PATHOLOGICAL AND IMMUNOLOGICAL STUDIES ON DIETARY T-2 TOXIN WITH CONCURRENT E.COLI INFECTION IN CHICKENS AND SOME RELEVANT CONTROL TRIAL

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ABSTRACT

This study was conducted to assess the effect of T-2 toxin on Escherichia coli –challenged broiler chickens from various aspects (health performance, biochemical, immunological, bacterial clearance rate, lymphocyte proliferation assay and histopathological alterations) and to study possible protective effects of antioxidant preparation. A total of 214 one day-old broiler chicks were used in two experiments. On day one, 190 chicks were separated into two groups, with one group fed a control commercial ration, and the other fed a commercial ration containing 2 ppm T-2 toxin. On day 14, each group was further separated into two subgroups, one subgroup supplemented with antioxidant preparations as 1 ml/l in drinking water and other subgroup was not supplemented. On day 21 each subgroup was further separated into two division, with one division inoculated with E. coli O78 (4 x 10^6 colony-forming units/0.5 ml), while the other division was not inoculated with E. coli. Five chickens from each group were randomly selected, weighed bled at 14, 21, 28 and 35 days. In experiment two, 24 day old chicks were randomly allotted to 4 dietary treatments as control, fed 2ppm T-2 toxin, fed 2ppm T-2 toxin plus antioxidant and antioxidant preparation 1ml/l of drinking water. At 3 weeks old, all groups were intravenously injected with E. coli 4 x 10^6 colony-forming units/0.5 ml. Blood sample were collected at 60, 120 and 180 min. post injection while, the liver, spleen and lung were collected 180 minutes P.I. Numbers of CFU per ml of blood and per gram harvested tissues were calculated.

Escherichia coli infection caused dullness, depression, huddling, and respiratory signs, wet eyes and diarrhea. Tracheitis, airsacculitis, pericarditis and periphlebitis was seen at postmortem. Mortality was (12%) in chicks infected with E. coli and increased up to 40% in chicks fed T-2 and infected with E. coli. Decreased body weight and reduced feed intake were observed in chicks fed T-2, and the effects were more pronounced in chicks fed T-2 and infected with E. coli. Increased serum levels of aspartate aminotransferase, alanine aminotrans-
ferase, urea, uric acid, and creatinine and decreased levels of total proteins, albumin, globulins and cholesterol were observed in T-2-fed birds. T-2 toxin significantly lowering lymphocyte proliferation and delay systemic clearance of *E. coli* from circulation with a significant increase persistence of *E. coli* in tissues. *Escherichia coli* infection alone or in combination with T-2 adversely affects all biochemical parameters and lymphocyte proliferation rate. The presence of T-2 toxin in ration even in a minimum amount could decrease the bird resistance and predisposed to certain diseases. In addition, will prolong the carrier state of chicken which results in prolonged shedding of the microorganism. T-2 toxin provokes dystrophic and necrotic changes in chicken's liver and kidney, different lesions in other internal organs as well as lymphocytic depletion and necrosis of the lymphoid tissue. These lesions increased in severity with *E. coli* infection and decrease in severity by using the antioxidant. The inclusion of Antioxidant preparation Toxnil as feed additives, might improve feed intake and body performance and could be able to counteract the T-2 adverse effects on biochemical parameters, immunity and pathological alterations. Poultry feed need to be screened regularly for the presence of mycotoxin T-2 under field conditions.

**INTRODUCTION**

Mycotoxins are a structurally diverse group of secondary metabolites produced by different genera of fungi. These mycotoxins are implicated in several animal and human toxicoses (*Surai and Dvorska, 2005*). More than 300 mycotoxins have been characterised and this number is growing quickly (*Fink-Gremmels, 1999*). The trichothecene group of mycotoxins accounts for over one hundred fungal metabolites, among those T-2 toxin was the first to be studied (*Bondy and Pestka, 2000*). T-2 toxin, a naturally occurring mycotoxin produced by several species of the genus Fusarium, is a 3-hydroxy 4, 15 diacetoxy-8 (3-methyl butyroxy), 12, 13-epoxy trichothec-9-ene metabolite (*Hollinger and Ekperigin, 1999*).

Several mycotoxins have been shown to suppress the immune responses and cause immunomodulation in domestic animals (*Sklam et al., 2001*). T-2 toxin is known to suppress both cellular and humoral mediated immune response. Other adverse effects are expressed in a diverse range of pathological alterations in the liver, kidney, lymphoid tissues, other internal organs and even cause cell death (*Hollinger and Ekperigin, 1999*).
Acute mycotoxicosis outbreaks in modern poultry production are rare. Although, low levels of mycotoxin contamination, which very often not detected, they are responsible for reduced efficiency of production and increased the susceptibility to infectious diseases (Krishnamoorthy et al. 2007).

Research in poultry has shown that, T-2 toxin causes reduced feed intake and weight gain, oral lesions, abnormal behavior, altered feathering, Impairs resistance to infectious diseases, reduces vaccination efficiency and induces pathologic damage to liver and other organs (Huff et al., 1988).

Various haematobiochemical parameters were resulted from feeding broiler chicken with T-2 toxin including, anaemia, hypoproteinaemia, hypoalbuminaemia, hypoglo-bulinaemia, hypoglycemia, hypcholesterolaemia and decreased ALT and increased AST (Kamalavenkatesh, 2003).

Escherichia coli on other hand, are normal inhabitant in the digestive and upper respiratory tracts of chickens and responsible for severe infections like collibacillosis. Certain predisposing factors are required to induce colibacillosis such as damage in the protective system of the respiratory tract, and the immunosupression (Saif et al. 2003). Colibacillosis causing a serious problem in poultry production, with mortality, condemnations and significant economical losses (Kumar et al., 2003). E.coli infection is known to damage the immune system of the chickens including lymphocyte depletion in both bursa and thymus (Nakamura et al. 1986). Moreover, Morgavi and Riley (2007) mentioned that T-2 toxin increased the susceptibility of animals to infection with E.coli. The immunity against E. coli can be evaluated by mortality rates, pathologic lesion scores, bacterial clearance rate, viable bacterial counts in internal organs, isolation frequencies, and weight loss after artificial challenge (Huang and Matsumoto, 1999).

The effects of T-2 toxin on poultry with infectious agents are obvious yet enigmatic. Therefore, the present study was conducted to:

Investigate the effect of T-2 toxin with concurrent E. coli infection on broiler chicken's health performance, biochemical, immunological and histopathological alterations.

Evaluate the immune response of chicks fed T-2 toxin and experimentally infected with E.coli through the bacterial clearance rate, lymphocyte proliferation assay.

Evaluate the protective and counteract effects of antioxidant preparation against T-2 toxin alone or in combination with E.coli infection.

MATERIAL & METHODS

Experimental birds:

A total of 214 day-old broiler chickens (Hubbard) were used. The chickens were maintained on commercial ration, feed and water were pro-
vided ad libitum. No medication or vaccinations were used during the entire period of the experiment.

**T-2 toxin:**
T-2 toxin was obtained from Sigma Chemical Company (St Louis, MO) USA (Sigma no:T-4887). Administration of T-2 toxicosis was induced according to Wyatt et al.(1973) and Ogunbo et al. (2007).

**Bacterial strain and Inoculum's preparation:**

E. coli strain was isolated from a field case of Colibacillosis, identified classified and serotyped as O78 according to Quinn et al .(1994) and (2002). The inoculums' was grown in nutrient broth for 24 hr at 37 °C and viable number adjusted to 4 x 10⁶ colony-forming units.

**Antioxidant preparation:**

Toxy-Nil™ plus (Nutri-Ad International, Belgium) was supplied from EGAVIT. This preparation composed of Neutralizing fermentation yeast extract, Saccharomyces cervisiae (4.125 X 10⁷ C.F.U.) per liter, Selected organic acids (citric acid, phosphoric acid, lactic acid, formic acid), Propyleneglycol, Beneficial bacterial count (1.0 X 10⁸ C.F.U. per liter) e.g. lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus delbrueckii subsp. Bulgaricus, Lactobacillus Enterococcus faecium, Bifidobacterium bifidum, Streptococci salivarius subsp. Thermophilus), -Amino acids (methionine, cystine, isoleucine, leucine, valine, phenylalanine, tyrosine, threonine, tryptophane, histidine, arginine, alanine, aspartic acid, glycine, proline, serine, glutamic acid), Selected vitamins (Bl, B2, B6, B12, pantothenic acid, biotin, PP and E) and Selected Minerals (selenium, magnesium, sodium, calcium, iron, iodine, copper, zinc and manganese).

**Experimental designs :**

Two experiment were conducted as follows:

**Experiment (1):** (Effect of feeding T-2 toxin alone or in combination with E.coli and antioxidnat on body performance, biochemical parameters , immunity and pathological alterations)

Hundred and ninety chickens of day old were used. On day 1, chicks were individually weighted, wing banded and randomly separated into two groups each of 95 each. First group was maintained on commercial ration alone (group C), the second group was fed on a commercial ration supplemented with 2 ppm T-2 toxin (group T). At day 14, five chicks from each group were randomly selected, weighed bled, and then subjected to postmortem examinations, the remaining chicks of each group (90 chicks/group) were subdivided into two subgroups, subgroup1 fed on commercial ration alone (n= 45), subgroup2 fed on commercial ration and drinking water mixed with antioxidant preparation as 1ml/l (n= 45). The chicks of group (T) were also separated into two subgroups
, commercial ration and given 2ppm T-2 toxin (n= 45) and commercial ration and given 2ppm T-2 toxin plus antioxidant preparation as 1 ml/l in drinking water. At day 21, five chicks from each group were randomly selected, weighed bled, and then subjected for postmortem examination. The remaining (40 chicks/group) were subdivided to two groups (15 and 25 chicks).

One group from each (n=25) was challenged with 0.5 ml 24 hrs broth culture of E. coli O78 serotype containing $4 \times 10^6$ colony-forming units by intraperitoneal route. The no. of birds in group infected with E.coli is more than those in other group because of expected mortality. The experiment design was illustrated in table (1):

Table (1): Showing design of experiment 1

<table>
<thead>
<tr>
<th>Day1 n=190</th>
<th>Group1</th>
<th>Group2</th>
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<tr>
<td>Number</td>
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<td>95</td>
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<tr>
<td>Commercial ration</td>
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<td>+</td>
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<tr>
<td>T-2 toxin</td>
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<td>Number</td>
</tr>
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<tr>
<td>T-2 toxin</td>
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<td>Antioxidant</td>
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<table>
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<tbody>
<tr>
<td>Subdivision</td>
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<tr>
<td>Number</td>
</tr>
<tr>
<td>Commercial ration</td>
</tr>
<tr>
<td>T-2 toxin</td>
</tr>
<tr>
<td>Antioxidant</td>
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<tr>
<td>E.coli inoculation</td>
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</tbody>
</table>

* Five chickens from each group were randomly selected, weighed bled, and then subjected for postmortem examination at days 14 and 21.

Clinical and post mortem finding:
The birds of all the groups, post-infection, were observed closely for assessing their health status. Mortality in different groups was also recorded after challenge. Re-isolation of inoculated E. coli was attempted from diseases and dead birds.

Weight of lymphoid organs:
Birds were randomly selected from each subgroup; slaughtered and lymphoid organs (thyroid, spleen and Bursa of Fabricius) were dissected out, weighed and calculated according to the formula of (Halouzka, 1991).
Serum biochemistry:
Blood samples were collected from birds in each group on 14th, 21th and 28th day of trial, allowed to clot and centrifuged at 1500 rpm for 20 minutes for serum separation. Serum total protein, albumin and globulin were estimated by modified Biuret and Dumas method (Varley et al., 1980). AST, ALT (Reitman’s and Frankel, 1957), cholesterol, glucose, urea, creatinine, uric acid were determined using ready made kit from (BioMeriux – France) by Auto analyzer Hitachi 912.

Lymphocyte proliferation assay:
Lymphocyte blastogenesis was applied to estimate the effect of T-2 toxin alone or in combination with E.coli infection on chicken lymphocyte proliferation in the presence of mitogens and the subsequent protection with antioxidant preparations. Briefly, the heparinized blood was layered carefully on the surface of lymphocyte separation medium, Ficol hypaque (1:1) in a 5 ml sterile centrifuge tube, then centrifuged for 40 min at 2400 r.p.m. at 4 °C and the separated Buffy coat was washed with RPMI-1640 medium three times each for 10 min at 2400, 2000 and 1500 r.p.m., respectively. The sediment washed lymphocytes were resuspended in one ml of RPMI-1640 medium containing 10 % fetal calf serum. Then to 100 µl of lymphocytes suspension, 100 µl of 0.4% trypan blue were added. The number of viable lymphocytes were adjusted at a final concentration of 2 X 10^6 cells/ml according to (Hanks and Wallace, 1985 and Husdan and Hay, 1980) and suspended in RPMT medium containing 10 % fetal calf serum. The phytohaemagglutinin (PHA) was reconstituted in RPMI-medium and the required concentration could be made to 10 µg/ml, then filtered through millipore filters (0.22 um pore size, Millipore, USA) and store at -20 °C. Flat bottom sterile tissue culture plates with 96 wells were used as follows: 3 wells containing 100 µl of suspended lymphocytes (2X10 cells) in 50 µl growth media (RPMI + 10 % fetal calf serum) served as cell control, 3 wells containing 100 µl of suspended lymphocyte + 50 µl of PHA mitogen (15 µl /ml) and 3 wells containing 150 µl of RPMT-1640 medium (medium control), then the plates were incubated at 37°C in a humid CO2 atmosphere (5-10% CO2), then blastogenesis was assayed after 48-72 hrs using residual glucose in the supernatant of tissue culture medium according to Decock et al.(1980).

Experiment (2): (Effect of feeding T-2 toxin on bacterial Clearance Rate)
To evaluate the ability of chicks fed T-2 toxin to clear bacteria from circulation Bacterial clearance rate was employed according to (Huang & Matsumoto, 1999 and Li et al. 1999).

Twenty four day-old chickens were used in this experiment. Feed and water was ad libitum. At day 14 of age
chickens were divided into 4 equal groups each of 6 as follow:

Gr.(1): obtained a commercial ration

Gr.(2): Fed commercial ration containing 2 ppm T-2 toxin

Gr.(3): Fed commercial ration and 2 ppm T-2 toxin + antioxidant preparation as 1ml/l in water

Gr.(4): Fed commercial ration + antioxidant preparation as 1ml/l in water. At day 21, chickens of all groups were intravenously inoculated with 4 x 10^6 E.coli.

Two milliliter of blood were collected on heparin from two chicks per infected groups at 60, 120 and 180 minutes post Escherichia coli challenge.

Then, the samples of liver, spleen and lungs were aseptically taken for quantitative bacterial determination. Whole blood samples were serially diluted with sterile PBS, and 100 μL of each dilution at each time point was plated onto MacConkey agar plates. Approximately 1 g of tissue samples was homogenized with 5 mL of sterile PBS. Serial dilutions of tissue suspension (100 μL) were plated onto MacConkey agar plates. Each sample was plated in duplicate. Plates were incubated at 37 C for 18 h, and the E. coli colony-forming units were enumerated. The final bacterial concentrations were calculated as the numbers of colony-forming units per milliliter of blood and as colony-forming units per gram of harvested tissue.

Histopathological examinations:
Tissue specimens of selected groups from liver, kidney, lung, intestine, heart, gizzard, thymus, spleen and bursa were collected, fixed in 10% neutral buffer formalin, dehydrated serial grades of ethyl alcohol, cleared by xylol, embedded in paraffin wax, sectioned at 3-5 microns, stained with Haematoxyline and Eosin (H&E) according to Bancroft et al. (1996) and then examined microscopically for recording the histopathological alterations.

Statistical analysis:
Data were Statistically analyzed using SPSS 11.0 (2001) for Windows.

RESULTS
Clinical signs and postmortems:
There was significant difference among groups, as seen in Table (1). Decrease growth rate, abnormal feathering (Fig.1), reduce weight gain in all T-2 toxin feeding groups than those feed commercial ration.

However, after infection with E.coli birds in group (fed T-2+E.coli) and (commercial ration+E.coli) showing signs of dullness, depression, huddling 24 hr post infection then 2nd day post infection respiratory signs were developed as rales, rhinitis and wet eyes (Fig.2). Some birds had watery diarrhea. Feed and water were drastically reduced and chickens were unable to stand. No signs were recorded
in all control groups or those received antioxidant preparation.

**Postmortem examination:**

Birds fed T-2 toxin showed reddens and inflammation of the intestine, palness, mottling and enlargement of the liver (Fig.3). Atrophy of the thymus, spleen and other lymphoid organs was seen, kidneys were swollen with urate deposits in the ureters.

Focal ulceration was noticed with inflammation of crop mucosa, and a thickened, rough lining in the gizzard and intestine (Fig. 4).

Tracheitis, perihepatitis, pericarditis and airsaccultis were observed after *E.coli* infection (Fig.5). Severe pericaditis, perihepatitis and airsaculitis were noticed in combined T-2 toxin and *E.coli* infected group (Fig.6)

**Growth rate response:**

The result was illustrated in table (2). Feeding T-2 toxin significantly retarded the growth of the chicks than other groups. Body weights were decreased drastically post *E. coli* infection in the T-2 fed group

**Biochemical alterations:**

Mean ±SE serum total protein, albumin, globulin and albumin / globulin (A/G) ratio, ALT, AST, creatinine, uric acid and cholesterol value in chickens fed T-2 toxin singly and in combination with antioxidant either non infected or infected with *E.coli* are presented in the Tables (3,4 and 5).

There was significant decrease in the total protein, albumin and globulin values in the T-2 fed birds when compared to the control. Albumin / globulin ratio increased in toxin fed groups. Comparison of means revealed significant (P<0.05) difference in AST,ALT Urea, creatinine, uric acid and cholesterol between groups fed T-2 toxin and control one tales (6 and 7).

*Escherichia coli* infection alone or in combination with T-2 adversely affects all biochemical parameters. The presence of T-2 toxin has much impact in these parameters than *E.coli* alone. Antioxidant preparation (Toxynil plus) was able to provide protection against adverse effects of dietary T-2 toxin on serum biochemical parameters.

**Lymphocyte proliferation assay:**

The effects of T-2 toxin alone or in combination with *E.coli* on the immune system of chicken were summarized in table (8). *Escherichia coli* inoculation resulted in significant reduction on the lymphocyte proliferation. Feeding T-2 toxin alone significantly ( P< 0.05) lowering the lymphocyte proliferation than control one. A combination of T-2 toxin and *E.coli* caused severe reduction in blastogenic response.

**Bacterial Clearance Rate and Counts of viable bacteria in organs:**

Bacterial clearance from the circulating blood was significantly affected by dietary T-2 toxin (table,9). T-2
Significantly delayed systemic clearance (P < 0.05) with persisting viable numbers of *E. coli* in the tissues until the end of the observation period in chicks fed 2 ppm T-2 toxin as compared to either control or antioxidant preparations fed groups (table, 10).

**Histopathological examination:**

**1-In T-2 fed group:**

The microscopical examination of the liver revealed diffuse edema, degeneration and necrosis of hepatocytes (Fig.7). Most of hepatocytes had unclear borders and granulated cytoplasm, others could be seen without nucleus and transformed into tiny eosinophilic detritus as well as fatty changes were recorded. Liver of other cases showed sever congestion and mild periportal fibrosis (Fig.8). The kidney showed edema and tubular nephrosis and vacuolar degeneration (Fig.16). The spleen showed moderate lymphoid depletion (Fig.18). Both thymus and bursa of fabricius revealed edema and mild lymphocytic depletion (Fig.19).

**2-In T-2 and antioxidant treated group:**

The addition of the antioxidant to the T-2 fed group decreased the number of affected birds and or the severity of the lesions in different organs. The liver showed edema and vacuolar degeneration (Fig.16). The kidney showed tubular nephrosis and vacuolar degeneration of some tubular epithelium (Fig.17). The spleen showed moderate lymphoid depletion (Fig.18).

**3-In *E. coli* infected group:**

The liver of *E. coli* infected birds revealed edema, severe congestion, vacuolar degeneration of some hepatocytes and necrosis (Fig.20). The kidney showed severe degeneration and necrosis of the renal tubules and gomerulei (Fig.21). The intestine showed mucinous degeneration, vacuolation of the glandular epithelium, edema and necrosis of the muscularis mucosa. Some lymphocytic infiltrations in lamina propria and submucosa, as well as sloughing and necrosis of the villar epithelium (Fig.22 & 23). The gizzard revealed shortening of villi, necrosis of the muscular layer and mononuclear cells infiltration in between the
muscle fibers (Fig.24 &25). The heart showed pericarditis, severe myocardial necrosis and edema (Fig.26). The lung showed degeneration of the alveoli, peribronchial lymphocytic infiltration as well as hyperplasia, mucinous degeneration and necrosis of the bronchial epithelium (Fig.27). The spleen revealed congestion of the splenic blood vessels, edema, lymphocytic depletion and necrosis (Fig.28). Bursa of fabricius showed lymphocytic depletion specially at the center of the follicles and slight interfollicular fibrosis (Fig. 29). The thymus also revealed lymphoid depletion.

4- In T-2 and E.coli infected group:

Presence of T-2 and E.coli in combination induce severe and marked pathological lesions in various organs. The liver showed edema, severe necrosis of hepatocytes as well as mononuclear cells infiltrations especially lymphocytes (hepatic lymphoma) (Fig. 30). The kidney revealed, severe diffuse edema and necrosis of the renal tubules and glomeruli (Fig. 31). The intestine showed hyperplasia, sloughing and necrosis of the villar epithelium, necrosis of glandular epithelium, edema and necrosis of the muscularis mucosa as well as mononuclear cells infiltration in the lamina propria and submucosa (Fig. 32 &33). The gizzard showed congestion, epithelial sloughing and necrosis together with edema and necrosis of the muscular layer (Fig. 34). The heart showed edema and necrosis of the myocardium as well as fibrinus pericarditis (Fig. 35). The spleen revealed congestion, edema, slight fibrous tissue proliferation, lymphocytic depletion and necrosis (Fig.36). The trachea of most cases revealed sloughing and necrosis of the epithelial cells, hyperplasia of the goblet cells as well as congestion, hemorrhages and mononuclear cells infiltrations (Fig. 37). The lung showed emphysema, congestion, hyperplasia of the alveolar epithelium and mononuclear cells infiltration (Fig.38). The bursa of fabricius revealed mild interfollicular fibrosis, edema and lymphoid depletion (Fig. 39). The thymus also showed lymphocytic depletion.

5- In T-2, antioxidant and E.coli treated group:

Birds treated with T-2, antioxidant and E.coli showed nearly more or less the same lesions as in E.coli inoculated group but mostly these lesions were less in severity. The liver showed edema, vacuolar degeneration and focal lymphocytic aggregation (Fig. 40). The kidney revealed edema and tubular nephrosis. The intestine revealed mucus degeneration. The lung showed congestion, emphysema and presence of mucopurelant exudates inside the bronchioles and the alveoli (Fig. 41). The spleen showed moderate edema and lymphocytic depletion. The thymus and bursa of fabricius showed slight lymphocytic depletion (Fig. 42).
Fig. (1): Chicken fed T-2 showing decrease growth rate, abnormal feathering.
Fig. (2): Chicken inoculated with *E.coli* showing respiratory signs, rhinitis and wet eyes
Fig. (3): Three-week-old broiler chicken fed T-2 toxin showing pale and enlarged liver.
Fig. (4): Chicken fed T-2 toxin showing inflammation in the gizzard and intestine.
Fig. (5): Chicken showing pericarditis and airsaccultis after *E.coli* infection
Fig. (6): Chicken exposed to combined t-2 toxin and *E.coli* infected showing severe pericaditis, perihepatitis and airsaculitis.
Fig. (7): Liver, of T-2 fed birds showing diffuse edema, degeneration and necrosis of hepatocytes. H&E stain. X 400.
Fig. (8): Liver, of T-2 fed birds showing severe congestion and necrosis H&E stain. X 400.
Fig. (9): Kidney, of T-2 fed birds showing, edema, degeneration and necrosis of the tubular epithelium and glomeruli. H&E stain. X 400.
Fig. (10): Gizzard, of T-2 fed birds showing, edema and necrosis of the muscular layer as well as severe necrosis of the epithelial layer. H&E stain. X 250.
Fig. (11): Intestine, of T-2 fed birds showing thickening and shortening of the villi, mucinous degeneration, necrosis of some villar and glandular epithelium as well as edema in lamina propria and submucosa. H&E stain. X 400.
Fig. (12): Heart of T-2 fed birds showing severe myocardial edema and necrosis. H&E stain. X 400.
Fig. (13): Spleen, of T-2 fed birds showing, congestion of the splenic blood vessels, focal lymphocytic depletion and necrosis. H&E stain. X 250.

Fig. (14): Bursa of fabricius, of T-2 fed bird showing edema, lymphoid depletion and mild interfollicular fibrosis. H&E stain. X 400.

Fig. (15): Thymus, of T-2 fed birds showing, severe medullary lymphocytolysis. H&E stain. X 250.

Fig. (16): Liver, of T2 and antioxidant treated birds showing, vacuolar degeneration. H&E stain. X 250.

Fig. (17): Kidney, of T2 and antioxidant treated birds showing, tubular nephrosis and vacuolar degeneration of some tubular epithelium. H&E stain. X 400.

Fig. (18): Spleen, of T2 and antioxidant treated birds showing, lymphocytic depletion. H&E stain. X 250.
Fig. (19): Bursa of fabricius, of T2 and antioxidant treated birds showing edema and mild lymphocytic depletion. H&E stain. X 400.

Fig. (20): Liver, of *E. coli* infected birds showing severe congestion, vacuolar degeneration of some hepatocytes and necrosis. H&E stain. X 400.

Fig. (21): Kidney, of *E. coli* infected birds showing severe degeneration and necrosis of the renal tubules and gomerulei. H&E stain. X 250.

Fig. (22): Intestine, of *E. coli* infected group showing, mucinous degeneration, edema and necrosis of the muscularis mucosa, some lymphocytic infiltrations in lamina propria as well as vacuolation of the glandular epithelium.

Fig. (23): Intestine, of *E. coli* infected birds showing, mucinus degeneration, sloughing and necrosis of the villar epithelium. H&E stain. X 400.

Fig. (24): Gizzerd, of *E. coli* infected birds showing, necrosis of The muscular layer. H&E stain. X 100.
Fig. (25): Gizzerd, of E. coli infected birds showing, severe mononuclear cells infiltrations in between the muscle fibers. H&E stain. X 400.

Fig. (26): Heart of E. coli infected birds showing, pericarditis, severe myocardial necrosis and edema. H&E stain. X 400.

Fig. (27): Lung, of E. coli infected birds showing degeneration of the alveoli, peribronchial lymphocytic infiltration as well as hyperplasia, mucus degeneration and necrosis of the bronchial epithelium. H&E stain. X 250.

Fig. (28): Spleen, of E. coli infected birds showing lymphocytic depletion and necrosis. H&E stain. X 250.

Fig. (29): Bursa of fabricius, of E. coli infected birds showing lymphocytic depletion specially at the center of the follicles. H&E stain. X 400.

Fig. (30): Liver of T-2 and E. coli infected birds showing, edema, necrosis of hepatocytes as well as focal mononuclear cells infiltrations especially lymphocytes. H&E stain. X 400.
Fig.(31): Kidney, of T-2 and *E. coli* infected birds showing diffuse edema and severe necrosis of the renal tubules and glomerulei. H&E stain. X 400.

Fig.(32): Intestine, of T-2 and *E. coli* treated group showing, hyperplasia, sloughing and necrosis of the villar and glandular epithelium, edema and necrosis of the muscularis mucosa. H&E stain. X 250.

Fig.(33): Intestine, of T-2 and *E. coli* infected birds, showing mononuclear cells infiltration in lamina propria and submucosa, edema, and glandular epithelial necrosis. H&E stain. X 400.

Fig. (34): Gizzard, of T-2 and *E. coli* infected birds showing, congestion, epithelial sloughing and necrosis as well as edema and necrosis of the muscular layer. H&E stain. X 250.

Fig. (35): Heart, of T-2 and *E. coli* infected birds showing, edema and necrosis of the myocardium as well as fibrinus pericarditis. H&E stain. X 250.

Fig.(36): Spleen, of T-2 and *E. coli* infected birds showing, congestion, edema, slight fibrous tissue proliferation, lymphocytic depletion and necrosis. H&E stain. X 250.
Fig. (37): Trachea, of T-2 and E. coli infected birds showing, sloughing and necrosis of the epithelial cells, hyperplasia of the goblet cells as well as congestion, hemorrhages and mononuclear cells infiltrations. H&E stain. X 100.

Fig. (38): Lung, of T-2 and E. coli infected birds showing, emphysema, congestion, hyperplasia of the alveolar epithelium and mononuclear cells infiltration. H&E stain X 400.

Fig. (39): Bursa of fabricius, of T-2 and E. coli infected birds showing, edema and lymphocytic depletion. H&E stain X 400.

Fig. (40): Liver, of T-2, antioxidant and E. coli infected birds showing, edema, vacuolar degeneration, necrosis of some hepatocytes and focal lymphocytic aggregations H&E stain X 400.

Fig. (41): Lung, of T-2, antioxidant and E. coli infected birds showing, congestion, emphysema and presence of exudates inside the bronchioles and the alveoli. H&E stain X 400.

Fig. (42): Spleen, of T-2, antioxidant and E. coli infected birds showing, moderate edema and lymphocytic depletion. H&E stain X 400.
DISCUSSION

Mycotoxin contamination of various feed and food commodities is a global problem (Schollenberger et al., 2006).

Clinically, birds fed T-2 toxin experimentally showed decrease growth rate, abnormal feathering and reduced weight gain. At postmortem, inflammation of the intestine, mottling of the liver, atrophy of the thymus, spleen, and other lymphoid organs, kidneys were swollen with urate deposits in the ureters. No oral lesion or ulcer could be observed in birds fed T-2 toxins. Similar finding were reported by Garcia et al. (2003). Also the differences in the results obtained when using pure mycotoxins or contaminated grain as a source of mycotoxins. Where, a contaminated grain contains non-identified metabolites which could be also involved. These unknown metabolites might strongly acted along with T-2 toxin and produce other toxic forms such oral ulcer (Rotter et al., 1989).

Birds experimentally infected with Escherichia coli showed depression, ruffling, rhinitis, wet eyes and respiratory signs from 2-3 days post infection. At postmortem examination, tacheitis, perihepatitis, pericarditis and airsaccultis were seen. These results are in accordance with Peighambari et al. (2000). While, chickens had concurrent Escherichia coli infection with T-2 toxin showed signs as early as 12-24 hr post infection with severe signs and high mortality rate than Escherichia coli infected birds alone. This may be due to the immunosuppressive effect of T-2 toxin which altered the ability of chickens to resist the infection and supported by bacterial clearance rate and immunological investigation in this study. Also the severity of the signs, postmortem lesions and mortality % were higher in T-2 fed group which agreed with similar findings reported by Kumar et al. (2003).

T-2 toxin alone at 2ppm/ kg ration had a significant effect on birds body weight and feed consumption. When E.coli infection was combined with T-2 toxin, an additive effect was observed. The reduction of body weight gain was higher than that obtained with each individually. This observation agreed with that reported by Garcia et al. (2003), Kumar et al. (2003). The decrease in body weight was mainly due to reduce feed intake which referred to presence of t-2 toxin.

Similar explanation reported by Hoerr., (1998). In addition, T-2 toxins are shown to alter the serotonin activity in the central nervous system, which is known to be involved in the regulation of appetite (Rotter et al., 1996).

These adverse effects on performance were completely overcome by the dietary supplementations of an-
tioxidant preparation which represented by improvement in feed intake and body weight gain and general health condition. Such improvement effect was not seen in other groups fed no antioxidant additives. No significance difference between the control group and the group received T-2 toxin combined with antioxidant preparation. These results confirm previous studies by Dalia, (2003) and Diaz et al.(2005).

Different strategies to combat mycotoxicosis have been developed, based on the addition of adsorbents to contaminated feed. (Karaman et al., 2005). Unfortunately, the toxic effects of T-2 toxin have not been found to be reduced by any adsorbents up to now (Garcia et al., 2003). On the other hand, antioxidants are organic molecules of either synthetic or natural origin, which can avoid or delay the progress of oxidative rancidity and oxidative stress caused by T-2 toxin. (Julia et al., 2007).

A Significant reduction in total protein, albumin and globulin value were observed in T-2 fed birds which agreed with the findings of Kamalavenkatesh, (2003), Krishnamoorthy et al.(2007). This hypoproteinemia, hypoaalbuminemia and hypoglobulinemia observed in T-2 fed group could attributed to the reduction in feed consumption and hepatic damage as observed in histopathological investigation of this study, since liver is the major organ of protein synthesis specially albumin (Kaneko et al., 1997). Moreover, Meloche and Smith (1995) found that T-2 toxin inhibit protein synthesis. T-2 toxin induce DNA damage in liver tissue and increase DNA fragmentation. The inhibitory effects of T-2 toxin have been attributed to the binding of the toxin to subcellular components, including sulphhydryl groups and ribosomes, with ensuing inhibition of RNA, DNA and protein synthesis (Atroschi et al. 1997). Significant reductions in serum concentrations of cholesterol levels probably can be attributed to a repression of cholesterol biosynthesis, due to the hepatotoxicity and possibly a shifting of the circulatory cholesterol back from the liver (Kubena et al., 1993).

It was shown that T-2 toxin inhibits hepatic protein synthesis, causing aminoacidemia. As a result, there will be greater degradation of free circulating amino acids for energy utilization, leading to excess uric acid synthesis Meloche and Smith. (1995). This could explain the increase in uric acid level associated with feeding T-2 toxin in this study.

Feeding T-2 toxin adversely affect urea, uric acid and creatinine. Similiar findings were reported by Bailey et al. (1998) and confirmed by histopathological alterations in kidney tissue.

Escherichia coli infection decrease the levels of total protein, glo-
bulin and albumin globulin ratio than control ones. However T.P contents in chickens fed T-2 and infected with *E. coli* were significantly less than in chickens infected with *E. coli* alone (other groups). This result agreed with Kumar *et al.* (2003) and abddel-fat-hah et al. (2003).

Blood plasma parameters might be used for an additional estimation of toxic effects on live birds (Schiefer, 1990). It is assumed that elevated activities of serum or plasma enzymes such as Alt and AST, might indicate recent organ damage (Coles, 1986), which confirmed by histopathological investigation.

The feeding of antioxidant alone improve body performance, weight gain without any adverse alterations in the biochemical parameters. Moreover, there were an improvement in biochemical parameters in T-2 fed groups which may be regarded to the counteract effects of antioxidant preparation. Such result agreed with Atroshi *et al.* (1997) who documented that administration of antioxidants prevented DNA damage caused by T-2 toxin through preventing formation of the fragmentation ladder as well as the appearance of other forms of damage to DNA. Also Dvorska & Surai, (2001) and Aravind et al. (2003) reported that the improvement in AST and Alt after feeding antioxidant may be regarded to the protective effect of antioxidant with inhibitory effect on liver antioxidant deletion.

Macrophages are mobile scavengers representing the first line of immuno-defense against infectious organisms. These cells play a role in both acquired and nonspecific immunity, by direct destruction of microbes cells and contribute to the clearance of bacterial pathogens (Novelli *et al.*, 1989 and Dietert *et al.*, 1991).

The bacterial clearance from the circulating blood in this study showed a significant delayed systemic clearance ($P < 0.05$) with persisting numbers of *E. coli* in the blood until the end of the observation period in chickens fed T-2 toxin in diet as compared to chickens either positive control, or those fed antioxidant preparations. This indicate the ability of chickens to eliminate bacteria from the blood was diminished by dietary T-2 toxins. This result agreed with Li *et al.* (1999).

Also the result indicated that diminished systemic blood bacterial clearance was associated with a significant increase of numbers of *E. coli* in tissues, suggesting both reduced phagocytosis and lysis capacity of the reticuloendothelial system. If the phagocytic system is compromised, the ability to resist infection is likely decreased (Neldon and Qureshi, 1992).

This could explained the earlier and severity of signs in T-2 fed group than other group, which may be
due to the T2-toxin may impaired phagocytosis thus leading to a reduction in bacterial clearance during infections. This explanation agreed with Qureshi and Hagler (1992).

Dealing with Lymphocyte proliferation assay, Escherichia coli inoculation resulted in significant reduction on lymphocyte proliferation. Such effect of E.coli can be caused by lymphocyte depletion of lymphoid tissue and supported by lymphocytic organ weight in this study. Similar results was reported by Abdel-Fattah et al. 2003. Nakamura et al. (1994) and Mona and Hussanein, (1998) and Hussanein et al. (2001) documented a marked depletion in both bursa and thymus following experimentally E.coli infection in chickens.

Several studies have been published on the effects of T-2 toxin on the proliferation of human or murine lymphocytes. Nevertheless, little attention has been paid to the effects of T-2 toxin on chicken lymphocytes (Pau-cod et al., 1990).

The amount of T-2 toxin required to inhibit lymphocyte blastogenesis (proliferation) was found to be at least 10 times less than the amount needed for inhibition of protein synthesis (Pestka and Bondy, 1990, 1994). This implies that lymphocyte proliferation could be the first parameter to be affected by T-2 toxin followed by the inhibition of protein synthesis and cell death at later stages.

The result showed that, chickens treated with T-2 toxin had significant reduction in blastogenesis response to phytohaemagglutinin (PHA).

This result goes parallel with the previous findings where, suppression of T lymphocyte or lymphocyte blastogenesis in animal treated with T-2 toxin was reported by Kamalavenkatesh et al. (2005) and Ziad et al. (2006).

Also Hurley et al. (1999) reported that T-2 toxin at level of 2 ppm significantly reduced blastogenic response of young mallard ducks to PHA. This appeared in full agreement with our results. A combination of T-2 toxin and E.coli caused severe reduction in blastogenic response. This indicated the additive effect of both T-2 toxin and E.coli infection in affecting the chickens' cellular immune system adversely.

Thymic atrophy and lymphoid depletion in all lymphatic organs in T-2 toxin fed birds indicated the immunosuppressive potential of this toxin. However, these findings concurred with lymphocytolysis observed histologically in the thymus, bursa and spleen of chickens treated with T-2 toxin. Kubena et al., (2001) reported a significant interaction resulted in increased mortality in chickens fed T-2 toxin and infected with Salmonella organisms.
An important field implications were come out as a result of modulation of the immune system (depletion of T-lymphocyte and decrease phagocytic, ability of bacterial clearance rate) that is exerted by T-2 toxin. Decreasing bird resistance, increase the carriers and prolonge shedding of the microorganism. This suggestion agreed with that reported by (Ziad et al., 2006).

With respect of the histopathological changes in chicken received T-2 toxin (either alone or in combination with E. coli), the most affected organs were liver and kidney which agreed with Seferd et al. (2003); Hammad et al. (2006) and Krishnamoorthy et al. (2007). These effects are due to selective suppression of the activity of natural killer cells (Pegram and Waytt, 1986). It also causes inhibition of DNA and protein synthesis leading to liver damage and nephrotoxicity (Tietz, 1976). Antonio, (2004) reported that, mycotoxins are metabolized in the liver and therefore the primary symptoms of mycotoxicosis are alterations of the liver tissue. Chi et al. (1977) recorded that the liver represents the central point of detoxification of mycotoxins, also the main way of secretion is through the bile so, it is logical to expect the most pronounced alterations would be in liver.

Coulombe, (1993) reported that, T2- toxin impairs the immune system and induces pathological damage to liver and other organs. The observed fatty change or vacuolar degeneration in the liver tissue is correlated to alterations in the carbohydrate metabolism resulting from mycotoxins which impair lipid transport, causing a reduction on the concentration of glucose and fat inside the hepatocytes (Naber & Wallace 1979). The hyperplasia of bile ducts which was seen in the present study was in agreement with Hoerr et al. (1982) might be caused by the fat alterations with signs of irreversible toxicity. Moreover; the observed vacuolization could be caused by T-2 toxicity. These types of cellular alterations, can cause irreversible lesions which could lead to cell death (Robbins et al., 1986).

The kidney of T-2 fed birds showed edema, degeneration and necrosis of the tubular epithelium and glomeruli. These results were in agreement with Antonio (2004); Grizzle et al. (2004) and Krishnamoorthy et al. (2007).

Histological examination of the lymphoid tissue (spleen, thymus, bursa of fabricius) in T-2 fed birds showed various degrees of hypocellularity (lymphoid depletion) and necrosis which were consistent with findings of previous reports in chickens (Hoerr et al., 1981; Sklan et al., 2001; Kamalavenkatesh 2003; Grizzle et al., 2004; and Krishnamoorthy et al, 2007).

In poultry T-2 toxin causes immunosuppression as were seen in rodents.
The ability of T-2 toxin to produce lymphoid depletion of thymus and spleen has been well documented in rodents (Hollady et al., 1993 and Hollady et al., 1995).

In the present study, T-2 toxin fed birds showed lymphocytolysis in the thymus of some birds. Kamalavenkatesh, (2003) reported that, broilers fed T-2 toxin from 0 to 4 weeks of age showed generalized lymphoid depletion and lymphocytolysis in bursa of fabricius, spleen, thymus and caecal tonsils.

In T-2 fed birds, the gizzard showed congestion, edema and necrosis of muscle layers, mononuclear cells infiltrations as well as sloughing and necrosis of the villar epithelium. These results agreed with (Kubena et al., 1990; Sklan et al., 2001; Kamalavenkatesh, 2003 and Krishnamoorthy et al. 2007).

The intestine of T-2 fed birds showed goblet cell hyperplasia (mucin degeneration), fusion of villi, edema and necrosis of the muscularis mucosa, degeneration and necrosis of the villar and glandular epithelium.

These results agreed with (Hoerr et al., 1981; Kamalavenkatesh 2003 and Krishnamoorthy et al. 2007).

Hoerr et al. (1998) recorded that, some toxins impart a radiomimetic injury to the intestine, such as that caused by T-2 toxin. With T-2 toxin, necrosis first affects the cells at the tips of the villi, probably a result of the caustic injury. With the addition of the antioxidant to the T-2 fed group, the severity of histopathological lesions decreased. The used antioxidant contain elements as copper, manganese, iron and various vitamins especially vitamin B complex and vitamin E which act as antioxidants which diminish liver and kidney damage (Awadallah et al., 1984).

The use of antioxidant had better weight gains and pathologic lesions when compared to chickens that fed T-2 toxins alone and the degree of reparation varied depending on the duration of exposure to toxin and on individual variation. On the other hand, E.coli infected birds either alone or with T-2 toxin showed different pathological lesions but, it was noticed that these lesions were more prominent with their combination.

With respect of birds received T-2, antioxidant and E.coli in combination, nearly the same histopathological lesions of E.coli infected birds were observed in all organs, but mostly these lesions were lower in severity. This is due to using of the antioxidant which diminish the tissue damage as observed by Awadallah et al., (1984).

All obtained results confirmed that T-2 toxin provokes dystrophic and necrotic changes in chicken's liver and kidney and different lesions in other internal organs, these lesions increased in severity with E.coli infection and decrease in severity by using the antioxidant.

It could be concluded that, feeding T-2 toxin even in minimum amount and the concurrent presence of Escherichia coli infection, negatively affect body performance, mortalities, serum biochemical parameters and bird diseases resistance. This is an important finding having practical implications. The inclusion of Antioxidant preparation Toxnil as feed additives, might improve feed intake, body performance and could be able to counteract the T-2 adverse effects on biochemical parameters, pathological alterations and immunity. Poultry feed need to be screened regularly for the presence of T-2 under field conditions.
Table (2): Effect of feeding T-2 toxin alone and in combination with *E.coli* and antioxidant on Body weights of broiler chickens. (Mean ± SE). n=190

| Age (days) | Control (commercial diet) | commercial diet + antioxidant preparation | T2-toxin | T2-toxin + antioxidant preparation
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>290 ± 7.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>310 ± 6.67&lt;sup&gt;a&lt;/sup&gt;</td>
<td>245 ± 11.68&lt;sup&gt;b&lt;/sup&gt;</td>
<td>275 ± 9.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>21</td>
<td>N=15</td>
<td>N=25</td>
<td>N=15</td>
<td>N=25</td>
</tr>
<tr>
<td></td>
<td>412 ± 14.30</td>
<td>410 ± 12.90</td>
<td>425 ± 11.83</td>
<td>420 ± 12.72</td>
</tr>
<tr>
<td>28</td>
<td>730 ± 14.32</td>
<td>628b ± 21.04</td>
<td>850 ± 12.50</td>
<td>725 ± 13.60</td>
</tr>
<tr>
<td>35</td>
<td>977 ± 21.50</td>
<td>795b ± 27.34</td>
<td>1082 ± 15.71</td>
<td>897 ± 25.21</td>
</tr>
<tr>
<td>mortality</td>
<td>0/15 (0%)</td>
<td>3/25 (12%)</td>
<td>2/25 (8%)</td>
<td>2/15 (13.3%)</td>
</tr>
</tbody>
</table>

Within a row, values with the same superscript letter don’t differ significantly (P>0.05).
Table (5): Effect of feeding T-2 toxin alone and in combination with *E.coli* and antioxidant on serum total protein, albumin, globulin and albumin/globulin ratio values Mean (±SE). (n=6)

<table>
<thead>
<tr>
<th>groups</th>
<th>Total protein (g/dL)</th>
<th>Albumin (g/dL)</th>
<th>Globulin (g/dL)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14th day</td>
<td>28th day</td>
<td>14th day</td>
<td>28th day</td>
</tr>
<tr>
<td>Control (commercial diet)</td>
<td>4.92 ±0.10</td>
<td>4.80 ±0.11</td>
<td>2.83 ±0.11</td>
<td>2.56 ±0.15</td>
</tr>
<tr>
<td>Control (commercial diet) + <em>E.coli</em> infection</td>
<td>------</td>
<td>4.20 ±0.12</td>
<td>------</td>
<td>2.29 ±0.11</td>
</tr>
<tr>
<td>Commercial diet + antioxidant preparation</td>
<td>4.93 ±0.17</td>
<td>4.84 ±0.15</td>
<td>2.86 ±0.11</td>
<td>2.52 ±0.15</td>
</tr>
<tr>
<td>Commercial diet + antioxidant preparation + <em>E.coli</em> infection</td>
<td>-------</td>
<td>4.79 ±0.13</td>
<td>-------</td>
<td>2.46 ±0.15</td>
</tr>
<tr>
<td>T2-toxin</td>
<td>3.40 ±0.09</td>
<td>3.86 ±0.15</td>
<td>1.90 ±0.05</td>
<td>2.21 ±0.05</td>
</tr>
<tr>
<td>T2-toxin + <em>E.coli</em> infection</td>
<td>-------</td>
<td>3.11 ±0.10</td>
<td>-------</td>
<td>1.75 ±0.03</td>
</tr>
<tr>
<td>T2-toxin + antioxidant preparation</td>
<td>4.15 ±0.11</td>
<td>4.70 ±0.12</td>
<td>2.35 ±0.10</td>
<td>2.32 ±0.04</td>
</tr>
<tr>
<td>T2-toxin + antioxidant preparation + <em>E.coli</em> infection</td>
<td>-------</td>
<td>4.33 ±0.20</td>
<td>-------</td>
<td>2.11 ±0.05</td>
</tr>
</tbody>
</table>

Means with same superscript letter within a column do not differ significantly (P>0.05)
Table (6): Effect of feeding T-2 toxin alone and in combination with *E.coli* and antioxidant on serum aspartate transaminase, alanine transaminase values. Mean (±SE). (n=6)

<table>
<thead>
<tr>
<th>groups</th>
<th>14&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>28&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>14&lt;sup&gt;th&lt;/sup&gt; day</th>
<th>28&lt;sup&gt;th&lt;/sup&gt; day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (commercial diet)</td>
<td>166.72a ±4.80</td>
<td>165.91a ±3.65</td>
<td>14.93a ±0.76</td>
<td>14.58a ±1.67</td>
</tr>
<tr>
<td>Control (commercial diet) + <em>E.coli</em> infection</td>
<td>-------</td>
<td>158.32b ±2.85</td>
<td>-------</td>
<td>12.90a ±1.1</td>
</tr>
<tr>
<td>commercial diet + antioxidant preparation</td>
<td>167.82a ±11.5</td>
<td>166.92a ±6.15</td>
<td>15.02a ±0.81</td>
<td>14.98a ±1.42</td>
</tr>
<tr>
<td>commercial diet + antioxidant preparation + <em>E.coli</em> infection</td>
<td>-------</td>
<td>162.47b ±3.01</td>
<td>-------</td>
<td>14.25a ±1.5</td>
</tr>
<tr>
<td>T2-toxin</td>
<td>233.07b ±9.41</td>
<td>245.98c ±2.71</td>
<td>22.90b ±1.20</td>
<td>24.36b ±2.07</td>
</tr>
<tr>
<td>T2-toxin + <em>E.coli</em> infection</td>
<td>-------</td>
<td>270.98d ±9.85</td>
<td>-------</td>
<td>26.39b ±0.03</td>
</tr>
<tr>
<td>T2-toxin + antioxidant preparation</td>
<td>180.50a ±12.21</td>
<td>191.71e ±4.52</td>
<td>16.95c ±1.57</td>
<td>17.71c ±1.51</td>
</tr>
<tr>
<td>T2-toxin + antioxidant preparation + <em>E.coli</em> infection</td>
<td>-------</td>
<td>195.95e ±5.11</td>
<td>-------</td>
<td>18.91c ±1.23</td>
</tr>
</tbody>
</table>

Means with same superscript letter within a column do not differ significantly (P>0.05)
Table (7): Effect of feeding T-2 toxin alone and in combination with E.coli and antioxidant on blood urea nitrogen, serum creatinine, uric acid and cholesterol values. Mean (±SE). (n=6)

<table>
<thead>
<tr>
<th>groups</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>Uric acid (mg/dL)</th>
<th>Cholesterol (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14th day</td>
<td>28th day</td>
<td>14th day</td>
<td>28th day</td>
</tr>
<tr>
<td>Control (commercial diet)</td>
<td>7.36±0.63</td>
<td>7.31±0.57</td>
<td>0.23±0.02</td>
<td>0.25±0.03</td>
</tr>
<tr>
<td>Control (commercial diet) + E.coli infection</td>
<td>-----</td>
<td>6.20±0.12</td>
<td>-----</td>
<td>0.29±0.01</td>
</tr>
<tr>
<td>commercial diet + antioxidant preparation</td>
<td>6.93±0.17</td>
<td>7.34±0.15</td>
<td>0.25±0.01</td>
<td>0.26±0.03</td>
</tr>
<tr>
<td>commercial diet + antioxidant preparation + E.coli infection</td>
<td>-----</td>
<td>6.79±0.13</td>
<td>-----</td>
<td>0.27±0.05</td>
</tr>
<tr>
<td>T2-toxin</td>
<td>5.75±0.62</td>
<td>5.55±0.34</td>
<td>0.31±0.02</td>
<td>0.35±0.02</td>
</tr>
<tr>
<td>T2-toxin + E.coli infection</td>
<td>-----</td>
<td>4.95±0.10</td>
<td>-----</td>
<td>0.40±0.03</td>
</tr>
<tr>
<td>T2-toxin + antioxidant preparation</td>
<td>7.15±0.11</td>
<td>6.43±0.12</td>
<td>0.27±0.01</td>
<td>0.30±0.01</td>
</tr>
<tr>
<td>T2-toxin + antioxidant preparation + E.coli infection</td>
<td>-----</td>
<td>5.33±0.20</td>
<td>-----</td>
<td>0.31±0.03</td>
</tr>
</tbody>
</table>

Means with same superscript letter within a column do not differ significantly (P>0.05)
Table (8): Mean ± (SE) stimulation index of lymphocyte proliferation assay.

<table>
<thead>
<tr>
<th>Groups</th>
<th>14th day</th>
<th>28th day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (commercial diet)</td>
<td>1.31 ± 0.02</td>
<td>1.51 ± 0.02</td>
</tr>
<tr>
<td>Control (commercial diet) + E. coli infection</td>
<td>----------</td>
<td>0.94 ± 0.01</td>
</tr>
<tr>
<td>commercial diet + antioxidant preparation</td>
<td>1.57 ± 0.02</td>
<td>1.56 ± 0.01</td>
</tr>
<tr>
<td>commercial diet + antioxidant preparation + E. coli infection</td>
<td>----------</td>
<td>1.01 ± 0.01</td>
</tr>
<tr>
<td>T2-toxin</td>
<td>0.77 ± 0.01</td>
<td>0.88 ± 0.01</td>
</tr>
<tr>
<td>T2-toxin + E. coli infection</td>
<td>----------</td>
<td>0.57 ± 0.01</td>
</tr>
<tr>
<td>T2-toxin + antioxidant preparation</td>
<td>1.26 ± 0.02</td>
<td>1.31 ± 0.02</td>
</tr>
<tr>
<td>T2-toxin + antioxidant preparation + E. coli infection</td>
<td>----------</td>
<td>0.95 ± 0.01</td>
</tr>
</tbody>
</table>

Table (9): Effect of T-2 toxin on Escherichia coli clearance from blood in chicks.

<table>
<thead>
<tr>
<th>groups</th>
<th>Time post infection (log_{10} cfu/mL blood)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 min</td>
</tr>
<tr>
<td>commercial diet + E. coli control</td>
<td>2.87 ± 0.03&quot;</td>
</tr>
<tr>
<td>T2 + E. coli</td>
<td>3.27 ± 0.06&quot;</td>
</tr>
<tr>
<td>T2 + antioxidant + E. coli</td>
<td>2.80 ± 0.04&quot;</td>
</tr>
<tr>
<td>antioxidant + E. coli</td>
<td>2.75 ± 0.05&quot;</td>
</tr>
</tbody>
</table>

Means with same superscript letter within a row do not differ significantly (P>0.05)

Table (10): Counts of viable bacteria in organs of chicks fed diets T-2 toxin

<table>
<thead>
<tr>
<th>groups</th>
<th>spleen</th>
<th>Liver</th>
<th>Lung</th>
</tr>
</thead>
<tbody>
<tr>
<td>commercial diet + E. coli</td>
<td>4.94 ± 0.05&quot;</td>
<td>4.71±0.02&quot;</td>
<td>4.75±0.03&quot;</td>
</tr>
<tr>
<td>T2 + E. coli</td>
<td>5.89 ± 0.06&quot;</td>
<td>5.35±0.03&quot;</td>
<td>4.85 ± 0.04&quot;</td>
</tr>
<tr>
<td>T2 + antioxidant + E. coli</td>
<td>5.12 ± 0.04&quot;</td>
<td>4.97 ± 0.04&quot;</td>
<td>4.35±0.03&quot;</td>
</tr>
<tr>
<td>antioxidant + E. coli</td>
<td>4.85 ± 0.04&quot;</td>
<td>4.78±0.04&quot;</td>
<td>4.35±0.03&quot;</td>
</tr>
</tbody>
</table>

Escherichia coli colonies were logarithmic transformed and expressed as log_{10} CFU/ g tissue
Acknowledgment:

The authors would like to thank Amin A.A. Prof. Animal Breeding and Genetics Dept. of Animal Prod. Fac. of Agri. Suez Canal Univ. Ismailia Egypt, for his statistical analysis helps in the present study.

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المملوكتين الباثولوجية ومناعية للسموم الفطرية في عينة الدم من الدجاج المتزامنة مع عدوى الميكروب القولونى وبعض طرق العلاج المناسبة

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تأممت هذه الدراسة لتقسيم اثر وجود السمنة الفطرية في عينة الدجاج المصاب بالميكروب القولونى (إيزيرشيا المعوية) وذلك من عدة جوانب تشمل (الأداء الصحي، والتغيرات البيوكيميائية، والمناعية، والقدرة على إزالة الميكروب المحسوس واستقرار معدل انتشار الخلايا المفيدة والتغييرات الباثولوجية المحتملة)، ودراسة التأثيرات الواعية الممكنة باستخدام مضاد للاكسدة أوكسينيل بلس. 

تم اجراء الدراسة على عدد 212 كتكوت عمر يوم في جرينتين. في التجربة الأولى اشتتملت على عدد 191 كتكوت قسمت الى مجموعتين، الأولى تم إضافة الميكروب القولوني (إيزيرشيا المعوية) على مقدار 4 x 10⁶ وحدة ميكروبية/مل، وتم تحميل كل مجموعة برديا بمضاد للاكسدة (أوكسينيل بلس) بمعدل 0.5 مل/لتر لترفيه مياه الشرب. 

وفي حين ان المجموعة الأخرى لم تتلق الميكروب القولوني. وقد عشوائية اختار خمسة كتاكيمات من كل مجموعة لتقسيم معدل الأوزان والتغيرات البيوكيميائية والباطولوجية وذلك في عمر 14 يوما، و35 يوما. أما في التجربة الثانية، فقد اجريت على 24 كتكوت عمر يوم قسمت بشكل عشوائي إلى 4 مجموعات (مضايقة، وعليفة تحتوي على 0.5 مل الميكروب القولوني، وعليفة تحتوي على 0.5 مل الميكروب القولوني مع مضاد للاكسدة، وعليفة تحتوي على 0.5 مل الميكروب القولوني مع مضاد للاكسدة وأوكسينيل بلس، وعليفة تحتوي على 0.5 مل الميكروب القولوني مع مضاد للاكسدة وأوكسينيل بلس وماء). 

وقد أظهرت النتائج في حالة تزامن العدوى مع السمنة الفطرية تأثير ملمؤن مع ارتفاع ووحيد في اعداد السمنة الفطرية وانخفاض وزن الجسم وانخفاض كمية الغذاء أكثر وضوءا في حالة تزامن العدوى مع السمنة الفطرية.

وقد لوحظ زيادة في حالات العدوى في جرينتين منزليتين للأسئلة، إميتيونافانيرجيز والثنين ترانسارفيز و البولياك وحمض البولياك، والكراتين وانخفاض مستويات مجمعة البروتينات الكلية، والزنال، الجلوبولين والكوليستيرول. كما لوحظت معدل تكاثر وانتشار الخلايا المفيدة وتأخير القدرة على إزالة
وتصفية الميكروبات المحفونة (الميكروب القولوني) مع زيادة أعداد الوحدات الميكروبية الموجودة لكل سم من الدم أو جرام من الأنسجة.

ومع زيادة أعداد الوحدات الميكروبية الموجودة لكل سم من الدم أو جرام من الأنسجة. كما وجد أن الإصابة بالميكروب القولوني وحده أو بالاشتراك مع ت2 قد أثر سلبياً على جميع المعايير البيوكيميائية وعدد تكاثر وانتشار الخلايا المهاوية. وان وجود ت2 ولو حتى في الحد الادنى له يمكن أن يؤدي إلى انخفاض مناعة الطيور ويجعلها غير قادرة على مقاومة بعض الأمراض. وبالإضافة إلى ذلك، سوف يزيد عدد الطيور الحاملة للعدوى مما يؤدي إلى زيادة أعداد الميكروبات. وبالرغم من أن ت2 كان له تأثيرات تطعتية ونخزية ألامية في كل من الكبد والكليتين في الدجاج، مع افات مختلفة في الأعضاء الداخلية الأخرى بالإضافة إلى نضوب الخلايا المحسودة ونخر النسيج المهاوي. هذه الافات قد زادت حدتها مع تزامن العدوى بالميكروب القولوني وان إضافة مضاد الأكسدة توكسيبيل بلس كان له مربع إيجابي في تحسن معدل التغذية والآثار السلبية المناعية والبيوكيميائية والباثولوجية المرتبة على تناول ت2 في العليقة. لذا فإن أعراض الدواجن تحتاج إلى فحص دوري لوجود السموم الفطرية في ظل الظروف الحالية.