

SOME IMMUNOSUPPRESSIVE EFFECT OF T-2 TOXIN IN BROILER CHICKS

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(Received February 15, 2005; Accepted July 3, 2005)

ABSTRACT

Male broiler 1-day-old chicks were fed T-2 toxin at a rate of 8 mg/kg for 3 weeks, to determine its effect on total leukocyte count; differential and absolute leukocyte count; stress ratio (Heterophils / Lymphocytes), (H/L); serum albumin and globulin's (α, β, δ) levels ; bursal weight index (BWI); bursal diameter index (BDI); Thymus length index (TDI); histological changes in both bursa of Fabricius and thymus. The results show that T-2 toxin had a significant negative effect on all of the above mentioned parameters, with cortical thinning effect on both bursa of Fabricius and thymus.

بعض جوانب التثبيط المناعي الذي يحدثه سم T-2 في أفراخ فروج اللحم

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الخلاصة

أعطيت ذكور فروج اللحم سم T-2 بمستوى 8 ملغم /كغم عليقة ولمدة 3 أسابيع لقياس تأثير السم على معدل اوزان الأفراخ ، العد الكلي لكريات الدم البيضاء ، النسبة المئوية والمطلقة لكريات الدم اللمفية والعدلة ، معامل الكرب (الخلايا العدلة / الخلايا اللمفية) قياس مستوى الالبومين والكلوبيولينات (ألفا ، بيتا ، كاما) معامل وزن وقطر غدة فابريشيا، معامل طول غدة الثايموس ،التغيرات النسيجية على هاتين الغدتين. أوضحت النتائج ان لسم T-2 تأثير معنوي سلبي على كل القياسات المدروسة وكذلك تأثير نسجي تمثل بضمور طبقة القشرة لكل من غدة فابريشيا والثايموس.

INTRODUCTION

The trichothecenes are group of sesquiterpenoides that produced mostly by members of the Fusarium genus (1). These mycotoxins include some of the most potent protein synthesis inhibitors known (2). There are about 148 trichothecenes isolated, but information on their occurrence in feed stuffs are Limited to the most frequent isolated vomitoxin, nivalenol, diacetoxyscirpenol, T-2 toxin and fumonisin (3). Trichothecenes have been implicated as causative agents in numerous episodes of fatal human and animal toxicosis (4, 5, and 6). Acute exposure to trichothecenes results in severe damage to actively dividing cells in tissues Such as bone marrow, lymph nodes, spleen, thymus and intestinal mucosa (7). There is substantial evidence that trichothecenes can have broad effect in host resistance, cellular function and humeral immunity (7).

T-2 toxin (type A trichothecenes) which is produced by many fusarium species (1) was the first trichothecene investigated for its toxicological properties as it may cause severe acute intoxication (8).

Experimentally, repeated intraperitoneal injection of animals with T-2 toxin results in markedly increased susceptibility to *candida* (9), *cryptococcus* (10), *Listeria* (11) and *mycobacterium* (12), increased susceptibility to acute herpes simplex virus type 1 (13).

T-2 toxin impairment of host resistance is dramatically illustrated by the observation that concurrent oral exposure to T-2 toxin lowers the oral LD₅₀ of *Salmonella typhimurium* from 5×10^6 to 5×10^0 organisms per mouse (14). It was also reported that T-2 toxin treated animals were exhibited close-dependant decrease in B and T cell mitogen responses (13,15 and 16).

The objective of this experiment was to study some of the immunosuppressive parameters in broiler chicks fed T-2 toxin.

MATERIALS AND METHODS

Forty day-old male broiler chicks were obtained from a Local commercial hatchery. The chicks were weighed and wing banded. They were randomly assigned and affixed to the wing. The chickens were maintained in electrically heated batteries under continuous fluorescent lighting with feed and water available *ad libitum*. The experimental design consisted of two dietary treatments :

1. Control with 0 mg T-2 kg⁻¹ diet .
2. T-2 toxin 8 mg kg⁻¹ diet .

There were 2 replicates of 10 broilers per dietary treatment and the broilers were maintained on these treatments to 3 wks of age. The chicks were fed a commercial, non-medicated, corn soybean meal basal diet which contained or exceeded the levels of critical nutrients recommended by the National Research Council (1984), (17) . The toxin; T-2 toxin (Kindly provided by the scientific research Laboratory of Ykraianian veterinary Institute , Ykraiania) was incorporated into the basal diet by dissolving the toxin in 95% ethanol and then mixing the appropriate quantities with 1 kg of the diet.

After drying, the dissolved toxin was mixed with the rest of the basal diet to produce the treatments containing T-2 toxin.

When the chicks reached 3 wks of age, trial was terminated .The live body weight was recorded.

Ten birds (5 from each replicate) of the two treatments were bled by cardiac puncture for hematological and serum biochemical analysis.

Total, differential and absolute leukocyte counts; serum total protein, albumin and globulin's were measured according to Coles (1986), (18).

The birds were then killed by cervical dislocation and the bursa of Fabricieous, thymus was weighed and their diameter was measured. Shank length and diameter were measured using a micrometer.

Measurement of immuno-suppressant parameters induced by feeding T-2 toxin to broiler chicks was conducted by using the formulas referred by Davison *et. al.*, 1986 (19).

$$1\text{-Bursal weight index (BM1)} = \frac{\text{Normal bursal weight}}{\text{Body weight ratio}} \times 1000$$

$$2\text{-Bursal diameter index (BDI)} = \frac{\text{Bursal diameter}}{\text{Shank diameter}} \times 10$$

$$3\text{- Thymus diameter index (TDI)} = \frac{\text{Thymus diameter}}{\text{Shank length}} \times 10$$

Samples for bursal and thymus histological examination were taken from two chicks (one chick from each replicate) immediately after cervical killing and were placed in 10% neutral buffered formalin. Paraffin sections were cut at 5μ and stained with hematoxylin and eosin.

Statistical analysis was performed using student's-t test with significance level of $P < 0.05$, (20).

RESULTS

Leukocyte counts:

Total, differential and absolute leukocyte counts (lymphocytes and heterophils) are represented in Table 1.

Table 1: Total leukocyte counts; differential and absolute (lymphocyte and heterophil) counts in chicks fed 8mg T-2 toxin kg⁻¹ feed.

Treatment group	Total leukocyte count 10 ³ /mm ³	Lymphocyte and Heterophils differential leukocyte counts %		Lymphocyte and Heterophils absolute leukocyte counts 10 ³ /mm ³		Stress ratio H/L
		Lymphocytes	Heterophils	Lymphocytes	Heterophils	
Control	28.7±1.08	73.2 ±1.55	21.9 ±2.64	21.0± 0.98	6.2 ±0.86	0.29
T-2	14.9±0.98*	65.8 ±3.31*	23.7± 0.56	9.8 ±0.62*	3.5 ±0.25*	0.35*

1- Values representing mean of 5 birds of replicate

*Significant at level of $P < 0.05$

From this table it is evident that 8 mg T-2 toxin kg⁻¹ feed induced leucopenia, since T-2 toxin was resulted in a significant ($P < 0.05$) reduction in total leukocyte counts when compared with control group .

It is also evident that leucopenia was merle reflection of lymphopenia, which was truly expressed by the significant ($P < 0.05$) reduction of the absolute number of lymphocytes compared with control group.

There were no significant changes in the remaining leukocyte types during T-2 feeding (not included in the table) when compared with control group.

Serum proteins:

The biuret method for determining total serum proteins revealed that feeding 8 mg T-2 toxin kg⁻¹ feed to broiler chicks was significantly (p < 0.05) reduced serum proteins. This reduction was mostly evident with globulin rather than albumin (Table 2).

Table 2: Serum proteins in chicks fed 8mg T-2 toxin kg⁻¹ feed

Treatment group	Serum proteins gm/dl					Total
	Serum total proteins	Albumin	Globulin's			
			α	β	γ	
Control	4.9±0.70	2.6±0.65	0.4±0.003	0.5±0.002	1.2±0.008	2.2±0.11
T-2	3.0±0.49*	1.1±0.48*	0.2±0.002*	0.4±0.002*	1.0±0.006*	1.8±0.09

1- Values representing mean of 5 birds of replicate

*Significant at level of P < 0.05

Immuno-suppressive parameters:

Feeding 8 mg T-2 toxin kg⁻¹ feed to broiler chicks for 3 wks was effective in significant (P < 0.05) reduction of live body weight compared with control chicks. The relative weights of lymphoid tissues (bursa of Fabricious and thymus) were also significantly (P < 0.05) reduced by feeding this toxin, lengths of these organs and the length of shanks were also affected by feeding the toxin (Table 3).

Table 3. Live body weight, bursal, thymus and shank measurements of chicks fed 8 mg T-2 toxin kg⁻¹ feed¹.

Treatment group	Live body weight (g)	Relative bursal weight (g)	Absolute organs diameter (cm)		Shank measurements(cm)	
			Bursa	Thymus	length	Diameter
Control	473.4±19.14	1.66±0.02	0.1±0.008	0.4±0.003	3.1±0.2	0.6±0.005
T-2	3.09±26.05*	0.67±0.04	0.003±0.008*	0.1±0.002*	2.3±0.9*	0.2±0.01*

1- Values representing mean of 5 birds of replicate

*Significant at level (P < 0.05).

Histological examination:

Lymphocyte atrophy of bursal and thinning of thymus cortical layer through lymphocytic depletion was evident in chicks fed 8 mg T-2 toxin kg⁻¹ feed when compared with control group (Figure 2 and 3)

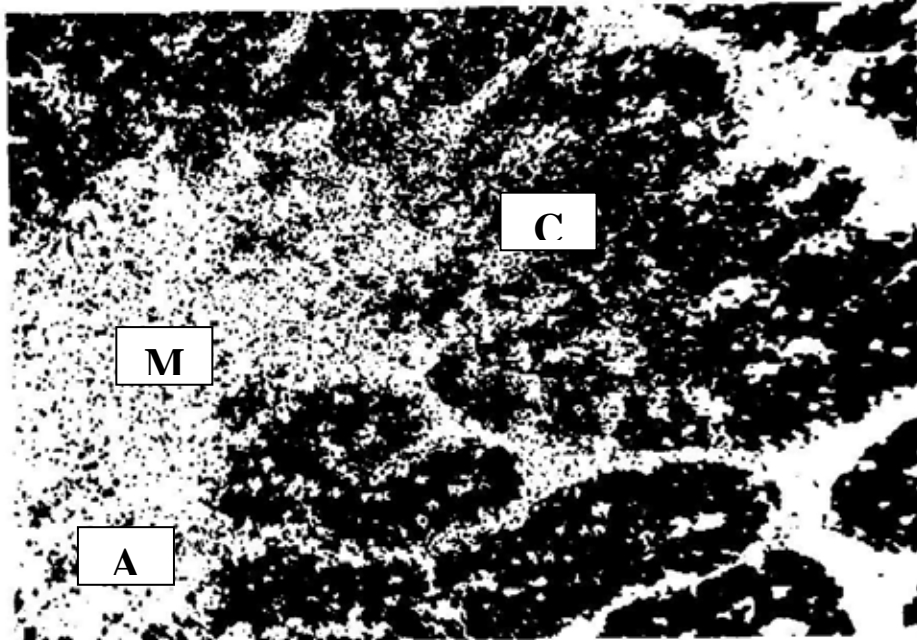


Figure 1: BW1, BDI and TDI of chicks fed 8 mg T-2 toxin kg⁻¹ feed

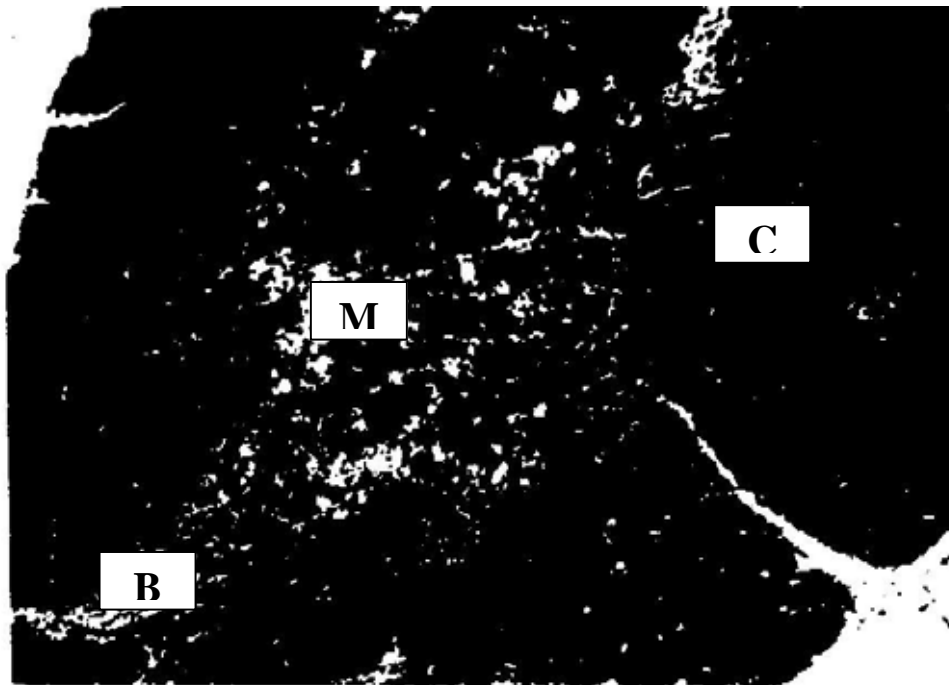


Figure2: photomicrographs of the thymus of broiler chick showing (A) cortical thinning in chick fed a diet containing 8 mg T-2 toxin kg⁻¹ feed (B) Normal cortical thickness in chick fed a control diet. HE stains (X180). C = cortex ; M = medulla

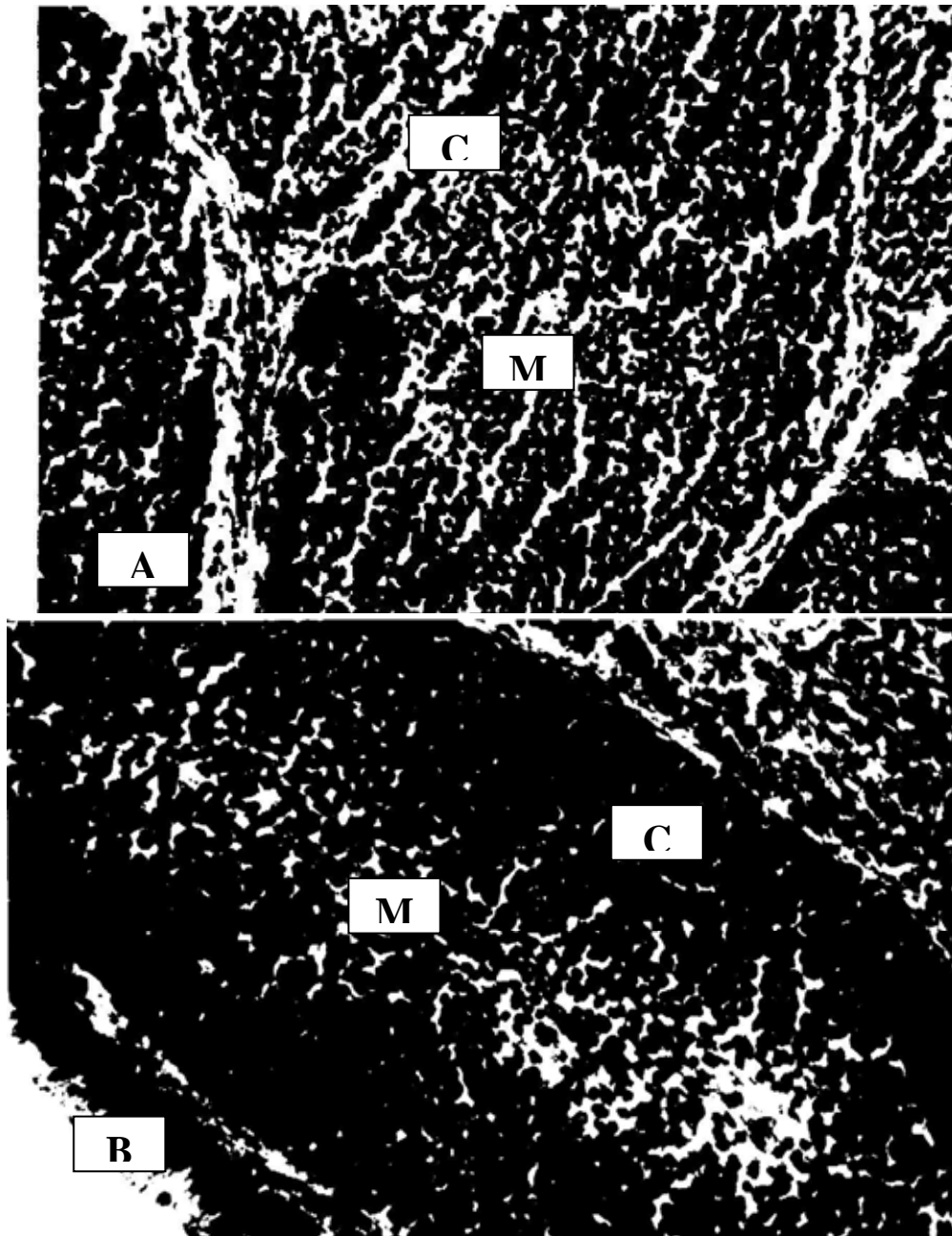


Figure 3: photomicrographs of the bursa of Fabricieous of broiler chicks showing (A) cortical thinning in chicks fed T-2 toxin kg ⁻¹ feed (B) Normal cortical thickens in chick fed control diet. HE stains (X180). C = cortex; M = medulla

DSCUSSION

Data of the current study confirms the immunosuppressive effect of T-2 toxin in broiler chicks. The first confirmation was evident through the redaction of total Leukocyte counts (leucopenia). Although it was not apanleucopenia, but precisely was aLymphocytic leucopenia (Redaction in the absolute number of lymphocytes) . This finding was also observed by previous studies (5, 21, and 22). The second prove

was came from the decreased lymphoid organs weights and sizes (burs a and thymus) . Reduction in lymphoid organ weights was supported by the histological lymphocytic depletion of these organs through lymphocytes depletion of their cortical layers. The cortical atrophy of lymphoid organs due to ingestion of T-2 toxin was also referred by others (2, 5, 21, and 23), who stated that T-2 toxin could induce reduction in bursal and thymal relative weights due to the severely damaged active dividing cells of these lymphoid tissues . This suggestion was supported by Cyongyossy-Issa and khachatourians (1984 and 1985), (24 and 25), who considered T-2 toxin as one of the most potent protein synthesis inhibitors, through the determination of T-2 toxin concentration required to Inhibit nucleic acid and protein synthesis in the murine lymphocyte which was approximately 1×10^5 molecules / cell and that this correlates to members of putative T-2 receptors estimated in accumulation studies. Another Support came from will (1988), (26) Who has determined that uptake of T-2 toxin is inhibited by anisomycin, an antibiotic that binds to ribosome's, suggesting that T-2 toxin accumulation may actually be a ribosomal –dependent event. Moreover, the third suggestion of immune suppressive effect of T-2 toxin in broiler chicks was clearly expressed in the studied, BW1, BD1 and TD1 indexes.

All these parameters were lower in T-2 treated chicks compared with control group. Normal value of these indexes is ranged from 2-4 for BW1 , 0.9-1.5 for BD1 and 1.6-2.0 for TD1 (19). The immunosuppressive effect of T-2 toxin was also referred by (27, 28, and 29), who stated that this toxin and other fusarium toxin could induce immune suppression in chicks.

The above mentioned results could give an answer to the reduced capacity of the reticuloendothelial system and suggest that feeding T-2 increased susceptibility of animals to infections with *mycobacterium bovis* , *Salmonella typhimurium* , *staphylococcus aureus* and *herpes simplex* type 1 (31). These results also could give an explanation to the effect of 8 mg T-2 kg-1 feed in reducing the viability or phagocytic activity (32). So, our finding imply that chicks fed T-2 toxin could be more susceptible to infectious diseases, resulting from dysfunction of mononuclear phagocytic system.

The fourth findings support the immumosuppression of T-2 toxin in this study, was the significant suppression in the total serum proteins and globulin's. These effects could be attributed to malabsorption syndrome caused by this toxin (18, and 21) and in the same time support the immunosuppressive effect of this toxin to the humeral immunity (30). Since, antibodies may migrate in both the beta and gamma globulin ranges IgG migrate primarily in the gamma globulin range, where as IgM migrate in the beta globulin (18).

In addition to all mentioned above, commercial poultry production constantly exposed to a variety of stresses that could adversely affect the immune system. Consumption of T-2 toxin together with these stresses may put the immune system at an added risk. In addition, a typical poultry ration is made up of several grain sources, each of which may be contaminated with a different mycotoxin or more than one mycotoxin. Thus, other mycotoxins in addition to T-2 toxin may be present simultaneously in a poultry ration. Toxicity of T-2 toxin has been shown to be enhanced when it present with other mycotoxins as Co – contaminants in feeds, like fumonisin (33) and aflatoxins (34) and in this context, T-2 toxin could be a potential concern to broiler industry and represent serious health risk to broilers.

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