Experimental pathology of T-2 toxicosis and mycoplasma infection on performance and hepatic functions of broiler chickens

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ABSTRACT This experiment was conducted using 192 day-old Ross 308 chicks, divided into 4 groups of 4 replicate consisting 48 birds. Group I was fed a control diet, Group II was fed control diet supplemented with 0.5 ppm T-2 toxin for 5 weeks, Group III was fed control diet supplemented with 8×10^8 cfu/mL of *Mycoplasma gallisepticum*, and group IV was fed control diet supplemented by T-2 toxin and *Mycoplasma gallisepticum*. Body weight and feed conversation ratio (FCR), relative organ weights, clinical signs, biochemical characteristics, and gross and histopathological lesions were recorded in the experimental groups at the end of the second and fifth weeks of age. Body weight and relative weights of bursa of Fabricius, thymus, and spleen decreased and FCR increased significantly ($P \leq 0.05$), but the relative weights of liver and kidney showed no significant decrease ($P \leq 0.05$) in the serum total proteins, albumin, and increase in aspartate aminotransferase and alanine transaminase were observed in T-2 toxin and T-2 accompanied with Mycoplasma fed birds when compared to the control group. Liver was enlarged, friable, and yellowish discoloration with distended gall bladder was noticed. Lymphoid organs such as bursa of Fabricius, thymus, and spleen were atrophied in group II and group IV throughout the study. Microscopically, liver showed vacuolar degeneration of hepatocytes, with increased Kupffer cell activity, bile duct epithelial hyperplasia, and infiltration of inflammatory cells. Kidney showed vacuolar degeneration of tubular epithelium along with pyknotic nuclei. Lymphoid organs showed lymphocytolysis and depletion with prominent reticuloepithelial cells. Proventriculus revealed desquamation of villous epithelial cells and lymphoid infiltration in submucosa. Heart showed mild hemorrhage with infiltration of inflammatory cells. Lung showed edema and inflammatory cells in the bronchioles. Trachea showed desquamation and erosions of mucosa. Proliferation of mucosal glands with increased mucous secretion was obvious. Air sacs showed thickening with presence of inflammatory cells and edema.

Key words: T-2 toxin, *Mycoplasma gallisepticum*, performance, pathological lesion, broiler

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INTRODUCTION

Poultry industry, especially broiler production, has made phenomenal growth over the past 2 decades contributing significantly to agri-based economy across the globe. Modernization of poultry industry with the main aim of gaining maximum profit has invariably increased "stress" on birds and predisposes for both infectious and noninfectious diseases. Among the noninfectious diseases mycotoxicosis is a common and persistent problem. *Fusarium* mycotoxin contamination was found to occur in wheat, corn, and other cereals including food products in certain regions of several countries based on the FAO report, 25% of world's cereals are contaminated with mycotoxins (Manafi, 2012). T-2 mycotoxins have been identified in many animal feed ingredients (Mirocha et al., 1967). The occurrence of T-2 toxin has been reported from America, Europe, Asia, and Africa. The toxin production has been shown to be highest in areas with high humidity and low to moderate temperature (6–24°C). The feed ingredients were found to be contaminated with T-2 toxin both in field and on storage with 14 to 25% moisture (Scott, 1989). Among 173 maize samples surveyed, 93 were found to be positive for a skin irritant factor, suspected to be T-2 toxin (Eppley et al., 1974). Irritation, extensive inflammation, and necrosis of oral mucosa with subsequent development of yellow caseous plaques were observed with T-2 toxicosis (Wyatt et al., 1972). Wyatt et al. (1973) also reported acute effects of T-2 toxicosis like digestive disturbances, decreased bone-marrow activity, immunodeficiency and neural disturbances in addition; abnormal feathering, hemorrhage, and regression of lymphoid organs were also observed in broiler (Wyatt et al., 1975). Many studies have considered impacts of T-2 toxicosis on growth parameters (Christensen et al., 1972),

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relative organ weights (Kubena et al., 1997), serum biochemical parameters (Manafi et al., 2011), clinical signs (Leeson et al., 1995), and histopathological lesions (Narayanaswamy, 1998).

Low levels of mycotoxins are known to produce immune suppression in broiler chickens. The T-2 toxin is ubiquitous in nature and always poses a threat to the poultry industry causing immunosuppression leading to outbreaks of diseases. The presence of toxins along with mycoplasmosis will lead to a severe and more dangerous problem in the infected flock. Thus, the present study throws light on immune suppression caused by low levels of mycotoxin and susceptible to mycoplasmal infection to see the response of birds to inclusion of toxin and microbial infections together.

Feeding diets contaminated with mycotoxin may reduce the immune function. This renders the animal more susceptible to any concurrent disease, such as *Mycoplasma gallisepticum* and secondary infections are common.

The findings will be helpful to the poultry industry to undertake appropriate preventive measures and effective treatment.

MATERIALS AND METHODS

Experimental Birds and Diets

One hundred and ninety-two day-old Ross 308 broiler chicks were obtained from a local hatchery, divided into 4 groups, i.e., control (group I); control plus 0.5 ppm T-2 toxin (group II), control plus 8×10^8 cfu/mL *M. gallisepticum* (group III) and control plus 0.5 ppm T-2 toxin and 8×10^8 cfu/mL *M. gallisepticum* (group IV), comprised of chicks in each group. Each group included four replicates with 12 chicks in each.

Broiler starter (1–17-day) and finisher (18–35-day) diets (Table 1) without mycotoxin binder supplements were prepared as the NRC (1994) requirements of broilers. T-2 toxin was produced on wheat using *Fusarium sporotrichoides* as suggested by Burmeister (1971). The birds were vaccinated against Newcastle disease and infectious bursal disease on the seventh and 11th day of age through oculo-nasal routes.

Table 1. Composition and nutrient levels of the basal diets (%, as-fed basis).

	Diet	
Item	1 to 17 d	18 to 35 d
Ingredient		
Corn	60.00	63.00
Soybean meal (46%)	31.20	28.17
Corn gluten meal	3.00	2.50
Soybean oil	1.14	2.27
Calcium carbonate	0.95	0.88
Calcium hydrophosphate	1.85	1.60
L-lysine hydrochloride	0.08	0.02
dL-methionine	0.16	0.09
Sodium chloride	0.50	0.40
Choline chloride	0.15	0.10
Vitamin and mineral premix	0.33[1]	0.32[2]
Rice hull	0.64	0.65
Total	100.00	100.00
Chemical Composition		
CP	20.83	19.37
ME,[3] MJ/kg	12.15	12.55
Calcium	0.97	0.87
Available phosphorus	0.44	0.39
Lysine	1.11	0.97
Methionine	0.48	0.39
Methionine + cysteine	0.86	0.72
Threonine	0.78	0.74

[1]Provided per kilogram of diet: vitamin A, 12,000 IU; cholecalciferol, 3,000 IU; vitamin E, 7.5 IU; vitamin K3, 1.5 mg; thiamine, 0.6 mg; riboflavin, 4.8 mg; pyridoxine, 1.8 mg; vitamin B12, 9 μg; folic acid, 150 μg; niacin, 10.5 mg; calcium pantothenate, 7.5 mg; iron, 100 mg; copper, 8 mg; manganese, 120 mg; zinc, 100 mg; selenium, 0.3 mg; iodine, 0.7 mg.

[2]Provided per kilogram of diet: vitamin A, 8,000 IU; cholecalciferol, 2,000 IU; vitamin E, 5 IU; vitamin K3, 1 mg; thiamine, 0.4 mg; riboflavin, 3.2 mg; pyridoxine, 1.2 mg; vitamin B12, 6 μg; folic acid, 100 μg; niacin, 7 mg; calcium pantothenate, 5 mg; iron, 80 mg; copper, 8 mg; manganese, 100 mg; zinc, 80 mg; selenium, 0.3 mg; iodine, 0.7 mg.

[3]The ME of the diet was calculated according to NRC (1994).

Fungal Culture

The *Fusarium sporotrichoides var sporotrichoides* MTCC 1894 culture was obtained from the Microbial Type Culture Collection, sector – 39, Chandigarh 160036, India. Group II and IV were fed with feed containing T-2 toxin (0.5 ppm) from starting the experiment.

Mycoplasma Gallisepticum (MG)

Pathogenic mycoplasma (*M. gallisepticum*) isolate was procured from contaminated broiler flock, from department of veterinary microbiology, Namakkal veterinary college, India and being used after positive result of RSA rapid test. Each bird in groups III and IV was inoculated with 0.3 mL of the cell sustentation containing 2.4×10^8 cfu via nasal on day 14th of the experimental study.

Body Weight, Feed Conversation Ratio, and Relative Organ Weights

The birds were weighed pen-wise on d 21 and 35. Feed consumption and feed conversion ratio (FCR) was determined for each of these periods. Upon obtaining permission from ethical committee of the university, 8 birds per treatment, 2 birds per pen (1 male and 1 female), were selected from all the groups at weekly intervals, weighed, fasted overnight, and then killed and processed to determine carcass yield and weight of internal organs. Liver, kidney, spleen, bursa of Fabricius, thymus, proventriculus, and gizzard were collected and weighed by using an electronic balance, and then relative weights of the organs were calculated. Feed:gain

ratio was corrected for mortality by including the weight of the dead birds in the body weight gain.

Serum Biochemical Parameters

During each weekly sacrifice, blood samples were collected separately in nonheparinized tubes. The serum was separated and stored at −20°C until further use. Serum was analyzed for total protein (TP), albumin (ALB) content; alanine transaminase (ALT) and aspartate aminotransferase (AST) enzyme activity were measured using commercial kits (Boehringer Mannheim Hitachi 704 automatic analyzer, Japan). The kits were obtained from Aspen Laboratories, Pvt. Ltd. Delhi, India.

Pathology

The sacrificed birds were subjected to detailed postmortem examination and gross lesions were recorded. The representative samples of different organs were collected in 10% neutral buffered formalin for histopathological examination. The tissues were fixed and processed by routine paraffin embedding technique. Sections of 5μm thickness were taken and stained with hematoxylin and eosin (H&E) as described by Luna (1968) for microscopic slides.

Statistical Analysis

Statistical analyses were computed with SAS (1990) software under a single 2 × 2 factorial experiment design. When the analysis of variance was significant, Duncan's multiple-range test was used to separate the means. Differences between treatment means were considered statistically significant at $P \leq 0.05$.

RESULTS

Growth Parameters

Mean body weight and relative organ weights of broiler chickens fed with T-2 toxin alone and in combination with *M. gallisepticum* culture was shown in Table 2.

There was no effect on the BW of broilers on d 14. However, the BW on d 35 of chickens in control group was significantly ($P \leq 0.05$) higher than others. Significant ($P \leq 0.05$) reduction in the body weight was observed in group II of birds that were fed with T-2 toxin fed treatments (groups II and IV) when compared to the control on d 35. There was a numerical increase in feed consumption and feed conversion ratio (FCR) in all treatment groups when compared to that of control and FCR was markedly increased in group II (T-2 toxin), but no significant difference was noticed among dietary treatments. The mean of the FCR was 1.23 and 1.84, on d 14 and 35, respectively (data not shown).

Relative Organ Weights and Serum Characteristics

As shown in Table 2, the relative weight of liver and kidney did not change among treatments on d 14 and 35. But, the relative weight of proventriculus on d 35 significantly ($P \leq 0.05$) was lower in groups II or IV). Same results were obtained for the relative weight of bursa of Fabricius and thymus on days 14 and 35. The relative weight of the spleen was significantly ($P \leq 0.05$) higher than all other treatments in group IV at day 14 of age, but was lower in both groups II and IV at 35 days of age.

The results of serum biochemical parameters are shown in Table 3. Results revealed that the mean values of TP on d 14 tend to reduce significantly ($P \leq 0.05$) in groups II or III, but it was lower in groups II and IV

Table 2. Effects of feeding T-2 toxin (0.5 ppm), *Mycoplasma gallisepticum* (8×10^8 cfu/mL) and their combinations on mean (±SE) BW and relative organ weights (g/100 g of BW) of broilers.

Item	Control (I)	T-2 (II)	*Mycoplasma gallisepticum* (III)	Combination (IV)
14 d				
BW (g)	446.66 ± 4.944	416.66 ± 8.432	441.66 ± 9.098	391.66 ± 8.333
Relative liver weight	4.13 ± 0.029	4.00 ± 0.025	4.16 ± 0.053	3.93 ± 0.085
Relative kidney weight	1.18 ± 0.034	1.19 ± 0.034	1.24 ± 0.054	1.15 ± 0.035
Relative bursa Fabricius weight	0.28 ± 0.006^a	0.19 ± 0.003^b	0.27 ± 0.008^a	0.19 ± 0.005^b
Relative spleen weight	0.06 ± 0.002^b	0.06 ± 0.001^b	0.06 ± 0.001^b	0.19 ± 0.005^a
Relative thymus weight	0.51 ± 0.007^a	0.36 ± 0.002^b	0.52 ± 0.009^a	0.40 ± 0.017^b
Relative proventriculus weight	0.41 ± 0.004	0.42 ± 0.003	0.41 ± 0.005	0.41 ± 0.004
35 d				
BW (g)	1,588.33 ± 26.257^a	1,305.00 ± 72.926^b	1,405.00 ± 100.589^b	1,290.00 ± 54.283^b
Relative liver weight	2.67 ± 0.087	2.73 ± 0.071	2.63 ± 0.075	2.61 ± 0.141
Relative kidney weight	1.15 ± 0.016	1.15 ± 0.018	1.15 ± 0.017	1.21 ± 0.050
Relative bursa Fabricius weight	0.23 ± 0.035^a	0.13 ± 0.024^b	0.22 ± 0.047^a	0.13 ± 0.008^b
Relative spleen weight	0.16 ± 0.002^a	0.11 ± 0.002^b	0.15 ± 0.003^a	0.10 ± 0.002^b
Relative thymus weight	0.46 ± 0.056^a	0.30 ± 0.004^b	0.41 ± 0.070^a	0.29 ± 0.003^b
Relative proventriculus weight	0.43 ± 0.014^a	0.51 ± 0.003^b	0.44 ± 0.017^a	0.51 ± 0.021^b

^{a,b}Different lowercase superscripts in the same row indicate a significant difference between treatments at ($P \leq 0.05$).

Table 3. Effects of feeding T-2 toxin (0.5 ppm), *Mycoplasma gallisepticum* (8×10^8 cfu/mL) and their combinations on mean (±SE) of serum biochemical parameters of broilers.[1]

Item	Control (I)	T-2 (II)	*Mycoplasma gallisepticum* (III)	Combination (IV)
14 d				
TP (g/dL)	2.80 ± 0.036[a]	2.50 ± 0.073[b]	2.58 ± 0.040[b]	2.36 ± 0.042[c]
ALB (g/dL)	1.58 ± 0.060[a]	1.46 ± 0.033[a,b]	1.51 ± 0.094[a,b]	1.30 ± 0.036[b]
ALT (IU/L)	46.08 ± 2.142	45.43 ± 2.543	46.01 ± 2.557	45.38 ± 2.403
AST (IU/L)	189.28 ± 6.445[b]	215.85 ± 12.970[a]	198.91 ± 3.623[b]	225.85 ± 11.767[a]
35 d				
TP (g/dL)	2.97 ± 0.049[a]	2.31 ± 0.047[b]	2.91 ± 0.048[a]	2.21 ± 0.048[b]
ALB (g/dL)	1.60 ± 0.057[a]	0.95 ± 0.175[b]	1.40 ± 0.057[a]	0.93 ± 0.173[b]
ALT (IU/L)	34.28 ± 1.725[b]	58.90 ± 1.073[a]	35.73 ± 1.616[b]	64.00 ± 1.620[a]
AST (IU/L)	127.16 ± 3.339[b]	174.70 ± 4.193[a]	131.51 ± 3.848[b]	186.46 ± 4.663[a]

[a–c]Different lowercase superscripts in the same row indicate a significant difference between treatments at ($P \leq 0.05$).

[1]TP = total protein; ALB = albumin; ALT = alanine transaminase; AST = aspartate aminotransferase.

on d 35, as ALB on d 35. The value of ALB in group I was significantly ($P \leq 0.05$) higher than group IV on d 14. The ALT activity did not change among the treatments on d 14, however, the activity of ALT at d 35 was increased in groups II and IV and its activity was lower significantly ($P \leq 0.05$) in groups I and III. The activity of serum AST in both periods was similar and its value highest ($P \leq 0.05$) in groups II and IV.

Gross Pathology

Grossly, liver exhibited enlargement, congestion and petechial hemorrhages in group II and IV throughout the experimental study (Figure 1a). Occasional pale and fatty livers were also observed. Gall bladder was distended with greenish thick bile. Kidneys were slightly enlarged and congested in all the treatment groups. The T-2 toxin-fed birds showed friable and tan-colored kidneys. Atrophy of bursa of Fabricius, thymus, and spleen were observed in both group II and group IV compared to control during the experimental period (Figure 1b and c). The proventricular lumen showed presence of slimy, thick mucus in toxin-treated group. The wall of the proventriculus appeared thickened. Duodenum showed catarrhal inflammation with increased mucus secretion, and it was more consistent in group II and group IV throughout the experimental period. The eyes showed congestion of conjunctival mucous membrane and edema of the eye lids in group III in final week of the experiment.

Histopathology

The liver showed mild to moderate vacuolar degeneration of hepatocytes, focal necrosis along with mononuclear cell infiltration (Figure 2a). Increased Kupffer cell nucleus was prominent during fourth week (Figure 2b). The portal area revealed infiltration of inflammatory cells around the vessels and bile duct along with periportal fibrosis. The bile duct epithelial hyperplasia was consistently noticed throughout the experimental period (Figure 2c). Glandular transformation of hepatocytes was conspicuous on d 35. In group III, liver showed focal areas of necrosis and vacuolar degeneration of hepatocytes along with stray infiltration of inflammatory cells. The lesions of the kidneys consisted of mild to moderate congestion and hemorrhages consistently throughout the experimental study. The tubular epithelium showed vacuoles in the cytoplasm with pyknotic nuclei. The bursa revealed lymphocytolysis leading to lymphoid cell depletion in the follicles with prominent reticuloepithelial cells and presence of apoptotic bodies were less frequently encountered (Figure 2d and e). The plical epithelium revealed corrugations,

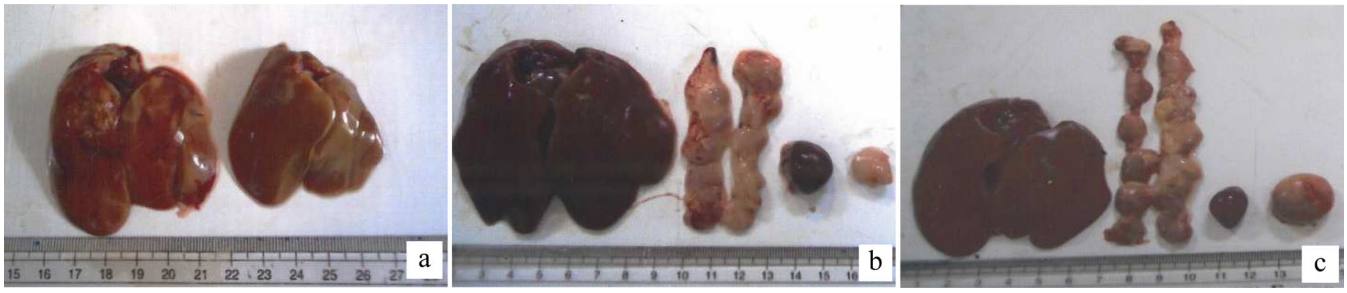

Figure 1. Morphology of affected and normal organs. (a) left side- group II, enlarged and congested liver, right-side normal liver on d 21; (b) group II, atrophy of thymus, bursa and enlarged liver on d 35; (c) Control, normal thymus, bursa, and liver on d 35.

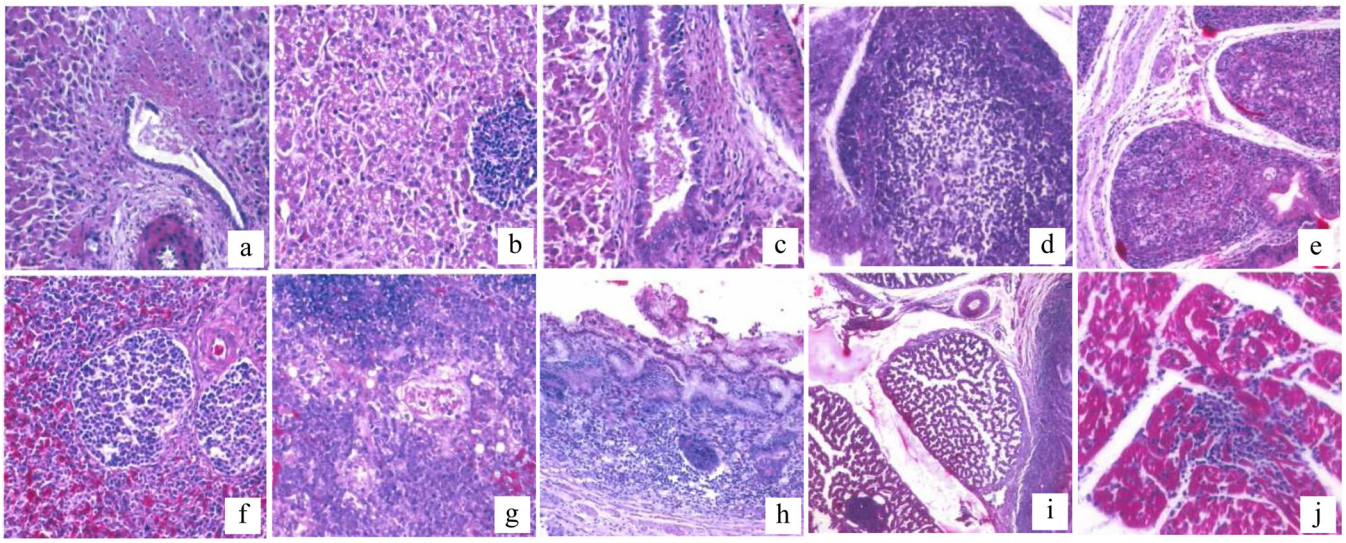

Figure 2. Morphology of different chicken organs All H.E. 200× except e and j 100× (a) liver, group II, periportal hepatocytes degeneration, and necrosis along with individualization of hepatocytes on d 14; (b) liver, group II, focal area showing degeneration and necrosis of hepatocytes with infiltration of multinucleated cells. Also note increased nucleus of Kupffer cells on d 28; (c) liver, group IV, periportal infiltration of inflammatory cells and fibrosis with mild bile duct epithelial hyperplasia on d 28; (d) bursa of Fabricius, group II, lymphocytolysis, and depletion of lymphocytes with prominent reticuloepithelial cells in medullary area on d 7; (e) bursa of Fabricius, group II, lymphocytolysis, and depletion of lymphocytes with prominent reticuloepithelial cells. Also note cystic transformation with infiltration of inflammatory cells on d 35; (f) spleen, group II, showing proliferation of lymphoblasts and formation of secondary lymphoid nodule on d 35; (g) thymus, group II, medullary area showing massive lymphocytolysis, esinophilic debris with hemorrhage on d 7; (h) proventriculus, group II, desquamation and degeneration of villus epithelium with infiltration of lymphoid cells into submucosa on d 28; (i) proventriculus, group IV, shortening of villi with increased mucus production on d 28; (j) heart, group II, degeneration of cardiac myocytes, and infiltration of mononuclear cells on d 35.

along with infiltration of mononuclear cells. These lesions were more severe in groups II and IV compared to that of the control group. Microcyst formation in the lining plical epithelium of the follicles was observed on d 35. Proliferation of interfollicular connective tissue was observed along with infiltration of mononuclear cells on d 35. In birds of group III, microcyst formation in medulla of the bursal follicle was noticed.

The histopathological lesions in the spleen included mild degree of congestion and areas of hemorrhages. Lymphoid cell necrosis and depletion were consistent features seen in the spleen especially around the arterioles. Formations of secondary lymphoid nodules were noticed during the fifth week of the study in groups II and IV (Figure 2f). Spleen in group III showed mild hemorrhage with formation of secondary nodules. Microscopic lesions observed in the thymus included mild to moderate degree of congestion and hemorrhages. Lymphoid cell necrosis and depletion of lymphocytes with prominent reticuloepithelial cells were consistently noticed. These changes were more marked in the groups II and group IV throughout the experimental study (Figure 2g). However, group III revealed apparently normal thymus. The microscopic Proventricular lesion includes shortening and desquamation of the villous epithelium with mononuclear cell aggregations in the lamina propria and submucosa (Figure 2h). Submucosal glands were partially destroyed due to massive infiltration of mononuclear cells and there was hyperplasia of glandular epithelium in later stages (Figure 2i). Heart revealed mild congestion and focal infiltration of inflammatory cells with the presence of eosinophilic fluid between myocardial fibers (Figure 2j). Duodenum revealed catarrhal changes characterized by increased goblet cell activity, fusion and desquamation of villous epithelium along with the presence of lymphoid cells in the lamina propria (Figure 3a). Microscopically the caecum showed catarrhal changes with increased goblet cell activity. Fusion of villi with mononuclear cell infiltration in the lamina propria was observed from third week onwards (Figure 3b). Cecal tonsils showed lymphoid hyperplasia observed in the later stages of experimental study in groups II and IV. Microscopically the lungs showed hemorrhages in all the treatment groups consistently. Infiltrations of mononuclear cells were noticed around the bronchioles and interstitium. Desquamation of bronchial epithelium, presence of edematous fluid in the alveoli and bronchioles along with increased goblet cell activity in groups III and IV after inoculation of *M. gallisepticum* (Figure 3c). The microscopic lesions in the trachea included focal erosions, loss of cilia and exfoliation of the tracheal epithelium with increased mucus secretion. Hyperplasia of tracheal epithelium and infiltration of inflammatory cells in the sub mucosa were found resulting in thickening of mucosal layer and desquamation of cells into lumen. These lesions were recorded consistently in groups III and group IV throughout the experimental study. The air sacs appeared edematous, thickened with infiltration of inflammatory cells. Also, congestion and hemorrhagic lesions were observed in the groups III and group IV birds.

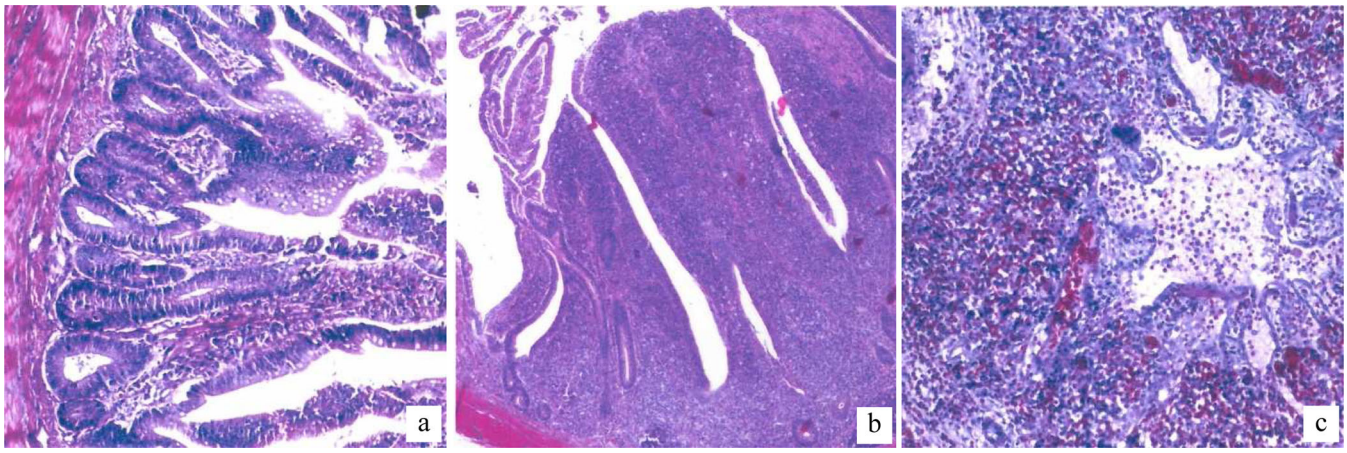

Figure 3. Morphology of intestine, cecum, and lung of broilers. (a) Intestine, group II, desquamation, and degeneration of villous epithelium with increased in mucous secretion, H.E. 200×, on d 21; (b) cecal tonsils, group II, lymphoid hyperplasia with destruction of villi due to infiltration of lymphoid cells, H.E. 50×, on d 28; (c) lung, group IV, alveolar, and bronchial epithelium showing degeneration and necrosis with cellular debris in the bronchiole, H.E. 200×, on d 35.

DISCUSSION

The results of present study revealed a significant reduction in the body weight of T-2 toxin-treated birds. The reduction in body weight due to T-2 toxin was in agreement with previous findings (Wyatt et al., 1972, 1973, 1975; Chi and Mirocha, 1978; Hoerr et al., 1982; Huff et al., 1988). Significant decrease in body weight gains were observed in T-2 toxin-fed birds from the second week onward. The decreases in body weight gain of broiler chickens have been reported by feeding of T-2 toxin 0.5 ppm (Krishnamoorthy et al., 2006). Similar findings were reported by various authors with T-2 levels of more than 0.5 ppm such as (Kubena et al., 1990, 1994; Narayanaswamy, 1998; Kamalavenkatesh et al., 2004a; Rezar et al., 2007). Reduction in the body weight was a characteristic feature of T-2 toxicosis. It may primarily be due to inhibition of protein synthesis followed by secondary disruption of RNA and DNA synthesis (Feinberg and McLaughlin, 1989). The decreased body weight gain in the group IV (T-2+MG) of the present study could be attributed to the combination effects of MG infection and T-2 toxin. This assumption clearly strengthens the present findings of T-2 toxicosis and MG infection in broiler chickens. In the present study, feeding of T-2 toxin and mycoplasma and their combinations showed increased feed conversion ratio as compared to control. The FCR of T-2 toxin-fed birds was higher than that of the control. Similar observations were reported in chicken by earlier workers (Chi and Mirocha, 1978; Coffin and Combs, 1981), 1 ppm for 28 days (Kamalavenkatesh et al., 2004b) and 0.5 ppm for 4 weeks (Krishnamoorthy et al., 2005). This could be due to inhibition of protein synthesis by T-2 toxin (Feinberg and McLaughlin, 1989).

The relative weights of liver, kidney, and proventriculus were not altered significantly in the present study. Pearson (1978) recorded significant increase in relative weight of liver, while Coffin and Combs (1981) observed a decrease in relative weight of liver. The relative weights of these organs were not influenced by the T-2 toxin even at higher doses (Huff et al., 1988 and Kubena et al., 1989b). The unaltered relative weights in the present study could be attributed to the low concentration of toxin used in the study. The present study revealed a general reduction in relative weights of thymus, spleen, bursa of Fabricius with that of the control group throughout the experimental period. The relative weights of lymphoid organs were significantly decreased as compared to control group; the relative weights of different organs did not differ significantly from those of group III. Wyatt et al. (1973) reported significant reduction in relative weights of spleen and bursa of Fabricius at 4 μg/g and above of T-2 toxin in the diet. The decrease in the relative weight of bursa of Fabricius and thymus may be due to the radiomimetic action of the toxin on dividing germinal and or blast cells such as lymphoid, erythroid and intestinal crypt cells, which contained numerous free polysomes leading to increased susceptibility of the cells to trichothecene toxicity resulting in lymphoid depletion and necrosis as observed by Terao (1983). This observation was recorded in the form of lymphoid depletion in these organs. The present study revealed elevated levels of serum AST and ALT, which were significant with that of control birds. These findings were similar to those of (Pearson, 1978; Mishra et al., 1987; Huff et al., 1988; Raina et al., 1991). They reported increased levels of serum AST and ALT in chickens experimentally fed with T-2 toxin.

The liver and heart were reported to be rich in these enzymes and the damage to these organs caused increased plasma membrane permeability releasing the enzymes into circulation resulting in elevated enzyme levels in serum (Ladue et al., 1954; Ramazzotto and Carlin, 1978). Histologically, degenerative changes in the liver and heart were observed in group II and group IV, which substantiates adequately the increased serum AST and ALT in the present study. In the present

investigation, the serum total proteins were significantly decreased in group II and IV (especially on d 14) compared to the control group. The decrease in serum total protein is in accordance with the other observations (Huff et al., 1988; Kubena et al., 1989a, b). In group III there was a numerical decrease in the values of total protein but it was not significantly different from the control group. Significant ($P < 0.05$) decrease in the levels of total protein has been reported due to T-2 toxicity of broiler chicks fed 4 ppm (Huff et al., 1988; Kubena et al., 1989a, b) and 8 ppm (Kubena et al., 1990; 1994) for 21 days of age. Kamalavenkatesh (2003) noticed significant reduction in the serum total protein in broiler chickens fed 1ppm of T-2 toxin from 0 to 28 days of age. Mishra et al. (1987) observed that the decrease in total serum proteins in T-2 toxicosis might be due to degeneration of endoplasmic reticulum and inhibition of protein synthesis in the hepatocytes. The T-2 toxin is a known inhibitor of eukaryotic protein synthesis by impairing initiation and termination steps of protein synthesis (Uneo, 1983; Feinberg and McLaughlin, 1989). Similar mechanisms may be responsible for histological alterations in the liver resulting in decreased serum protein levels. The levels of serum albumin showed significant decrease in group II and IV as compared to control birds. In group III the values were not significantly different with that of control. Krishnamoorthy et al. (2006) fed 0.5 ppm of T-2 toxin to broiler chicks from day 1 to 4 weeks of age and observed a significant reduction in albumin, globulin, and albumin/globulin ratio. Similar reductions in albumin levels were also found with T-2 toxin in chickens by Huff et al. (1988) and Kubena et al. (1990, 1994). Mishra et al. (1987) reported decreased levels of albumin in broiler chickens, which may be due to degeneration of endoplasmic reticulum resulting in inhibition of protein synthesis.

The clinical signs noticed in T-2 toxin fed birds in the present study revealed inappetence, decreased growth rate, ruffled feathers, droopy wings, and economic loss. Similar signs were described by different workers (Wyatt et al., 1973, 1975; Chi and Mirocha, 1978; Bitay et al., 1981; Coffin and Combs, 1981; Mishra et al., 1987). The birds in group III showed clinical signs, like swollen head, keratoconjunctivitis, swollen eyelids, coughing, and rales. These clinical signs were very well described in MG infection of broiler chickens (Soeripto et al., 1989; Nunoya et al., 1995). Similarly the characteristic clinical signs of chronic respiratory disease (CRD) and myasthenia gravis (MG) were well documented in OIE (2008). Thus, the observations made in the present study are supported by the previous findings of researchers. The clinical signs of MG in infected poultry can vary from subclinical to obvious respiratory signs including coryza, conjunctivitis, coughing and sneezing. Nasal exudate, rales, and breathing through the partially open beak respiration (OIE, 2008). Johnson (1954) reported that the respiratory disease in chickens of 6–10 weeks of age showed tracheal rales, nasal discharge, persistent cough, retarded growth, loss of body weight, reduced feed consumption, and remarkable thickening of air sac membranes. In group IV (T-2 with Mycoplasma) birds, the clinical signs were decreased feed intake, depressed growth, feather malformations, keratoconjunctivitis, swollen eyelids, and coughing were noticed. Though the literature pertaining to T-2 toxin and MG infection was not available, the findings in the present study were similar to the clinical signs of T-2 toxicosis and MG infection per se. Though the development of clinical signs in group IV were independent of T-2 toxin and *M. gallisepticum*, the immunosuppression caused by T-2 toxin and subsequent development of MG infection can not be excluded. Hence it can be construed that the T-2 toxin induced immunosuppressed birds are more susceptible for MG infection.

In the present study, the T-2 toxin fed birds revealed enlargement of liver with congestion and petechial hemorrhage. Similar features were observed by Wyatt et al. (1973). Krishnamoorthy et al. (2007) observed enlarged and pale livers in broiler chickens fed with 0.5 ppm of T-2 toxin for four weeks. They opined that the hemorrhage was a consequence of exposure of T-2 toxin, which appeared to be associated with number of mechanisms *viz* tissue necrosis, thrombocytopenia, platelet dysfunction, decreased activity of coagulation factors, and possibly altered vascular integrity.

The lymphoid organs such as bursa of Fabricius, thymus, and spleen were atrophied in T-2 toxin alone and its combination with Mycoplasma groups. Similar findings due to T-2 toxicosis have been well documented (Bitay et al., 1981; Hoerr et al., 1982). This change was probably due to lymphoid depletion, followed by mild fibroblast proliferation and or reticular supporting network, which may be responsible for atrophy of lymphoid organs. In the Mycoplasma alone inoculated group, the birds showed keratoconjunctivitis, swollen eyelids, swollen heads, slight thickening of air sacs. These finding have been well documented by OIE (2008). McKay and Taylor (1954) reported that the pleuropneumonia-like organism (PPLO) caused CRD in chickens and infectious sinusitis in turkeys. In the present study, birds inoculated with MG through the nasal route, this might be responsible for the lesions in the respiratory tract. These lesions appeared similar to those described previously in the trachea, air sacs, and lungs (Siccardi, 1972; King et al., 1973). Based on the previous findings it can be presumed that the variable lesions were produced by MG depending upon the route of infection and other environmental factors, which culminate the disease process. Liver showed mild to moderate vacuolar degeneration of hepatocytes and focal areas of necrosis along with mononuclear cell infiltration. Increased activity of Kupffer cells and mild bile duct epithelial hyperplasia were also observed. These changes were more pronounced in group IV compared to group II and group III. In addition, fatty changes and lymphoid cell infiltration in periportal areas were also noticed.

Tendency of hepatic cords to form glandular or tubular structures during the fifth week of the experiment was more conspicuous. Many workers reported corresponding histological lesions in the liver due to T-2 toxicosis (Raina et al., 1991). Hoerr et al. (1981) reported mild bile ductular hyperplasia, necrosis at portal triads, while Hoerr et al., (1982) recorded vacuolar degeneration and bile ductular hyperplasia. The histopathological lesions of the present study were parallel to the observations of previous workers indicating that the T-2 toxin was a hepatotoxic. Further liver was reported to be the major organ of T-2 toxin excretion in chickens (Chi and Mirocha, 1978). The hepatic and biliary lesions appeared to be closely related to absorption and excretion of T-2 toxin as reported by Chi and Mirocha (1978) and Hoerr et al. (1981). T-2 toxin fed birds showed predominant lesions such as renal congestion, hemorrhages along with vacuolar degeneration of tubular epithelium and pyknosis of the nuclei. Similar lesions were recorded in chicken fed with T-2 toxin by previous workers (Hoerr et al., 1981; Raina et al., 1991). In group III, mild infiltration of lymphocytes in the interstitium was observed in the present study and was also reported earlier by Lockaby et al. (1998). However, Kerr and Olson (1970) recorded swelling of tubular epithelial cells by 3 days postinfection along with swollen and hyperplastic glomeruli in the contact-infected birds. The kidney lesions of the present study are comparable to the previous reports. Similar lesions were also noticed in the T-2 and MG groups. But there was no variation in the occurrence of the lesions compared to group II and III. This indicates that the kidney is less frequently affected in MG infection. In the present study, bursa of Fabricius showed predominantly lymphocytolysis and depletion in the medullary region. Corrugations of plical epithelium, infiltration of lymphoid cells in the sub epithelial area, and cyst formation were noticed. Increase in the interfollicular stromal tissue and occurrence of apoptotic bodies in the follicles were less commonly encountered lesions in the bursa of Fabricius. Similarly the thymus in T-2 toxin-fed birds showed mild-to-moderate lymphocytolysis with increase in number of histiocytes leading to decreased thickness of cortex, mild degree of congestion, hemorrhages and heterophil infiltration in medulla. Several workers have reported similar lesions in the lymphoid organs in T-2 toxicosis of birds (Wyatt et al., 1973; Hoerr et al., 1982). Hoerr et al. (1981) gave a detailed account of lymphoid lesions characterized by necrosis and depletion of lymphoid cells in bursa of Fabricius, spleen, and thymus. In addition, they also recorded necrosis of follicular epithelium of bursa of Fabricius. It could be postulated that the trichothecenes were identified as potent immunosuppressive toxins including T-2 toxin, which was responsible for the cytotoxic radiomimetic like activity in the rapidly dividing cells of lymphoid organs (Terao, 1983; Uneo, 1983). The depletion of lymphocytes in the various organs in the present study might reflect the above hypothesis. In group III, spleen showed depletion of lymphocytes as reported earlier by Lockaby et al. (1998) but did not show the changes as of group II. Group IV showed similar lesions as in the group II. These lesions may be due to the effect of T-2 toxin. No comparable literature was available. The microscopic lesions of proventriculus in broilers due to T-2 toxicosis were fusion of villi, submucosal infiltration of heterophils, and hyperplastic changes in the lining epithelium. Desquamation of villous epithelium and sub mucosal glands were partially destroyed because of infiltration of mononuclear cells. Hoerr et al. (1981) recorded the necrosis of superficial epithelium, exfoliation of epithelial cells, and mucosal folds in birds given a single dose of T-2 toxin by crop gavage, which amply supports the findings of the present study. In group IV also the proventriculus showed similar lesions as in group II, which were attributable to the effect of T-2 toxin. Such lesions were not reported in CRD in the available literature. Hence, it may be construed that the T-2 toxin alone may be responsible for proventriculus lesions.

Myocardium in the present study showed infiltration of mononuclear cells between the cardiac fibers, mild edema and occasional heterophil accumulation. Yarom et al. (1983) in left ventricle of T-2 toxin-treated rats encountered similar lesions, which could be due to increased lipid peroxidation of plasma membrane. In the T-2 toxin-fed birds, duodenum showed mild catarrhal changes along with hyperplasia of goblet cells, fusion of villi with mononuclear cell infiltration in the lamina propria, and decreased crypt length. Cecal tonsils relieved lymphoid necrosis and depletion. Hoerr et al. (1981) observed necrosis of villous tips and mononuclear cell infiltration in the lamina propria and atrophy of villi in both small and large intestine in chickens treated with T-2 toxin. The radiomimetic activity of the T-2 toxin on rapidly dividing cells of intestinal tract might be attributed to development of intestinal lesions (Terao, 1983; Uneo, 1983). In the birds fed with T-2 toxin and Mycoplasma group duodenum showed catarrhal changes due to hyperplasia of goblet cells, decreased length of crypts third week onward until the end of the experiment, which could be probably due to the action of T-2 toxin as similar changes were noticed in the duodenum of group II but not in group III. In lungs, pulmonary edema hemorrhages and mononuclear cell infiltration were observed in all the treatment groups. The lesions were pronounced in group III and group IV. Lutsky et al. (1978) recorded pulmonary edema in cats treated with T-2 toxin. These changes might be due to reduction in the activity of coagulation factors as opined by Doerr et al. (1981). Based on the previous findings, it can be concluded that MG produces respiratory infection and induces a variety of lesions depending upon the stage of infection. In the present study, the microscopic lesions in trachea include focal erosion and exfoliation of the tracheal epithelium with loss of cilia, excessive mucus production. Hyperplasia of tracheal epithelium, infiltration of inflammatory cells,

and desquamation of epithelium was observed in the group III and group IV. In the respiratory tract, studies have identified Mycoplasma colonization on tracheal epithelial surface resulting in loss of cilia, erosion of ciliated epithelial cells, and hypertrophy of nonciliated basilar epithelial cells (Dykstra et al., 1985). Similar lesions were found in the present study. These lesions were in parallel with observation of previous scientists, indicating that membrane-associated attachment proteins (cytadhesins) have been characterized in a number of Mycoplasma species, especially *M. pneumoniae,* and are responsible for tissue damage (Razin and Jacobs, 1992). The epithelial cells lining of the air sacs showed extensive edema, thickening with heterophilic infiltration. In the present study, congestion and hemorrhagic lesions were observed in groups III and IV. Air sac lesions were histologically similar to previously reported by Siccardi (1972) due to Mycoplasma synoviae (MS) in chickens. The lesions were similar to descriptions of air sac lesions caused by MG infection of chickens (Kerr and Olson, 1970). The histological changes observed in the air sacs were reflections of the ways in which this tissue responds to injury or infection and cannot be interpreted as specific lesions for MG, although the histological lesions were not specific. MG was the likely cause since similar lesions were absent in the air sacs of control birds. Similar lesions were observed by Springer et al. (1974).

In conclusion, our results showed that T-2 toxin was a potent immunosuppressive toxin and also that MG produces respiratory infection and induces a variety of lesions depending upon the stage and route of infection and other environmental factors that culminate the disease process.

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