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Effect of T- 2 toxin on pathological alterations in broiler chicks

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The fungal contamination of feedstuffs occurs at various stages of production, harvesting, handling, processing and storage (Anjum *et al*. 2011). T–2 is a highly toxic trichothecene mycotoxin produced by different *Fusarium* species, mainly *Fusarium sporotrichoides* and to a lesser extent by *F. poae* (Pozzo *et al*. 2013). The effects of T-2 toxins in poultry are quite pronounced in young broiler chicks poor growth rate, poor feed efficiency and immunosuppression thereby resulting in economic losses to the poultry farmers (Indresh and Umakantha 2013). Hence, an attempt was made in this study to evaluate the effect of T-2 toxin on performance, haemato biochemical parameters and pathological changes in broiler chicks.

The experimental trials were approved by the Institutional Animal Ethics Committee, and conducted under its guidelines at the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Jabalpur, Madhya Pradesh. T-2 were produced in crushed maize using the pure culture of *Fusarium sporotrichioides* var *sporotrichioides* (MTCC No, 1894, Strain 3) (Source: Microbial Type Culture Collection and Gene Bank, IMT, Chandigarh, 160036, India) and incubated for 21 days. T-2 toxin was extracted as per Pande *et al*. (2006) and quantified by thin layer chromatography (TLC;Tapia 1985).

Basal diet was formulated and compound to meet the nutrient requirements of toxin free and mycotoxin binder free pre starter and starter ration were used as standard diet. The chicks were kept on prestarter ration having 22.5% crude protein (CP) and 3,000 MEkcal/kg of diet from day 1 to 7 and starter feed having 21% crude protein and 3,100 MEkcal/kg of diet from day 8 to 28. Chicks were provided *ad lib.* feed and water throughout the study. To this basal diet, required quantities of contaminated culture materials were added to attain dietary levels of 4.0 ppm of T-2 toxin containing diets. Feeding of test diets commenced at first day of age and continued till the termination of experiment at 28 days of age.

Apparently healthy, unsexed commercial 36 broiler chicks, day-old were wing banded, weighed individually and housed in cages. All the birds were reared and maintained standard managemental condition with *ad lib.* supply of feed and water for 28 days. Birds were randomly divided into 2 groups of 18 chicks each i.e. control (T-2, 0.0 ppm) and experimental group (T-2, 4.0 ppm). Feed consumption for each birds was measured daily by the difference between the daily feed supplied and refusal, and live weight changes of birds were taken weekly throughout the experimental period. In both the groups 6 birds were sacrificed by the cervical dislocation method on day 14th, 21st and 28th, same time blood samples were collected in sterile vials with and without anticoagulants for complete blood count and serum biochemistry (Calnek *et al*. 1997). For observing the gross and microscopic pathology detailed necropsy examination was conducted and representative pieces of tissue from lung, liver, heart, kidney, spleen, thymus, bursa, crop were collected, fixed in 10% buffer formalin solution and were processed and stained by the standard technique (Gridley 1960).

The experimental data were analyzed statistically by using Duncan's multiple range tests using Hierarchical design (Snedecor and Cochran 1994).

The data of effect of dietary treatments on growth performance, hematological and biochemical parameters are presented in Tables 1–3.

Growth performance: Dietary T-2 produced lower body weight and feed consumption significantly ($P \leq 0.05$) by third week, when compared to control group of chicks, similar findings were recorded in T-2 toxicities by different scientists (Nesic *et al*. 2011, Manafi *et al*. 2012). This growth depression effect of T-2 appears to have primarily resulted by their inhibitory action on protein synthesis and nutrient utilization.

Experimental group birds showed significantly ($P \leq 0.05$) reduced hemoglobin concentration, packed cell volume and total erythrocyte count at all intervals in comparison to control birds indicative of anemia. In chicks, severe anemia is one of the earliest signs of foliate deficiency therefore, it remains to be determined if reductions in chick RBC and haematocrit observed in this study may have occurred as a

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Table 1. Weekly feed consumption (g/bird) and body weight (g/bird)

Groups	Feed consumption				Body weight				
Control	1st week	2nd week	3rd week	4th week	1 day	1st week	2nd week	3rd week	4th week
	114[a]	338[a]	450[a]	510[a]	48.50[a]	116.15 [a]	375.20[a]	625.75[a]	900.50[a]
Contaminated diet	108[a]	315[ab]	350[bc]	425[c]	48.50[a]	112.50[b]	315.20[c]	530.15[c]	800.75[b]

Means in columns with similar superscripts indicate nonsignificant differences (P≥0.05) and those in columns with different superscripts indicate significant differences (P≤0.05).

Table 2. Mean values of haematological parameters in broiler chicks

Groups	Treatments (day)	Hb (g/dl)	PCV (%)	TEC (10^6/ mm)	TLC (10^3/ mm)	H (%)	L (%)	M (%)	E (%)	B (%)
Control	14th day	9.4[a]	27.5[a]	3.1[a]	28.7[b]	35.2[c]	63.8[a]	2.0	0.8	0.8
	21st day	10.2[a]	29.6[a]	3.8[a]	29.0[b]	33.5[c]	65.0[a]	2.2	0.2	0.2
	28th day	10.3[a]	30.4[a]	3.9[a]	29.9[b]	32.4[d]	66.8[a]	2.1	0.9	0.9
Contaminated diet	14th day	8.4[bc]	24.8[c]	2.9[b]	20.1[d]	45.8[b]	53.1[b]	1.2	0.9	0.9
	21st day	8.0[c]	24.5[d]	2.8[b]	20.0[d]	46.9[b]	52.8[b]	1.5	1.0	1.0
	28th day	7.9[c]	24.4[d]	2.7[c]	19.9[d]	49.8[c]	49.1[c]	1.5	1.0	1.0

Means in columns with similar superscripts indicate nonsignificant differences (P≥0.05) and those in columns with different superscripts indicate significant differences (P≤0.05).

Table 3. Mean values of biochemical parameters in broiler chicks

Groups	Treatments (day)	ALP (u/l)	AST (u/l)	ALT (u/l)	BUN (mg/dl)	CRET (mg/dl)	TP (g/dl)	SA (g/dl)	G (g/dl)
Control	14th day	234[d]	144.2[c]	37.2[c]	1.2[cd]	1.1[c]	2.1	1.1	1.0
	21st day	348[d]	142.6[c]	39.0[d]	1.3[c]	1.1[c]	2.2	1.1	1.0
	28th day	365[d]	141.4[c]	32.2[a]	1.2[c]	1.2[c]	2.3	1.2	1.1
Contaminated diet	14th day	424[ab]	278.2[b]	125.8[a]	4.8[a]	1.9[ab]	2.03	1.0	0.9
	21st day	588[b]	302.2[a]	136.5[a]	4.9[a]	1.9[ab]	2.0	1.0	0.9
	28th day	640[b]	334.0[a]	132.2[b]	5.0[a]	2.0[a]	2.0	0.9	1.0

Means in columns with similar superscripts indicate nonsignificant differences (P≥0.05) and those in columns with different superscripts indicate significant differences (P≤0.05).

result of mycotoxin-induced foliate deficiency. Similar reduction in haematocrit was reported in chicks by Pandey *et al*. (2006) and Pozzo *et al*. (2013).

A significant (P≤0.05) decrease in leukocyte count was recorded in birds fed contaminated diet in comparison to the control group at all stages. Differential cell count revealed that mean per cent of heterophils increased whereas lymphocytes showed a significant (P≤0.05) decrease at all intervals. An insignificant variation was observed in eosinophils, monocytes and basophils. These results are in agreement with Girish *et al.* (2008) who recorded that total lymphocyte counts were significantly (P≤0.05) reduced at third week. Total white blood cell counts and other differential leukocyte counts, however, were not significantly (P≥0.05) affected by diet. Chowdhury *et al*. (2005) observed transient changes in differential leukocyte counts including basophil and monocyte counts. The workers opined that a reduction in total lymphocytes during the early growth phase in the absence of hematological parameters indicated the sensitivity of the immune system to such a challenge. This effect may be attributable to reduced proliferation of lymphocytes or direct lymphocyte cytotoxicity caused by *Fusarium* mycotoxins (Indresh and Umakantha 2013). However, a decrease in leukocyte counts would be more likely to occur because leukopenia is frequently associated with anemia caused by foliate deficiency.

Serum biochemistry: A significant (P≤0.05) increase in alkaline phosphatase (ALP), aspartate amino transferase (AST), alanine amino transferase (ALT), blood urea nitrogen (BUN) and creatinine at all stages due to contaminated diet are indicative of hepatic and renal damage and thus reflect the severe hepatopathy and nephropathy. The findings of the present study are in agreement with

Chowdhury and Smith (2007). Serum ALP activity increases in birds with hepatic disorders and biliary obstruction. Inhibition of protein synthesis reduces the activity of the enzymes necessary for the metabolism of toxic substances, induces lipid peroxidation, and increases the activity of glutathione reductase. In the present study nonsignificant ($P \geq 0.05$) decrease in the total protein and albumin concentration was observed in birds fed with contaminated diet throughout the experiment. In birds fed contaminated diet the reduction in serum globulin and reduced albumin globulin ratio was significant at 21 days interval. The main toxic effect of T-2 toxin appears to be primary inhibition of protein synthesis followed by a secondary disruption of DNA and RNA synthesis (Manafi *et al.* 2012, Indresh and Umakantha 2013). Taken together, the present findings clearly indicated that contamination of poultry diet by *Fusarium* mycotoxins leads to hepatic and renal damage reflected by abnormalities in the blood and biochemical profile of birds.

Necropsy findings in birds fed with contaminated diet included as pale carcass, enlarged and pale yellowish tan friable liver, distended gallbladder with thick bile, bilaterally enlarged and congested kidneys. Generalized atrophy of lymphoid organs such as thymus, spleen and bursa was noticed at all intervals. Microscopically at 14th day liver sections revealed infiltration of round cells, granular degenerative changes in cytoplasm of hepatocytes and congestion of portal vein. The sinusoids were dilated and vacuolation of hepatocytes was prominently seen. On 21st day liver was enlarged and liver sections showed congested sinusoidal space, billiary hyperplasia and dilated portal vein. Granular degenerative changes, focal areas of necrosis and marked proliferation of Kupffer cells were also evident. On 28th day grossly pale yellowish discoloured enlarged liver histologically extensive degenerative changes with necrosis of hepatocytes and bile duct hyperplasia. Pande *et al.* (2011) also described that discrete foci of hepatocyte necrosis were followed by mild proliferation of bile ductules in T-2 toxicosis of broilers. Madheswaran *et al.* (2005) reported vacuolar degeneration and biliary hyperplasia in Japanese quail fed T-2 toxin. Sokolovic *et al.* (2008) opined that T-2 is cytotoxic to hepatocytes and our findings endorse their views. In kidney sections interstitial hemorrhage and tubular epithelial granular degenerative changes along with detachment of tubular epithelial membrane in proximal convoluted tubules were observed. The renal degenerative changes increased on 21st and 28th day. Most of the earlier studies on T-2 induced toxicity have reported very mild effects of the toxin on renal tissue (Madheswaran *et al*. 2005). The extensive renal degenerative changes observed in present study can be attributed to the concurrent presence of other mycotoxins produced by *Fusarium sporotrichioides.* Spleen sections revealed mild atrophy of white pulp. Sections of thymus revealed mild depletion of lymphocytes in the medulla and congestion at few places with increased number of Hassal's corpuscles and proliferation of reticular cells. Sections of bursa revealed mild atrophy of bursal follicles with normal distribution of lymphocytes. The depletion of lymphocytes increased with passage of time in all organs. Manafi (2012) observed acute lethal oral intoxication with T-2 toxin causes necrosis of lymphoid and hemopoeitic tissue. Kamalvenkatesh *et al.* (2005) observed that T-2 toxin and related mycotoxins can induce apoptosis in thymus. Madheswaran *et al.* (2005) hypothesized that the severe affection of lymphoid organs in mycotoxin fed birds indicated their immunosuppressive potential. Epithelial hyperplasia and keratinization of crop mucosa during the 14th and 28th day of the trial is in agreement with reports by Pande *et al*. (2011) for T-2 toxicosis.

Thus, on the basis of our results it can be concluded that broiler chickens are susceptible during extended feeding of grains naturally contaminated with fusarium mycotoxins. *Fusarium sporotrichioides* contaminated diet can cause severe toxicity affecting different organ systems in broiler birds.

SUMMARY

The present work was undertaken to study the effects of feeding diets mixed with *Fusarium sporotrichioides* culture with 4 mg T-2 toxin/kg of diet, from 0 to 28 days of age and studied various haemato-biochemical parameters and sequential pathological changes on 14th, 21st and 28th day of experiment. Experimental group birds showed a significant decrease in total erythrocyte count, haematocrit values and leukocyte count at all stages. Significant increase in ALP, AST, ALT, BUN and creatinine and insignificant decrease in the total protein and albumin concentration were observed. Gross pathological changes observed as anemic carcasses, enlarged liver, kidneys and generalized atrophy of lymphoid organs. Histologically, extensive degenerative changes of hepatocytes, bile duct hyperplasia, degenerative changes in kidney sections, depletion of lymphocytes in different lymphoid tissues and glandular necrosis with MNC infiltration of muscles of glandular organ was uniformly observed. These changes are of more or less similar but of increased severity with the duration of experiment.

REFERENCES

Anjum M A, Sahota A W, Akram M and Ali I. 2011. Prevalence of mycotoxins in poultry feed ingredients in Punjab. *Journal of Animal and Plant Science* **21** (**2**): 117–20.

Calnek B W. 1997. *Diseases of Poultry.* pp.97–112. Mosby Wolfe, New York.

Chowdhury S R, Smith T K, Boermans H J and Woodward B. 2005. Effects of feed-borne *Fusarium* mycotoxins on hematology and immunology of turkeys. *Poultry Science* **84**: 1698–1706.

Chowdhury S R and Smith T K. 2007. Effects of feed-borne *Fusarium* mycotoxins on performance, plasma chemistry and hepatic fractional protein synthesis rates of turkeys. *Canadian Journal of Animal Science* **87**: 543–51.

Girish C K, Smith T K, Boermans H J and Karrow N A. 2008. Effects of feeding blends of grains naturally contaminated with *Fusarium* mycotoxins on performance, hematology,

metabolism and immunocompetence of turkeys. *Poultry Science* **87**: 421–32.

Gridly 1960. *Manual of Histologic and Special Staining Techaniques*. 2nd edn, pp. 202. Mc Graw-Hill Book Company Inc., New York, Torento, London.

Indresh H C and Umakantha B. 2013. Effects of ochratoxin and T-2 toxin combination on performance, biochemical and immune status of commercial broilers. *Vetworld*: 945–49.

Kamalvenkatesh P, Vairamuthu S, Balachandran C, Murlimanohar B and Dhinakar G R. 2005. Immunopathological effect of mycotoxins cyclopiazonic acid and T-2 toxin on broiler chicken. *Mycopathology* **159**: 273–79.

Madheswaran R, Balachandran C and Murlimanohar B. 2005. Pathological effects of feeding aflatoxin and T-2 toxin in Japanese quail. *Indian Journal of Veterinary Pathology* **29** (**1**): 23–26.

Manafi M, Umakantha B, Mohan K and Narayana Swamy HD. 2012. Synergistic effects of two commonly contaminating mycotoxins (aflatoxin and T-2 toxin) on biochemical parameters and immune status of broiler chickens. *World Applied Science Journal* **17** (**3**): 364–67.

Manafi M, Umakantha B, Noor Ali M and Narayana Swamy H D. 2012. Study of the Combination Effects of Aflatoxin and T-2 Toxin on Performance Parameters and Internal Organs of Commercial Broilers. *Global Veterinaria* **8** (4): 393–96.

Nesic K, Resanovic R, Jakic-Dimic D and Nesic V. 2011. Efficiency of various feed additives on performance of broilers treated with T-2 toxin. *Biotechnology in Animal Husbandry* **27** (3): 705–11.

Pande V V, Kurkure N V and Bhandarkar A G. 2006. Effect of T-2 toxin on growth, performance and haematobiochemical alterations in broilers. *Indian Journal of Experimental Biology* **44**: 86–88.

Pande V, Kurkure N, Bhandarkar A, Kalorey D and Nagamallewshwari Y. 2011. Histopathological and electron microscopic changes in T-2 toxicosis in broiler chicks *Indian Journal of Veterinary Pathology* **35** (1): 21– 26.

Pozzo L, Salamano G, Mellia E, Gennero M S, Doglione L, Cavallarin L, Tarantola M, Forneris G and Schiavone A. 2013. Feeding a diet contaminated with ochratoxin A for chickens at the maximum level recommended by the EU for poultry feeds (0.1 mg/kg). 1. Effects on growth and slaughter performance, haematological and serum traits. *Journal of Animal Physiology and Animal Nutrition* **97** (1): 13–22.

Snedecor G W, Cochran W G. 1994. *Statistical Methods.* Oxford and I.B.H. Publishing Co., New Delhi.

Sokolovic M, Verica G V and Borka I. 2008. T-2 toxin incidence and toxicity in poultry. *Arh Hig Rada Toksikology* **59**: 43–52.

Tapia M O. 1985. A quantitative thin layer chromatography method for the analysis of aflatoxin, ochratoxin A, zearalenone, T-2 toxin and sterigmatocystin in foodstuffs. *Revista Argentina de Microbiologia* **17**: 183–86.