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# Effect of T-2 toxin on haematological and serum biochemical parameters and immune response status in turkey poults

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## ABSTRACT

*Babu Prasath, N., Rao, Ganne Venkata Sudhakar, Balachandran, C. and Murali Manohar, B. (2009). Effect of T-2 toxin on haematological and serum biochemical parameters and immune response status in turkey poults. Indian J. Vet. Pathol., 33(2): 173-176.*

The present work was undertaken to study changes in certain haematological and serum biochemical parameters and immune response in T-2 toxin fed turkey poults. Known amount of T-2 toxin containing powdered wheat culture were incorporated into the toxin free turkey prestarter mash to yield 1 and 3 ppm T-2 toxin. Thirty newly hatched Beltsville Small White turkey poults were randomly allotted to three groups of 10 poults each (0, 1 and 3 ppm) and fed toxin mixed diets from day 1 to 28 days of age. PCV and Hb levels decreased significantly ($P<0.05$). Serum glucose level increased and lipid profiles decreased significantly ($P<0.05$) in toxin fed poults. Serum AST, ALT and ALP values increased significantly ($P<0.05$) in T-2 toxin groups, when compared to control. The mean ± SE ELISA titres were 4.883 ± 0.25, 4.568 ± 0.31 and 3.099 ± 0.15 and stimulation index 0.448 ± 0.10, 0.315 ± 0.27 and -0.195 ± 0.10 in the control, 1 ppm and 3 ppm T-2 toxin groups, respectively. Thus T-2 toxin was found to cause anaemia, hyperglycaemia, hypolipidaemia, elevation of serum AST, ALT and ALP levels and exert immunosuppressive effects in turkey poults.

**Keywords:** Cell-mediated immunity, haematology, humoral immunity, serum biochemistry, T-2 toxin, turkey poults



## INTRODUCTION

T-2 toxin is 3-α-hydroxy-4-β, 15 diacetoxy-8α-isovaleroxy-12, 13-epoxy-trichothec-9-ene type A non-macrocyclic trichothecene produced by *Fusarium sporotrichioides*. In poultry, mycotoxicosis reduces growth rate, lowers feed efficiency, impairs immunity, predisposes to infectious diseases, produces lesions in many organs and causes death[8]. Feeding day-old hybrid male turkey poults fed *Fusarium* mycotoxins contaminated feed for four weeks showed reduction in PCV and Hb but increased after eight weeks[6]. Gounalan *et al*.[11] reported that feeding 0.5 ppm T-2 toxin to layer chicks caused hypoproteinaemia, hypoalbuminaemia, hypoglobu-linaemia, hypoglycaemia, hypertri-glyceridaemia, hypocholesterolaemia, hypocalcaemia, hypopho-sphataemia, hyponatraemia, hypokalaemia and hypolipidaemia (HDL and LDL) and increase in the AST, ALT, ALP, creatinine and uric acid levels in layer chicks. There was scanty literature on the effects of T-2 toxin in turkey poults. Hence, the present work was undertaken to study the haematobiochemical and immunological changes in turkey poults exposed to T-2 toxin at different dose levels.

## MATERIALS AND METHODS

T-2 toxin was produced in wheat substrate using *Fusarium sporotrichioides var sporotrichoides* MTTC 1894[4] and quatified by using thin layer chromatography[23]. Known amount of T-2 toxin containing wheat material were incorporated in the turkey prestarter mash to yield 1 and 3 ppm T-2 toxin. Thirty newly hatched, unsexed, Beltsville Small White turkey poults procured from Poultry Research Station, Nandanam, TANUVAS, Chennai were wing banded, weighed and housed in battery brooders with *ad libitum* supply of feed and water. They were randomly allotted to three groups of 10 birds each. The control (0 ppm) and toxin mixed diets, containing 1 and 3 ppm T-2 toxin, were fed to different groups for 28 days from the day of hatch. All poults were vaccinated with Newcastle disease vaccine D58 strain by oculonasal route on the 11th day of age. Vaccine was procured from Department of Veterinary Microbiology, Madras Veterinary College, Chennai, Tamil Nadu.

**Haematology:** Blood samples were collected by intracardiac puncture in Heller and Paul anticoagulant mixture on 28th day of experiment. Haematological studies were conducted to determine the haemoglobin (Hb) by acid haematin method and packed cell volume (PCV) by microhaematocrit method[7].

**Serum biochemistry:** Samples of blood collected in test tubes were allowed to clot and centrifuged at 3000 rpm for 30 min to separate sera. Serum total protein and albumin were estimated by modified Biuret and Dumas method, glucose by glucose oxidase method, total cholesterol (TC) by CHOD/POD (Cholesterol dehydrogenase/peroxidase) method, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) by IFCC (International Federation of Clinical Chemistry) method, blood urea nitrogen (BUN) by glutamate dehydrogenase (GLDH)

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method, creatinine by Jaffe's kinetic method and triglycerides (TG) by colorimetric enzymatic method[5] by using semiauto analyzer (MISPA Excel) and VLDL using the formula TG/5, LDL using the formula (TC-HDL-VLDL) and TC/HDL.

**Humoral immunity:** Sera were separated from all 30 poults on 28th day of age to estimate ELISA titre against Newcastle disease virus.

**Cell mediated immunity:** Cell mediated immunity to NDV was measured by using colorimetric blastogenesis assay in spleen at the end of fourth week of age[21]. The formazan crystals formed after 4 hr incubation of cells with MTT were dissolved by adding 150 µL of dimethylsulphoxide (DMSO) to each well. The mean optical density was read on an ELISA reader. The blastogenic responses for the MTT assay were expressed as a mean stimulation index (SI) by dividing mean absorbance of stimulated culture (Cs) minus mean absorbance of unstimulated culture (Cu) by mean absorbance of unstimulated culture.

**Statistical analysis:** The data generated from different parameters of the experimental study were subjected to one way analysis of variance (ANOVA) by using SPSS software version 10 for windows.



## RESULTS AND DISCUSSION

**Haematology:** No significant differences were observed between the T-2 toxin treated groups. Significant (P<0.05) decrease in the PCV and Hb values were observed in the T-2 toxin treated birds when compared to the control group indicating anaemia (Fig. 1). Similar observations were made in turkey poults fed diet containing *Fusarium* mycotoxins for four weeks[6]. Marginally reduced feed consumption and alimentary tract lesions affecting digestion and absorption of nutrients observed in this study might have contributed to anaemia at 3 ppm level. Besides, it was postulated that the anaemia observed in T-2 toxicosis could be due to its direct cytotoxicity to erythropoietic marrow or inhibition of uptake of iron into the erythropoietic cells[9].

The AST, ALT and ALP values did not differ significantly between the control and 1 ppm T-2 toxin fed groups and 1 and 3 ppm T-2 toxin treated groups for ALT and ALP. There was significant (P<0.05) increase in the AST, ALT and ALP in 3 ppm toxin fed group when compared to the control and 1 ppm T-2 toxin fed groups (Fig. 2-4). The effect was dose dependent. This agreed with the findings of Ogunbo *et al.*[20] who reported a significant increase in the AST level in turkey poults. No comparable reports were available for ALT and ALP in turkey poults for T-2 toxicosis. However, increased levels of ALT and ALP were reported in Japanese quails fed 1, 2 and 3 ppm T-2 toxin[1], layer chicken fed 0.5 ppm[16] and broiler chicken fed 0.5 ppm[12]. The elevated levels of AST and ALT corroborated with the hepatic, skeletal and cardiac muscle degeneration observed in this study.

Mean (± SE) BUN, serum creatinine and uric acid values (mg/dL) of turkey poults fed T-2 toxin were 7.40 ± 0.24, 6.81 ± 0.29 and 6.93 ± 0.28 for BUN, $0.54^{a}$ ± 0.00, $0.35^{b}$ ± 0.02 and $0.35^{b}$ ± 0.01 for creatinine and 5.83 ± 0.59, 4.64 ± 0.16 and 5.57 ± 0.37 mg/dL for uric acid for 0, 1 and 3 ppm levels respectively. No significant differences were observed between the control and T-2 toxin fed groups for BUN and uric acid levels. The creatinine levels differed significantly (P<0.05) between the control and T-2 toxin treated groups. Significant (P<0.05) decrease in the creatinine level was observed in the T-2 toxin treated groups when compared to the control group. Madheswaran *et al.*[19] did not find any significant changes in the BUN, creatinine and uric acid levels in Japanese quail fed 4 ppm T-2 toxin for 35 days. However, feeding 1, 2 and 3 ppm T-2 toxin to Japanese quail significantly increased the creatinine in 3 ppm and uric acid in 2 and 3 ppm fed birds[2]. BUN and creatinine are more specific and sensitive indicator of renal function. The observation of significant decrease in creatinine and no change in the BUN levels corroborated with the muscular dystrophy found in the present study.

Feeding 1 and 3 ppm T-2 toxin in turkey poults for four weeks caused no significant difference in the serum proteins between the control and T-2 toxin fed groups (data not shown). The lack of T-2 toxin effect on serum total protein, albumin and globulin is in contrast to several reports indicating hypoproteinaemia, hypoalbuminaemia and hypoglobulinaemia in broilers fed 1 ppm of T-2 toxin for 28 days[12] and Japanese quails fed 1, 2 and 3 ppm of T-2 toxin for six weeks[2]. Liver, being the major organ of protein synthesis, was not significantly affected to produce a change in the total protein at the dose level up to 3 ppm used in this study.

Feeding 1 and 3 ppm T-2 toxin in turkey poults from 0 to 28 days of age significantly increased the serum glucose level in 1 ppm toxin fed group (211.77±6.85 mg/dL) but not at 3 ppm. No comparable reports were available in turkey poults for T-2 toxicosis. However, reduction in the serum glucose level was reported in broiler chicken fed 6 ppm[17,18] and 1 ppm[12] of T-2 toxin for 21 and 28 days, respectively.

The study revealed decrease in the serum total cholesterol, HDL, VLDL, LDL and triglycerides in turkey poults fed 1 and 3 ppm T-2 toxin for four weeks (Fig. 5,6). Though no comparable studies were available, Madheswaran *et al.*[19] reported hypocholesterolaemia in Japanese quail fed 4 ppm T-2 toxin for 35 days. Balachandran *et al.*[3] reported similar changes in lipid

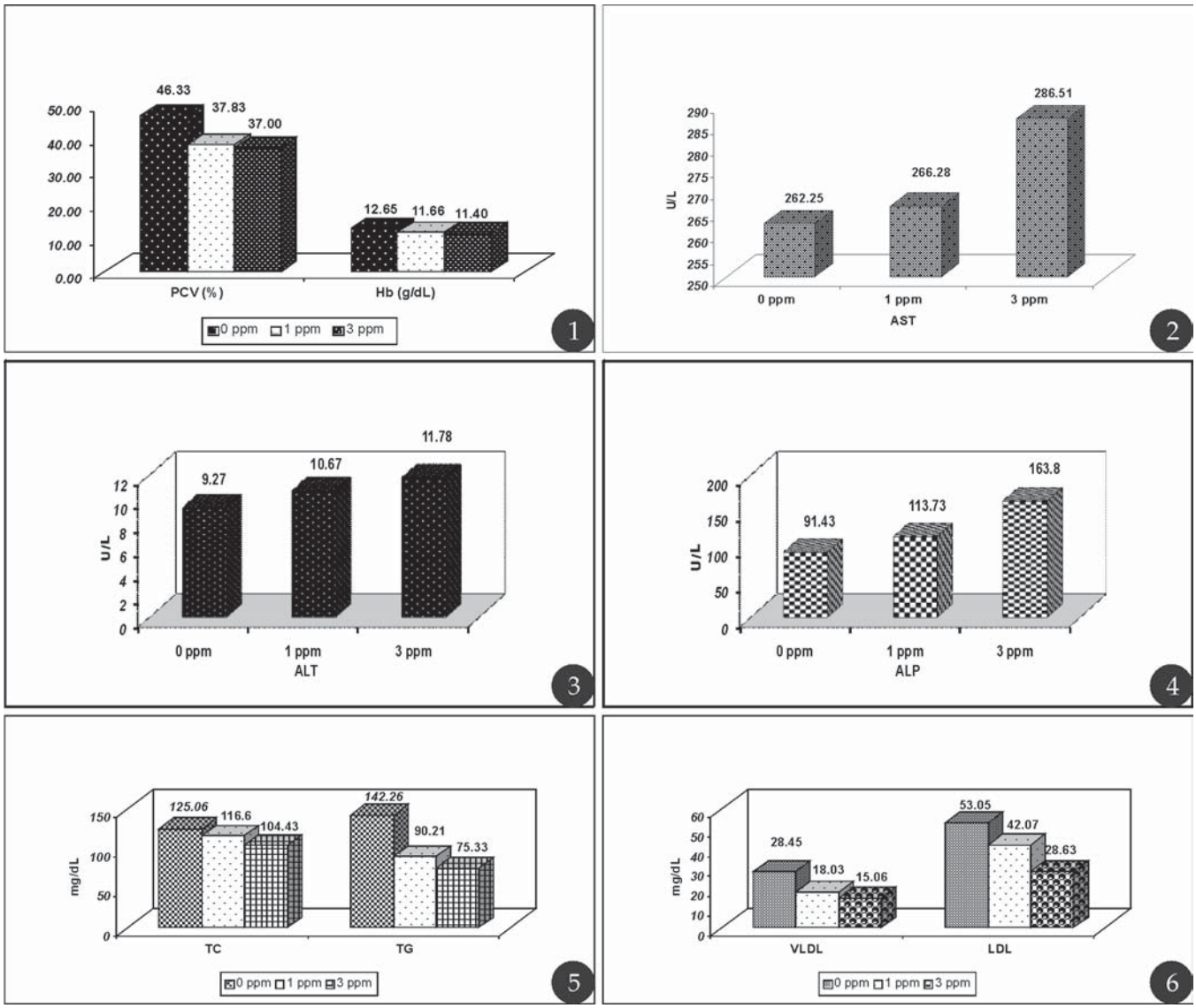


**Fig.1.** Effect of T-2 toxin on PCV and Hb values in turkeys poults. **Fig. 2.** Effect of T-2 toxin on the serum AST levels in turkeys poults. **Fig. 3.** Effect of T-2 toxin on the serum ALT levels in turkeys poults. **Fig. 4.** Effect of T-2 toxin on the serum ALP levels in turkeys poults. **Fig. 5.** Effect of T-2 toxin on the serum total cholesterol and triglyceride levels in turkeys poults. **Fig. 6.** Effect of T-2 toxin on the serum VLDL and LDL levels in turkeys poults.

profile of broiler chicks fed 1 ppm T-2 toxin for 28 days. Similar observations were also made by Gounalan *et al.*[11] in layer chicken fed 0.5 ppm T-2 toxin except for TG. Arun Prasath *et al*.[2] also found similar changes in lipid profile of Japanese quails fed 1, 2 and 3 ppm T-2 toxin for six weeks. Circulating lipids are derived from intestinal absorption of fat, hepatic synthesis and mobilization of fat from fat depots. The dietary lipids absorbed in the intestine enter via portal vein as VLDL. Decrease in serum cholesterol and total lipids occurs because of decreased intestinal absorption due to enteritis or decreased hepatogenic lipogenic activity[7,14]. Hence, decreased levels of serum lipid observed in this study could be ascribed to enteritis and hepatic damage.

The respective overall mean (± SE) ELISA titre against NDV for control, 1 ppm and 3 ppm T-2 toxin were $4.883^{a}$ ± 0.25, $4.568^{a}$ ± 0.31 and $3.099^{b}$ ± 0.15. Comparison of means revealed no significant difference between the control and 1 ppm T-2 toxin treated groups. However, there was significant ($P<0.05$) decrease in the ELISA titre to NDV in 3 ppm T-2 toxin treated group**.** This agreed with the findings of Sklan *et al*.[22] who did not observe reduction in antibody titre to NDV in turkey poults fed 1 ppm T-2 toxin for 32 days. However, significant decrease in the HI titre against NDV was also reported in 0.5 and 1 ppm T-2 toxin fed broiler chicks[13, 15] and 1, 2 and 3 ppm T-2 toxin fed Japanese quails[1]. The mean (±

SE) SI were $0.448^{a} \pm 0.10$, $0.315^{a} \pm 0.27$ and $-0.195^{b} \pm 0.10$ for 0, 1 and 3 ppm levels, respectively.

A significant reduction in lymphocyte stimulation index was observed in 3 ppm T-2 toxin fed birds when compared to the control and 1 ppm T-2 toxin fed turkey poults. Though, no comparable literature was available on such studies in turkeys, similar findings were reported in broiler chicks[13, 15] and 1, 2 and 3 ppm T-2 toxin fed Japanese quails[1].

T-2 mycotoxin is extremely toxic to leucocytes and other rapidly dividing cells resulting in *in vivo* and *in vitro* immunosuppressing effects. Indeed, lymphocytes are more sensitive to T-2 toxin than other cell types and either DNA or protein synthesis inhibition were sensitive endpoints in cell systems when compared to general cytotoxicity[10] and therefore the capacity of the mitochondrial enzyme succinate dehydrogenase to transform the tetrazolium salt of MTT into blue coloured formazan is inhibited. Hence, significant depression in the cell mediated immunity observed in this study could be due to functional impairment of splenocytes and inhibition of mitochondrial enzyme synthesis at 3 ppm T-2 toxin level.

Thus T-2 toxin was found to cause anaemia, hyperglycaemia, hypolipidaemia and elevation of serum AST, ALT and ALP levels in turkey poults. The present study indicated that T-2 toxin at 3 ppm level affected both humoral and cell mediated immunity which might result in vaccine failures and predispose the birds to various infectious diseases.



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