CTN, AF and CTN+AF diets (n=12)

Groups	Relative weight of spleen (g)		
	3rd week	6th week	Overall mean
Control	0.10 ± 0.01	0.16 ±0.01	0.13^{b} ± 0.01
CTN(5 ppm)	0.22 ± 0.02	0.16 ± 0.02	0.19^{a} ± 0.01
AF (0.5 ppm)	0.15 ± 0.00	0.33 ± 0.06	0.24^{a} ± 0.04
CTN+ AF	0.22 ± 0.02	0.21 ± 0.02	0.21^{a} ± 0.01

Overall means with different superscripts differ significantly (P<0.05)

Table 3

Mean (±SE) relative weight of bursa of Fabricius in broiler chicken fed with control, CTN, AF and CTN+AF diets (n=12)

Groups	Relative weight of bursa of Fabricius (g)		
	3rd week	6th week	Overall mean
Control	0.14 ± 0.01	0.29 ± 0.03	0.22^{a} ± 0.02
CTN(5 ppm)	0.13 ± 0.01	0.21 ± 0.01	0.17^{b} ± 0.01
AF (0.5 ppm)	0.18 ± 0.01	0.26 ± 0.01	0.22^{a} ± 0.01
CTN+ AF	0.14 ± 0.01	0.22 ± 0.02	0.18^{b} ± 0.01

Overall means with different superscripts differ significantly (P<0.05)

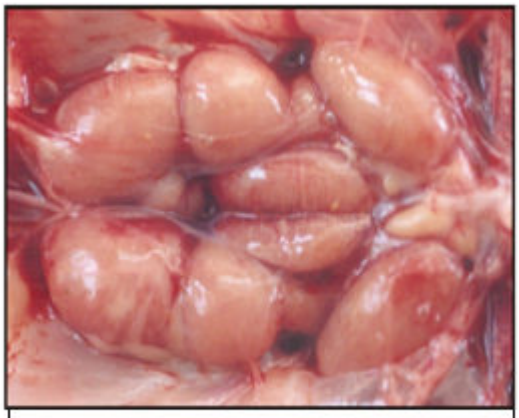

Figure 1. Chick - 3 weeks (wks) - CTN+AF: Marked enlargement and pale kidneys

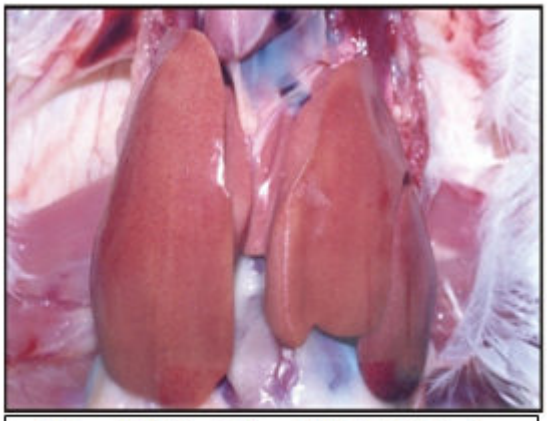

Figure 2. Chick - 6 wks - CTN+AF: Markedly enlarged and pale liver

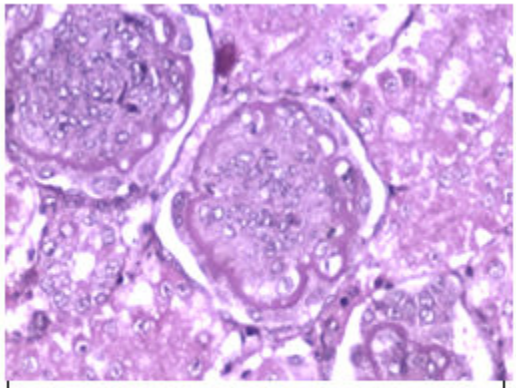

Figure 3. Chick-3 wks- CTN- Kidneys: Glomerular basement membrane thickening H&E x1000

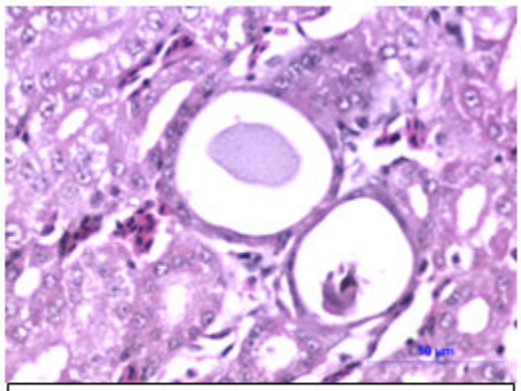

Figure 4. Chick - 3 wks - CTN - Kidneys: Baso-eosinophilic fluid in the tubular lumen H&E x 400

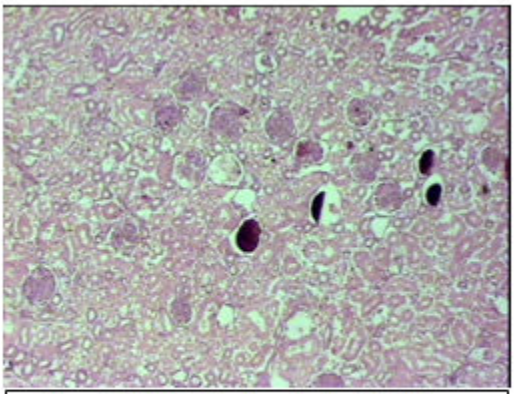

Figure 5. Chick - 3 wks – CTN+AF- Kidneys: Glomerular Atrophy and calcification H&E x 100

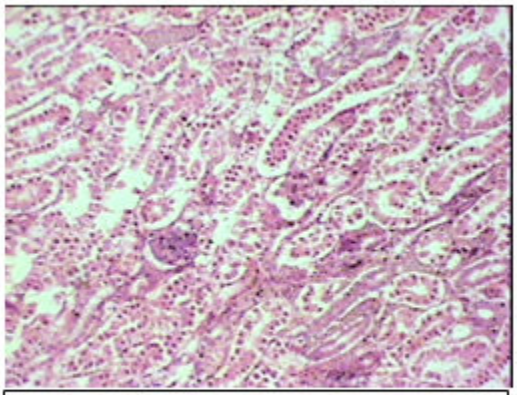

Figure 6. Chick - 6 wks- CTN+AF- Kidneys: Tubular epithelial cell degeneration and necrosis H&E x200 100

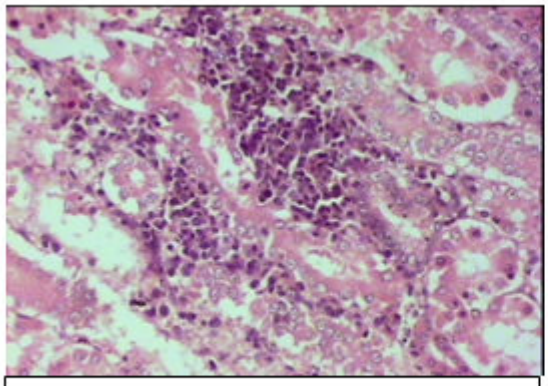

Figure 7. Chick- 6 wks-CTN+AF- Kidneys: Interstitial Mononuclear cell infiltration H&E x200

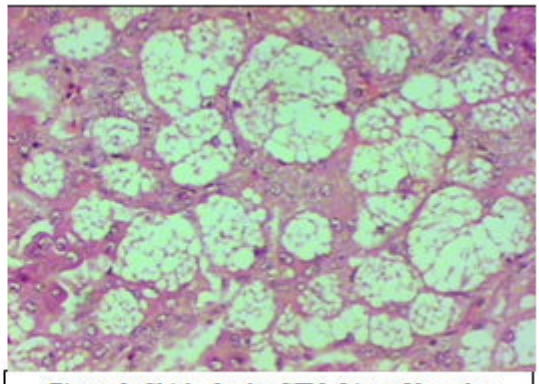

Figure 8. Chick- 3 wks-CTN- Liver: Vacuolar degeneration of hepatocytes H&E x400

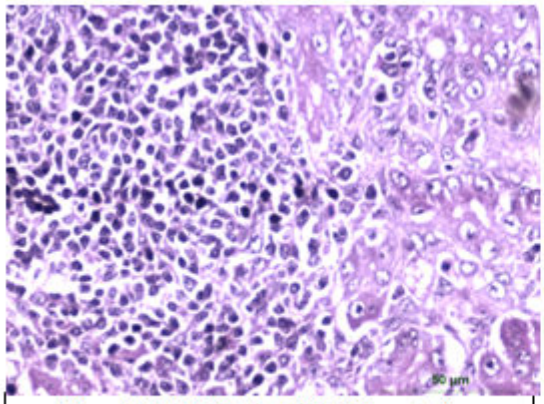

Figure 9. Chick- 3 wks-CTN- Liver: Acinar formation and microgranuloma H&E x 400

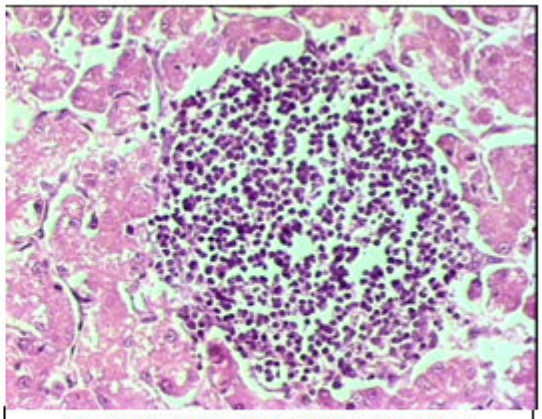

Figure 10. Chick- 3 wks-CTN+AF- Liver: Microgranuloma H&E x 200

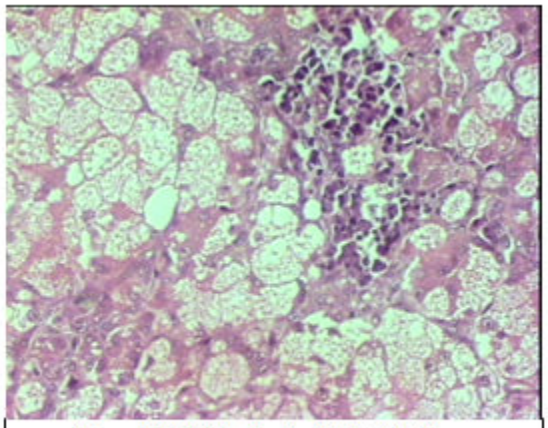

Figure 11. Chick- 3 wks-CTN+AF- Liver: Mononuclear cell infiltration in the parenchyma H&E x 200

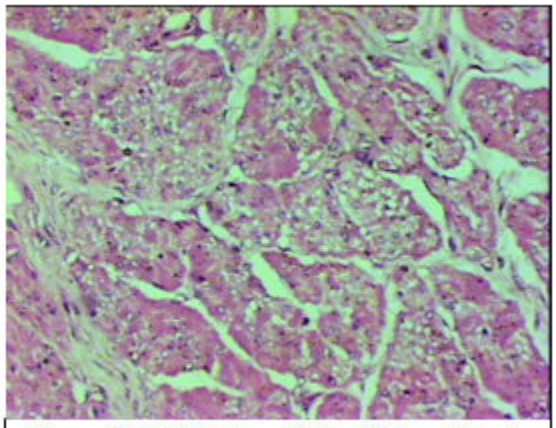

Figure 12. Chick- 3 wks - CTN+AF- Crop: Muscle degeneration H&E x 400

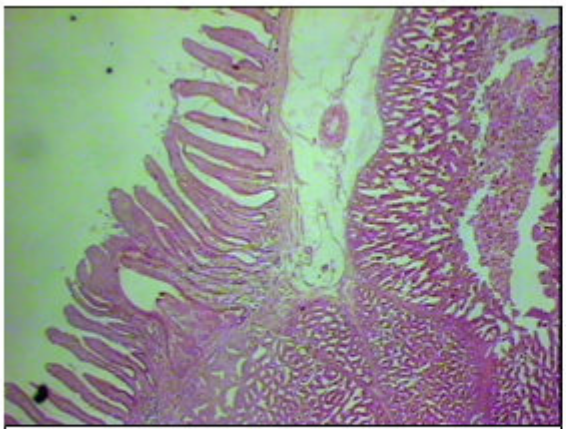

Figure 13. Chick- 3 wks-CTN+AF- Proventriculus: Shortening of the villi and oedema in the lamina propria H&E x 100

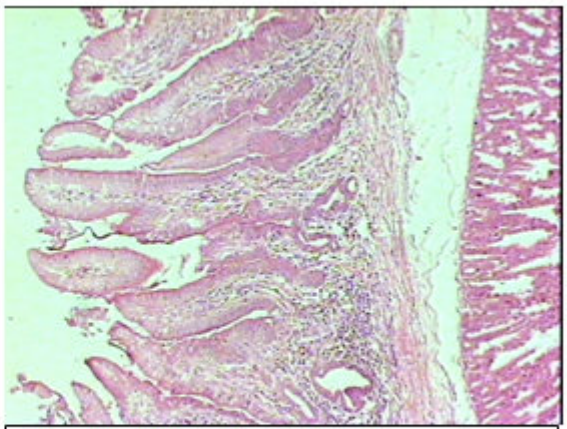

Figure14 . Chick- 6 wks-CTN+AF- Proventriculus : Mononuclear cell infiltration in the mucosa and oedema H&E x 200

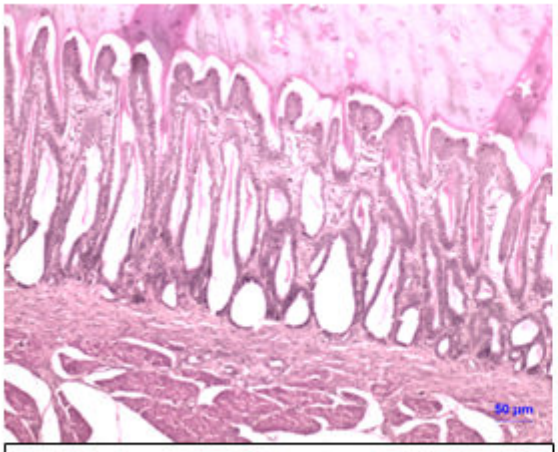

Figure 15 . Chick- 3 wks- AF- Gizzard: Dilatation of the glands H&E x 200

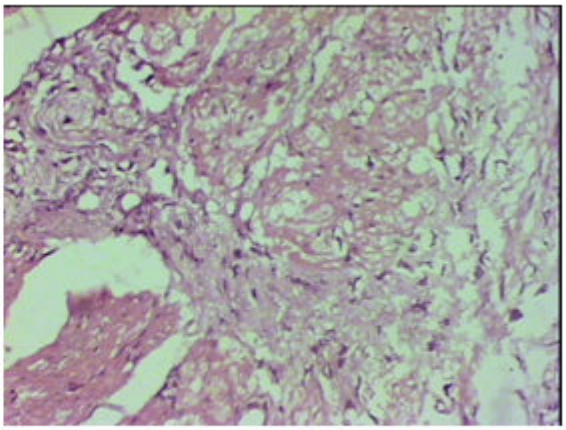

Figure 16. Chick- 3 wks-CTN+AF- Gizzard: Muscle degeneration H&E x 200

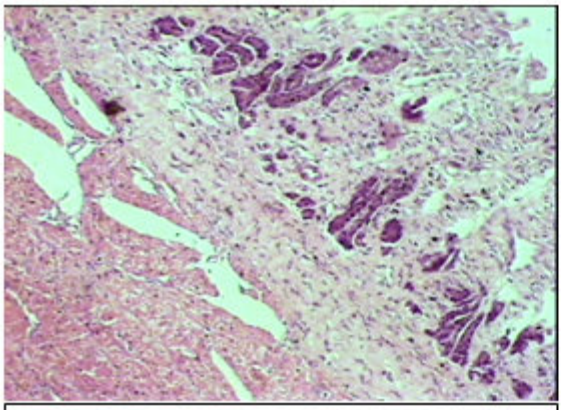

Figure 17. Chick- 6 wks- CTN+AF- Gizzard: Fibrosis and glandular atrophy H&E x 200

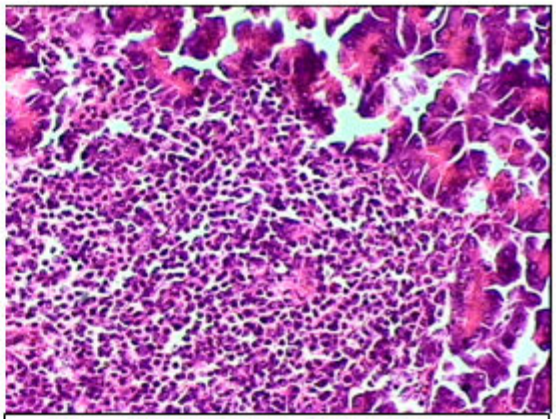

Figure 18. Chick- 3 wks- CTN+AF- Pancreas Mononuclear cell infiltration H&E x 200

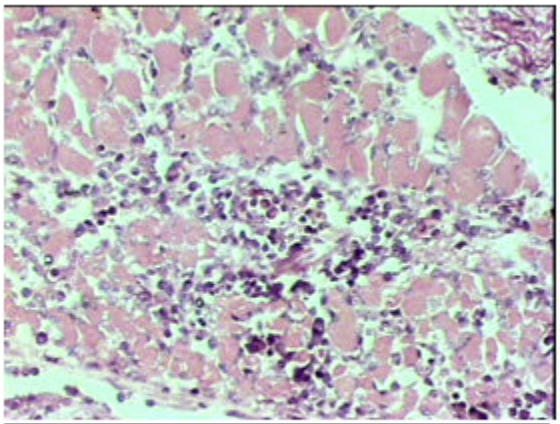

Figure 19. Chick- 3 wks- CTN+AF- Myocarditis H&E x 400

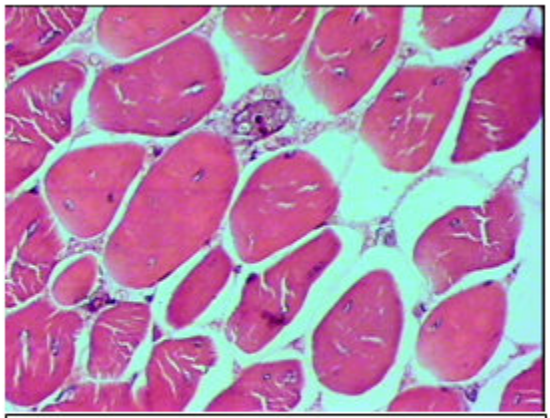

Figure 20. Chick- 3 wks- CTN+AF- Pectoral muscle Hyaline degeneration H&E x 400

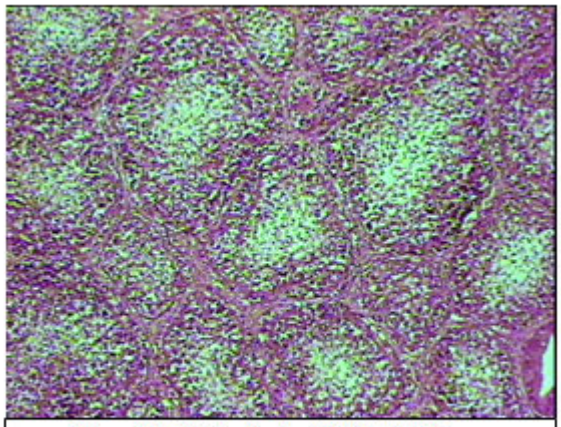

Figure 21. Chick- 6 wks- CTN+AF- Bursa: Lymphocytolysis in the medulla H&E x 100

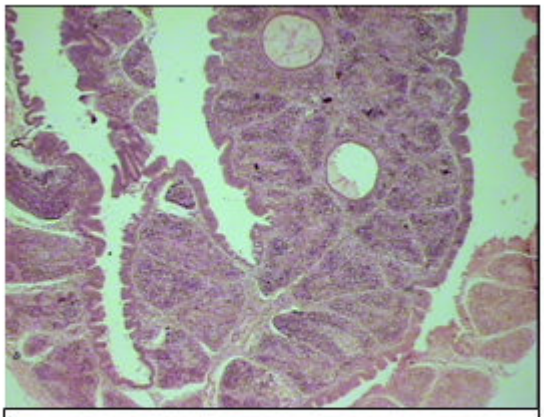

Figure 22. Chick- 6 wks- CTN+AF- Bursa: Atrophy and multiple follicular cysts H&E x 40

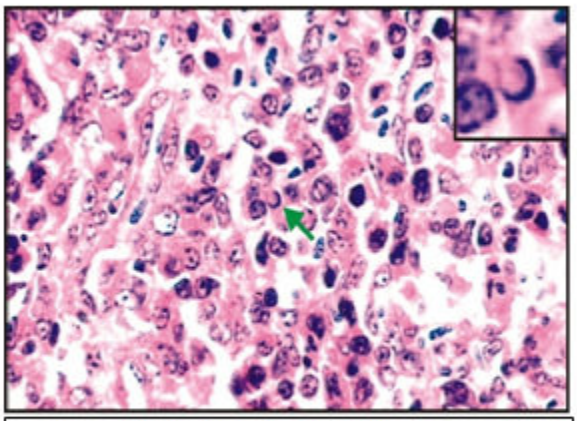

Figure 23. Chick- 3 wks- CTN-Spleen: Apoptotic body (arrow/inset) H&E x 1000

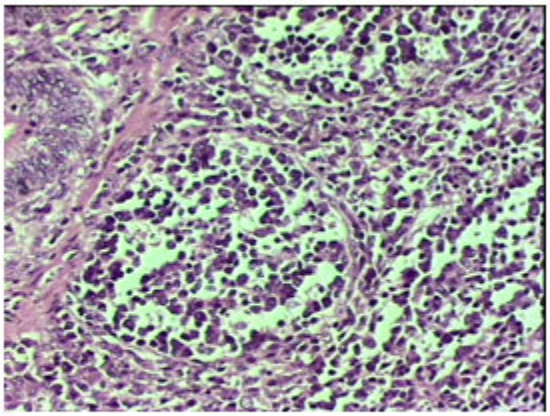

Figure 24. Chick- 3 wks- CTN+AF- Caecal tonsil : Severe lymphoid depletion H&E x 400

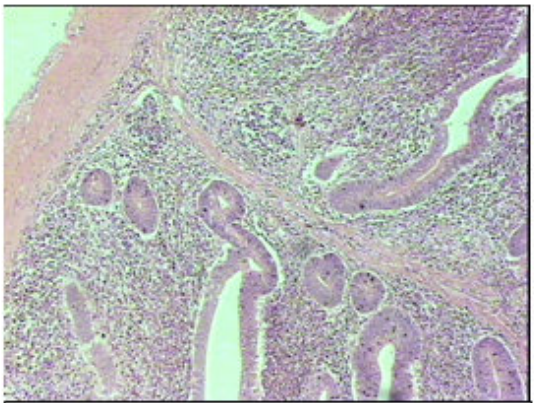

Figure .25. Chick- 6 wks- CTN+AF- Caecal tonsil: Severe lymphoid depletion H&E x 100

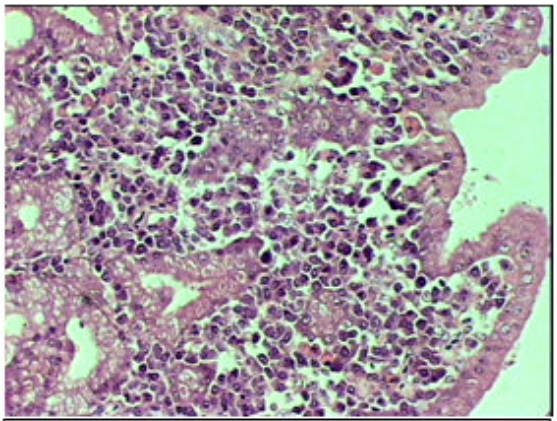

Figure 26 . Chick- 3wks- CTN- Harderian gland: Plasma cells with Russell bodies H&E x 200

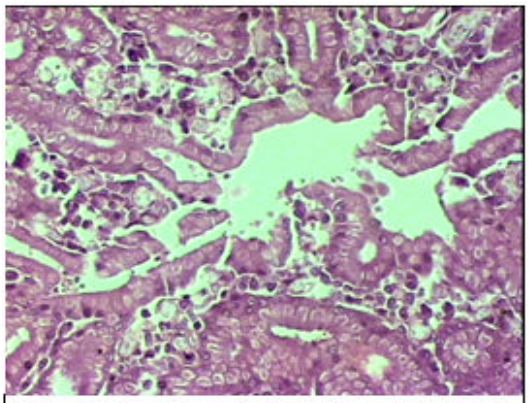

Figure 27. Chick- 3 wks- CTN+AF- Harderian gland: Severe plasma cell depletion H&E x 400

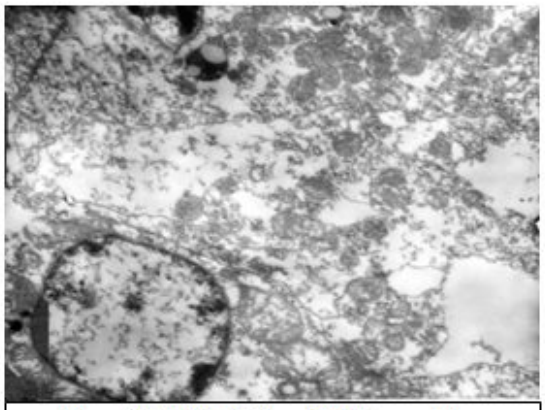

Figure 28. Chick- 6 wks- CTN-Transmission electronmicrograph - Kidney- Vacuolation and swollen mitochondria (arrow) with disruption of cristae- Uranyl acetate- Lead citrate stain x 7000

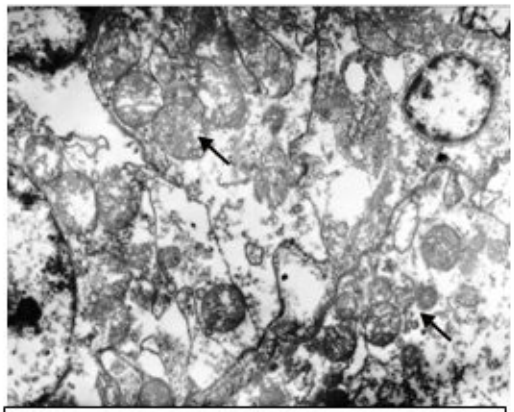

Figure 29. Chick- 6 wks- CTN+AF-Transmission electronmicrograph - Kidney- Vacuolation and markedly swollen mitochondria (arrow) - Uranyl acetate- Lead citrate stain x 7000

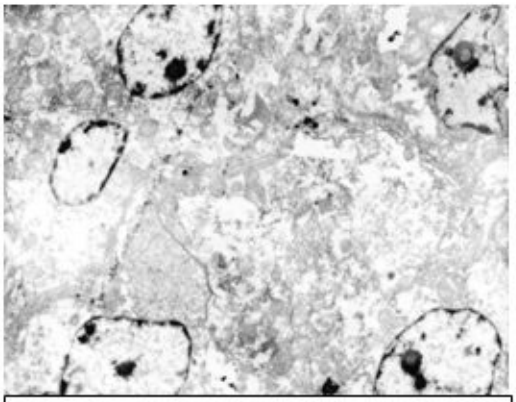

Figure 30. Chick- 6 wks- CTN+AF-Transmission electronmicrograph - Liver- Vacuolar degeneration- Uranyl acetate- Lead citrate stain x 4200

DISCUSSION

The increase in the relative liver weights of the CTN fed groups concurred with the findings in broiler chicken fed 150 ppm CTN for 28 days of age. The toxicosis leading to liver enlargement and the hypertrophy of liver to enhance its detoxification role, were attributed for the increase in the liver weight due to CTN (Swaminathan, 2002). Lipid accumulation as seen in this study must have also contributed to the increase in weight of liver. The increase in the liver weights of AF group was significantly higher than CTN group. This indicates that AF caused comparatively more hepatic damage than CTN which concurred with the findings of Kumar *et al.* (2005) in broiler chicks fed 0.5 ppm AF for 35 days. The increase in the relative weight of liver in the CTN+AF group concurred with the findings of Ahamad and Vairamuthu (2001). The authors attributed the increase in liver weights to the congestion and lipid accumulation, which were also observed in this study. Significant increase in the relative weight of spleen was observed during sixth week. However, Swaminathan (2002) observed no significant change in the relative weight of spleen in broiler chicken fed 125 ppm CTN for three weeks. In the present study also no significant difference was seen in spleen weights up to three weeks. Hence, a

longer duration of exposure to CTN was required for splenic enlargement. The subsequent proliferation of the reticuloendothelial cells as observed in histopathology might have increased the spleen weight. The weight of spleen during sixth week was the highest in the AF group. This indicated that AF caused more damage to spleen than CTN on prolonged exposure. Similar findings were reported in broiler chicken when AF was fed 0.5 ppm up to 35 days (Kumar *et al.,* 2005). In the CTN+AF group, broiler chicks showed increase in weight of the spleen which contradicted the findings of Ahamad and Vairamuthu (2001) who reported no significant change in the spleen weight when broiler chicks were fed 150 ppm CTN and 0.5 ppm AF from 3 to 30 days of age. The increase in the spleen weights in the mycotoxin fed groups and especially in AF group might be attributed to the splenitis and reticulum cell hyperplasia as observed in this study. Significant decrease in bursal weight as noticed in the sixth week of this study was reported in broiler chicks fed AF 1 ppm for five weeks (Natraja *et al.*, 2004) and 0.5 ppm for 35 days (Kumar *et al.,* 2005). The increase weight of bursa of Fabricius in the AF group during third week might be due to the mild oedema and on prolonged exposure the bursal atrophy, as observed in the histopathology in this study, must have reduced the weight at sixth week. No literature was available to compare the reduction of overall mean relative bursal weights of CTN+AF group. Both toxins caused lymphoid depletion and atrophy, which correlated with findings in the combined toxicity. In the CTN fed birds, the gross lesions in kidney and liver and the catarrhal enteritis, splenomegaly and atrophy of bursa of Fabricius during sixth week concurred with the earlier findings in broiler chicks fed 125 and 250 ppm CTN for six weeks (Uma and Reddy, 1995; Ahamad and Vairamuthu, 2001. However, Swaminathan (2002) reported slight congestion of bursa in broiler chicks fed with 150 ppm CTN from 1 to 28 days of age. The lesions observed in the kidneys and liver and splenomegaly in AF fed birds concurred with the observations of Kumar and Balachandran (1998) and Ahamad and Vairamuthu (2001). The more pronounced lesions in liver, kidneys, spleen and lymphoid organs of the CTN+AF group concurred with the findings in broiler birds fed with 150 ppm CTN and 0.5 ppm AF from 3 to 30 days (Ahamad and Vairamuthu, 2001). In the CTN fed birds the histopathological lesions in kidney, liver and intestines during sixth week concurred with the earlier findings in broiler chicks fed 125 and 250 ppm CTN for six weeks (Uma and Reddy, 1995) and in broiler chicks fed 150 ppm CTN for 3-30 days (Ahamad and Vairamuthu, 2001). In AF fed birds, the lesions observed in the kidneys, liver, crop, proventriculus, pancreas, heart concurred with the observations of Kumar and Balachandran (1998) in broiler chicks fed 1 ppm AF up to 28 days of age and in muscle and intestine with the findings of Balachandran and Ramakrishnan (1987).

In histopathological lesions, the thickening of basement membrane of the glomerular tuft in the kidneys of all the mycotoxin fed groups was observed only in this study. The degeneration of tubules and stasis of fluid might have caused the enlargement and bulging of kidney surface as observed grossly. The more pronounced lesions in liver, kidneys, spleen and lymphoid organs of the CTN+AF group concurred with the findings of Ahamad and Vairamuthu (2001) in broiler birds fed with 150 ppm CTN and 0.5 ppm AF from 3 to 30 days and opined that the severity in the renal and hepatic lesions of CTN+AF group could be due to the combined effect of CTN and AF when fed at 150 ppm CTN and 0.5 ppm AF in broiler chicks from 3 to 30 days. The degenerative lesions in the heart might have resulted in venous congestion in the systemic circulation leading to anoxia and poor supply of nutrients to the vital organs like liver and kidneys, which would have further reduced their function and caused degeneration of their cells. The crop in the CTN treated birds revealed hyperplasia and colonies of bacilli embedded on the mucosal surface. In the AF fed birds, hyperplastic epithelium projected into the lumen. In the CTN+AF group, the lesions were marked with degeneration of muscle. The finding in the AF group concurred with those of Kumar and Balachandran (1998) in broiler chicks fed 1 ppm AF for 28 days and the lesions may be attributed to the local irritant action of CTN and AF on the crop mucosa. The lesions in proventriculus, gizzard and intestines concurred with the findings of Swaminathan (2002) in broiler chicks fed with 150 ppm CTN from 1 to 28 days of age. The lesions in the gizzard could interfere with the grinding of feed and affect digestion and absorption in the intestine. In pancreas, the reduced zymogen granules and mononuclear cell infiltration observed in the AF and

CTN+AF group correlated with the observations of Kumar and Balachandran (1998) in broiler chicks fed 1 ppm AF up to 28 days of age and with Ahamad and Vairamuthu (2001) in broiler chicks fed 150 ppm CTN and 0.5 ppm AF. The reduced zymogen granules might have led to decreased pancreatic enzyme secretion and thereby interfered with digestion and absorption of nutrients. In the muscle, the AF and CTN+AF fed groups showed hyaline degeneration of pectoral muscle fibres, which concurred with the finding of Balachandran and Ramakrishnan (1987) in broiler chicks fed 1 and 3 ppm of AF for 28 days. Although no alterations were observed in the CTN group, CTN might have played a minor role in the combined toxin group as Roberts and Mora (1978) have also reported hyalinization of pectoral muscle fibres in broiler chicks fed with 62 per cent of CTN contaminated corn for four weeks of age. In the lymphoid organs, the lymphoid cell depletion and lymphocytolysis were the consistent lesions observed in the bursa of Fabricius, spleen, thymus, caecal tonsils and Harderian gland in all the mycotoxin fed groups. The lesions were mild to moderate in the CTN group, moderate to severe in AF group and marked in the CTN+AF group. The epithelial corrugations, atrophic and cystic changes observed in the bursa of Fabricius in the CTN+AF group were more diffuse involving many or all the follicles when compared to the individual CTN and AF fed groups. These findings were in accordance with Ahamad and Vairamuthu (2001) for CTN and CTN+AF who fed 150 ppm CTN and 0.5 ppm AF in broiler chicks from 3 to 30 days of age. The findings in the AF group are in accordance with Kumar and Balachandran (1998) who observed lymphoid depletion and necrosis in the bursa of Fabricius and spleen and increased germinal centres with reticular cell hyperplasia in spleen of broiler chicks fed 1 ppm AF up to 28 days of age. Similar to the present findings, bursa of Fabricius, spleen, thymus and caecal tonsils evinced lymphoid depletion and lymphocytolysis when layer chicks were fed 0.5 ppm AF from 0 to 12 weeks of age (Gounalan *et al*., 2006). The decrease in plasma cell population in the Harderian glands in AF fed group is in accordance for experimental aflatoxicosis in broiler chicks fed 0.5 ppm AF for eight weeks (Mundas *et al.,* 2001). The ultrastructural lesions in liver were in agreement with those of Brown *et al.* (1986) for CTN fed group, Mollenhauer *et al.* (1989) for AF fed group and though there was no literature for the ultrastructural pathological changes in CTN+AF mycotoxicoses the lesions were comparable to the individual toxicities.

CONCLUSION

The CTN group showed mild pathological changes. The AF treated groups showed moderate changes during third and sixth week. The CTN+AF group birds showed severe changes during third and sixth week. The ultra structural lesions of liver and kidneys revealed degenerative and necrotic changes in toxin fed groups. In CTN+AF mycotoxicoses these were comparable to the individual toxicities. Though the citrinin and aflatoxin in combination significantly affected the health of broiler chicken the effect was less than additive. The effect of aflatoxin prevailed over citrinin.

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REFERENCES

1. Ahamad, D.B. and S. Vairamuthu. 2000a. Occurrence of citrinin mycotoxin in compounded feeds and various feed ingredients of poultry ration in Namakkal area (Tamilnadu). *Indian J Env Toxicolo,* 10(2): 84-86.
2. Ahamad, D.B. and S. Vairamuthu. 2001. Individual and combined effects of citrinin and aflatoxin B_1 in broiler chicks: A pathomorphological study. *Indian J. Vet. Pathol*, 25(1&2): 32-34.
3. Anandkumar, C.T., G. V. Sudhakar Rao, V. Titus George and C. Balachandran. 2005. Co-occurrence of aflatoxicosis and infectious bursal disease in chicken. *Indian Vet. J.*, 82: 935-937.

4. Balachandran, C. and R. Ramakrishnan. 1987. An experimental study on the pathology of aflatoxicosis in broiler chicken. *Indian Vet. J.*, 64: 911-914.
5. Bancroft, J.D. and A. Stevens. 1996. Theory and Practice of Histological Techniques. 4th Edn. Churchill Livingstone, New York. pp. 47-68, 99-113.
6. Brown, T.P., R.O. Manning, O.J. Fletcher and R.D. Wyadd. 1986. The individual and combined effect of citrinin and ochratoxin A on renal ultrastructure in layer chicks. *Avian Dis*., 30(1): 191-198.
7. Carlton, W.W., G. Sansing, G.M. Szczech and J. Tuite. 1974. Citrinin mycotoxicosis in beagle dogs. *Food Cosmet. Toxicol.*, 12: 479-490.
8. Coulombe, R.A. 1993. Biological action of mycotoxins. *J Dairy Sci*., 76: 880-891.
9. Gounalan, S., C. Balachandran and B. Murali Manohar. 2006. Immunopathological effects and induction of apoptosis in spleen and thymus of layer chicken in aflatoxicosis. International Conference on Advanced Veterinary Practice in Medicine and Surgery – Augmenting Health and Production, MSU and TANUVAS, 21-25, June, Chennai, Compendium of Lead Papers and abstracts, p. 65.
10. Kumar, R. and C. Balachandran. 1998. Pathological changes in broiler chicken fed aflatoxin and cyclopiazonic acid. XV Annual Conference of Indian Association of Veterinary Pathologists, November 9-11,
11. Mannuthy. Kumar, P.A., M.L. Sathyanarayana, S.K. Vijayasarathi, R.N.Sreenivas Gowda and Suguna Rao. 2005. Effect of vitamin E and selenium on serum biochemical parameters in broiler chicken fed with aflatoxin and ochratoxin. *Indian Vet J.*, 82: 522-525.
12. Mollenhauer, H.H., D.E. Corrier, W.E. Huft, L.F. Kubena, R.H. Harvey, M.S. Robert and E. Droleskey. 1989. Ultrastrucutre of hepatic and renal lesions in chickens fed aflatoxin. *Am J Vet Res.*, 50: 771-777.
13. Mundas, S., D.G.K. Rao and N. Prakash. 2001. Pathological changes in Harderian gland of broiler chicks following concomitant exposure to aflatoxin B_1 and Fenvalerate pesticide. *Indian J Vet Pathol.*, 25(1&2): 71-72.
14. Natraja, T.H., H.D. Narayanaswamy, B. Santhosh Kumar and G.C. Prakash. 2004. Performance of broiler chickens in experimental aflatoxicosis and T-2 toxicosis. *Indian Vet Med J.*, 28: 40-44.
15. Natarajan, A., D. Chandrasekaran, T.K. Sundaram and B. Mohan. 1999. Aflatoxin B_1 incidence in cereals, oil cakes and feeds in Namakkal. 18th Annual Conference of Society of Toxicology, India, November 11-13, Namakkal, p.22.
16. Nelson, T.S., J.N. Beasley, L.K. Kirby, Z.B. Johnson and G.C. Ballam. 1980. Isolation and identification of citrinin produced by *Penicillium lanosum. Poultry Sci.*, 59: 2055-2059.
17. Roberts, W.T. and E.C. Mora. 1978. Toxicity of *Penicillium citrinum* AVA 532 contaminated corn and citrinin in broiler chicks. *Poultry Sci*., 57: 1221-1226.
18. Shotwell, O.L., C.W. Hesseltine, R.D. Stubblefield and W.G. Sorenson. 1966. Production of aflatoxin on rice. *Appl Microbiol.*, 14(3): 425 - 428.
19. Sundaram, T.K., D. Chandrasekaran, A. Natarajan and B. Mohan. 1999. Multimycotoxins: An overview of their occurrence in feed ingredients and feed in Namakkal. 18th Annual Conference of the Society of Toxicology, India, November 11 -13, Namakkal. Abstract and Souvenir, p. 21.
20. Swaminathan, B. 2002. Individual and combined effect of ochratoxin A and citrinin toxicity in broilers. M.V.Sc. thesis submitted to Tamilnadu Veterinary and Animal Sciences University, Chennai.
21. Tapia, M.O. 1985. A quantitative thin layer chromatography method for analysis of aflatoxins, ochratoxin and citrinin in foodstuffs. *Revista Argentina de Microbiologia*, 17(4): 183-186.
22. Uma, M. and M.V. Reddy. 1995. Citrinin toxicity in broiler chicks. Haemato-biochemical and pathological studies. *Indian J Vet Pathol.*, 19(1): 11-14.