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Chlorpyriphos and T-2 Toxin Induced Haemato-Biochemical Alterations in Broiler Chicken*

P. Krishnamoorthy¹, S. Vairamuthu, C. Balachandran² and B. Muralimanohar Department of Veterinary Pathology, Madras Veterinary College, Chennai–600 007, Tamilnadu, India ¹Dr. ALM. Postgraduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai-600113, Tamilnadu, India ²Centralised Clinical Laboratory, Madras Veterinary College, Chennai 600007, Tamilnadu, India

Abstract: The present work was undertaken to study the haematobiochemical changes in chlorpyriphos and T-2 toxin fed broiler chicken. Known amounts of chlorpyriphos and T-2 toxin containing powdered wheat substrates were incorporated into the toxin free broiler starter mash, singly and in combination to yield 45 ppm chlorpyriphos and 0.5 ppm T-2 toxin. Forty eight day-old broiler chicks were randomly allotted into four groups of 12 each i.e., control, chlorpyriphos, T-2 and chlorpyriphos+T-2 groups and fed with toxin mixed diets from 0 to 4 weeks of age and studied various haematobiochemical parameters on 14th and 28th day of age. PCV and Hb levels decreased significantly (P<0.01) in toxin fed birds indicating anaemia. Serum total protein, albumin, globulin and albumin/globulin ratio decreased significantly (P<0.01) in T-2 fed group. Serum glucose and cholesterol values decreased significantly (P<0.01) in chlorpyriphos group. AST, ALT and ALP values increased significantly (P<0.01) in T-2 and chlorpyriphos+T-2 groups. Chlorpyriphos fed birds showed increased ALT. Chlorpyriphos and chlorpyriphos+T-2 fed groups showed significant (P<0.01) increase in serum amylase values. Decreased serum lipase values were observed in T-2 and chlorpyriphos+T-2 groups. No significant differences were observed in values of blood urea nitrogen, creatinine, uric acid and GGT between the control and toxin treated groups. Chlorpyriphos and T-2 toxins affected functions of various organs in broiler chicken, the effect being less than additive.

Key words: Broiler, chlorpyriphos, haematobiochemical changes, T-2 toxin

Introduction

Poultry feed ingredients are exposed to number of pesticides of residual potential. Chlorpyriphos (O,Odiethyl-o-[3, 5, 6-trichloro-2-pyridyl] phosphorothioate), an organophosphorus compound, which particularly affects the cholinesterase enzyme system, is a broad spectrum systemic insecticide widely used for the control of pests, mites, flies and lice affecting the livestock and poultry (Loomi et al., 1972) and detected in poultry egg, meat and cow milk and milk products (Rawat et al., 2003). The chlorpyriphos decreased the body weight gain and increased haemoglobin, total erythrocyte count, total leucocyte count, heterophils, glucose, total protein, Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), cholesterol and uric acid in broiler chicken fed 35. 70 and 140 parts per million parts (ppm) from 2 to 8 weeks of age (Yadav et al., 2003). The T-2 toxin, a naturally occurring mycotoxin produced by Fusarium 3hydroxy 4.15 diacetoxy-8(3is a methylbutryloxy), 12,13 epoxy tricothec-9-ene metabolite. Feeding broiler chicken with 1 ppm of T-2 toxin from 0 to 4 weeks of age caused decreased body weight gain,

anaemia, hypoproteinaemia, hypoalbuminaemia, hypoglobulinaemia, hypoglycemia, hypocholesterolaemia and decreased ALT and ALP and increased AST (Kamalavenkatesh, 2003). There was lack of literature on the combined effects of chlorpyriphos and T-2 toxin in broiler chicken. Hence, the present work was undertaken to study the haemato-biochemical changes in broilers exposed to these toxins either individually and in the combination.

Materials and Methods

Newly hatched, unsexed forty-eight commercial broiler chicks (VENCOBB) procured from M/s. Venkateshwara Hatcheries (P) Ltd, Chennai were wing banded, weighed and housed in battery brooders with *ad libitum* supply of feed and water. They were randomly distributed into four groups of 12 chicks each (i.e. control, chlorpyriphos, T-2 and chlorpyriphos+T-2). The chlorpyriphos pesticide technical grade (96.4%) was procured from M/s. De-Nocil Crop Protection Pvt. Ltd, Mumbai, India. The T-2 toxin was produced in wheat using *Fusarium sporotrichioides var sporotrichioides* Microbial Type Culture Collection 1894 (Burmeister, 1971) and

Table 1: Mean (±SE) haematological values in broilers fed chlorpyriphos and T-2 toxin singly and in combination (n=6)

Chlorpyriphos (ppm)	T-2 (ppm)	Packed cell vo	olume (%)		Haemoglobin (g /dL)			
(PP)	(PP)	14th day	28th day	O∨erall mean	14th day	28th day 9.00°±0.20	O∨erall mean	
0	0	28.00±1.26	29.00°±0.99	28.50°±1.10	8.16±0.13	9.00°±0.20	8.58°±0.44	
45	0	25.33±0.71	26.66ab±0.66	26.00 ^{bc} ±0.71	7.80±0.10	7.93ab±0.15	7.86ab±0.12	
0	0.5	25.66±0.66	26.83ab ±0.90	26.33ab±0.76	8.11±0.21	7.50°±0.25	7.80°±0.26	
45	0.5	24.83±0.83	24.66b±0.84	24.75°±0.80	7.75±0.37	7.15b±0.25	7.45b±0.33	

Means with same superscripts within a column do not differ from each other (P>0.01)

quantitified by using thin layer chromatography (Tapia, 1985).

The experimental trials were approved by the Institutional Animal Ethics Committee, India and conducted under its guidelines at Poultry Research Station, Chennai 600035. Broiler mash containing no toxin binders and tested to be free from aflatoxins, T-2 toxin, ochratoxins, cyclopiazonic acid, penicillic acid, citrinin and zearalenone and pesticides. The analyses were carried out at our university Central Animal Feed and Food Residue Laboratory, Chennai 600051. Known amounts of chlorpyriphos and T-2 containing wheat culture materials were incorporated in the broiler mash to yield 45 ppm chlorpyriphos and 0.5 ppm T-2 respectively and fed for 28 days from the day of hatch.

Haematology: Blood samples were collected from six birds in each group by intracardiac puncture in Heller and Paul double oxalate anticoagulant mixture on 14th and 28th day of trial. Haematological studies included the determination of haemoglobin (Hb) by acid haematin method and packed cell volume (PCV) by microhaematocrit method (Coles, 1986).

Serum biochemistry: Samples of blood collected from six birds in each group on 14th and 28th day of trial were allowed to clot in nitric acid washed test tubes and centrifuged at 1500 rpm for 20 minutes to separate the sera. Serum total protein and albumin were estimated by modified Biuret and Dumas method (Varley et al., 1980), glucose by glucose oxidase method, cholesterol by cholesterol dehydrogenase/peroxidase method. AST. ALT and ALP by IFCC (International Federation of Clinical Chemistry) method on 14th and 28th day of trial and blood urea nitrogen by glutamate dehydrogenase method, creatinine by Jaffe's kinetic method, uric acid and amylase by enzymatic photometric test by IFCC method, lipase by turbidimetric Ultra Violet method (Burtis and Ashwood, 1996) and gamma glutamyl transferase (GGT) by Szasz method (Szasz, 1976) on 28th day of trial by using semiauto analyzer, Biosystems (BTS 320).

Statistical analysis: The results of the study were subjected to one or two way analysis of variance (Snedecor and Cochran, 1989) and interactions were

found out (Steel and Torrie, 1960). Data were shown as mean±standard error. A value of P<0.05 was judged to be significant and P<0.01 to be highly significant.

Results

Haematology: Mean±SE of PCV and Hb values in broilers fed chlorpyriphos and T-2 toxin singly and in combination are presented in the Table 1. Highly significant (P<0.01) difference was observed for PCV and Hb values between the control and chlorpyriphos+T-2 groups. The PCV and Hb values decreased in the treatment groups when compared to the control.

Serum biochemistry: Mean±SE serum total protein, albumin, globulin and albumin/globulin (A/G) ratio in broilers fed chlorpyriphos and T-2 toxin singly and in combination are presented in the Table 2. When compared to the control and chlorpyriphos groups total protein and albumin values differed significantly (P<0.01) in the T-2 and chlorpyriphos+T-2 groups. Chlorpyriphos group differed significantly (P<0.01) from other groups in globulin values. A/G ratio showed significant (P<0.05) difference between the control and chlorpyriphos groups. There was significant decrease in the total protein, albumin and globulin values in the T-2 and chlorpyriphos+T-2 groups when compared to the control. Albumin/globulin ratio increased in toxin fed groups. There was significant (P<0.05) interaction in the chlorpyriphos+T-2 group for albumin values. Mean±SE glucose and cholesterol levels in broilers fed chlorpyriphos and T-2 toxin singly and in combination are presented in the Table 3. Comparison of means revealed highly significant (P<0.01) difference between the chlorpyriphos with other groups in glucose and cholesterol values. Serum glucose and cholesterol levels decreased significantly in the chlorpyriphos group. There was significant (P<0.01) interaction in the chlorpyriphos+T-2 group in cholesterol value. Mean±SE AST, ALT and ALP values in broilers fed chlorpyriphos and T-2 toxin singly and in combination are presented in Table 4. The T-2 and chlorpyriphos+T-2 groups showed significant (P<0.01) difference for the AST values when compared to the control and chlorpyriphos groups. Highly significant elevation of AST values was observed in T-2 and chlorpyriphos+T-2 group when compared to the control and chlorpyriphos groups. There was marked

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Table 2: Mean (±SE) serum total protein, albumin, globulin and albumin/globulin ratio values in broilers fed chlorpyriphos and T-2 toxin singly and in combination (n=6)

Chlorpy-	T-2	Total protein (g/dL)			Albumin (g/dL)			Globulin (g/dL)			A/G ratio		
riphos (ppm)	(ppm)	ppm) O∨erall				Overall			Overall		14th	28th	Overall
		14th day	28th day	mean	14th day	28th day	mean	14th day	28th day	mean	day	day	mean
0	0	4.98°±0.10	4.60 ^b ±0.11	4.79°±0.13	2.86°±0.11	2.46b±0.15	2.66°±0.15	2.11°±0.02	2.13°± 0.08	2.12°±0.06	1.35°	1.17 ²	1.26 ^z
45	0	4.75°±0.12	4.93°±0.17	4.84 ^{ab} ±0.15	2.75°±0.08	3.05°±0.07	2.75° ±0.10	2.00°±0.05	1.88b±0.12	1.92ab±0.09	1.37°	1.65 ^x	1.51 ^x
0	0.5	3.40b±0.09	3.66 ^d ±0.15	3.53°±0.13	1.90°±0.05	2.11b±0.05	2.00b±0.06	1.50°±0.06	1.55b±0.11	1.52°±0.08	1.27°	1.39 ^y	1.33 ^{yz}
45	0.5	4.55°±0.17	4.33°±0.20	4.44 ^b ±0.21	2.61°±0.13	2.63b±0.15	2.62 ^{a1} ±0.14	1.93°±0.16	1.70b±0.10	1.81 ^b ±0.14	1.42°	1.57 ^x	1.49 ^{xy}

Means with same superscripts within a column do not differ from each other (P>0.01). x, y, z superscripts within a column differ significantly (P<0.05). 1Represents significant (P<0.05) chlorpyriphos and T_2 toxin interaction

Table 3: Mean (±SE) serum glucose and cholesterol values in broilers fed chlorpyriphos and T-2 toxin singly and in combination (n=6)

Chlorpyriphos (ppm)	T-2 (ppm)	Glucose (g/dL)			Cholesterol (mg/dL)			
		14th day	28th day	Overall mean	14th day	28th day	O∨erall mean	
0	0	133.40°±9.36	176.98±21.64	155.19°±18.41	261.66°±19.66	140.41b±18.07	201.04°±11.28	
45	0	117.01°±4.96	146.46±18.09	131.74b±14.12	146.63b±16.81	145.65b±14.34	146.14b±16.32	
0	0.5	183.90°±18.90	185.91±22.85	184.90°±20.00	313.00°±12.54	82.96°±10.33	197.98°±18.74	
45	0.5	155.06°±9.99	215.91±13.68	185.52°±17.28	180.95b±15.13	175.63°±13.64	178.29°1±15.09	

Means with same superscripts within a column do not differ from each other (P>0.01). 1Reprsents significant (P<0.01) chlorpyriphos and T2 toxin interaction

Table 4: Mean (±SE) serum aspartate transaminase, alanine transaminase and alkaline phosphatase values in broilers fed chlorpyriphos and T-2 toxin singly and in combination (n=6)

Chlorpy-	T-2	AST (U/L)			ALT (U/L)			ALP (U/L)		
riphos	(ppm)									
(ppm)		14th day	28th day	Overall mean	14th day	28th day	Overall mean	14th day	28th day	Overall mean
0	0	166.72°±14.84	65.91b±3.65	116.05°±9.24	14.93±0.76	14.58°±1.67	14.75°±1.24	3702.66°±580.82	1565.66°±186.25	2634.16°±613.75
45	0	159.07°±16.55	69.71b±2.48	114.39°±9.51	16.60±1.35	21.90°±1.63	19.25ab ±1.82	3495.66°±710.49	3480.16b±192.82	3487.95° ±627.42
0	0.5	233.07°±9.41	45.98°±2.71	139.52b±6.06	16.90±1.20	24.36°±1.83	20.63°±2.17	5238.50°±671.02	2123.83°±285.88	3696.16° ±825.14
45	0.5	166.95°±7.68	106.30°±4.11	136.62b±5.89	14.43±0.67	18.23b±1.57	16.33 ^{bc} ±1.41	6073.00°±571.30	4360.00°±284.03	5216.50b1±564.27

Means with same superscripts within a column do not differ from each other (P>0.01). ¹Represents significant (P<0.05) chlorpyriphos and T₂ toxin interaction

Table 5: Mean (±SE) blood urea nitrogen, serum creatinine, uric acid, gamma glutamyl transferase, amylase and lipase values in broilers fed chlorpyriphos and T-2 toxin singly and in combination (n=6)

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Chlorpyriphos (ppm)	T-2 (ppm)	Blood urea nitrogen (mg/dL)	Creatinine (mg/dL)	Uric acid (mg/dL)	Gamma glutamyl transferase (U/L)	Serum amylase (amylase units/dL)	Serum lipase (U/L)
0	0	6.55±0.62	0.21±0.01	1.98±0.31	29.33±3.30	272.81c±11.95	30.98a±2.80
45	0	7.36±0.63	0.31±0.02	2.53±0.43	25.50±1.25	399.66a±6.41	25.70a±1.06
0	0.5	5.95±0.34	0.25±0.03	3.18±0.36	20.66±2.23	237.28c±7.87	16.74b±0.96
45	0.5	7.31±0.57	0.23±0.02	2.98±0.29	28.00±3.08	301.58b±22.89	17.52b±0.82

Means with same superscript within a column do not differ significantly (P>0.01)

elevation of ALT values in treatment groups when compared to control group. Highly significant (P<0.01) elevation was observed in the chlorpyriphos+T-2 group when compared to other groups for ALP values. Significant (P<0.05) interaction was found for ALP value in chlorpyriphos+T-2 group. Mean±SE serum blood urea nitrogen, creatinine and uric acid levels in broilers fed chlorpyriphos and T-2 toxin singly and in combination are shown in the Table 5. No significant differences were observed between the control and treatment groups and also among the treatment groups. Mean±SE serum GGT, amylase and lipase values in broilers fed chlorpyriphos and T-2 toxin singly and in combination are presented in Table 5. Comparison of means revealed no significant differences between the control and treatment groups and also among the treatment groups in GGT values. Highly significant (P<0.01) differences were observed between the chlorpyriphos and chlorpyriphos+T-2 groups when compared with control and T-2 groups for serum amylase values. Increased serum amylase values were observed in the chlorpyriphos and chlorpyriphos+T-2 fed birds. Serum lipase values differed significantly (P<0.01) in T-2 and chlorpyriphos+T-2 groups when compared to the control and chlorpyriphos groups. Serum lipase levels decreased in T-2 and chlorpyriphos+T-2 groups.

Discussion

Haematology: Toxin treated birds revealed reduction in PCV and Hb values indicating anaemia. However, increased PCV and Hb levels were reported in broilers fed 30, 60 and 120 ppm chlorpyriphos for 6 weeks (Malik et al., 2002) and 35, 70 and 140 ppm chlorpyriphos from 2 to 8 weeks of age (Yadav et al., 2003). Anaemic changes have been reported in broilers fed 1 ppm of T-2 toxin onwards (Chi et al., 1981; Huff et al., 1988; Raju and Devegowda, 2000 and Kamalavenkatesh, 2003). Reduced feed consumption, digestive tract lesions and hypoproteinaemia observed could have contributed to anaemia. Further, T-2 toxin could cause membrane changes resulting in an increase in cell volume and dramatic alteration in the red cell morphology (Gongyossy et al., 1986) leading to anaemia.

Serum biochemistry: Chlorpyriphos fed broiler chicks showed a marginal increase in total protein, albumin and globulin values, which concurred with the previous reports (Yadav *et al.*, 2003). Significant reduction in total protein, albumin and globulin values were observed in T-2 fed birds, which agreed with the findings of earlier workers (Boonchuvit *et al.*, 1975; Huff *et al.*, 1988 and Kamalavenkatesh, 2003). Chlorpyriphos+T-2 fed birds showed significant reduction in total protein and globulin values. Hypoproteinaemia, hypoalbuminaemia and hypoglobulinaemia observed in T-2 and

chlorpyriphos+T-2 groups could be attributed to the reduction in feed consumption and hepatic damage as observed in this study, since liver is the major organ of protein synthesis especially albumin (Kaneko *et al.*, 1997).

Feeding 45 ppm chlorpyriphos in broilers showed significant reduction in glucose and cholesterol values. However, Yadav *et al.* (2003) reported significant increase in glucose and cholesterol values. The T-2 and chlorpyriphos+T-2 fed birds did not differ significantly from the control birds. However, reduction in glucose and cholesterol values in T-2 fed birds was reported by earlier workers at a higher dose level (Raina *et al.*, 1991 and Kamalavenkatesh, 2003). The hypoglycaemia and hypocholesterolaemia observed in chlorpyriphos group could be attributed to hepatic damage and pancreatic damage observed in this study.

Feeding 45 ppm chlorpyriphos and 0.5 ppm T-2 toxin in broilers from 0 to 4 weeks of age resulted in significant increase of AST levels in T-2 and chlorpyriphos+T-2, ALT values in all toxin fed birds and in combined toxin fed birds. These findings concurred with earlier reports (Yadav *et al.*, 2003; Raina *et al.*, 1991, Raju and Devegowda, 2000 and Kamalavenkatesh, 2003). The increased AST, ALT and ALP values might be attributed to the liver damage in the toxin fed birds.

No significant change was observed in the blood urea nitrogen, serum creatinine and uric acid levels in toxin fed birds. However, increased uric acid levels were reported in broilers fed chlorpyriphos (Yadav et al., 2003). No significant difference was observed in the toxin fed groups for GGT values. Increased serum amylase values were observed in chlorpyriphos and chlorpyriphos+T-2 fed birds. Literature on serum amylase values in chlorpyriphos and T-2 toxicoses are not available. These increased levels could be attributed to the pancreatic damage. Serum lipase values showed significant decrease in T-2 and chlorpyriphos+T-2 fed birds. No comparable literature was available for the serum lipase values in broiler chicken on this study. However, pancreatic lipase levels decreased in broilers fed 4 ppm T-2 toxin from 0 to 3 weeks of age (Osborne et al., 1982). Decreased levels could be attributed to the hypoproteinaemia as observed in this study affecting the synthesis of the lipase.

The chlorpyriphos and T-2 toxins individually and in combination in broilers caused anaemia and affected the functions of liver, pancreas and other organs. Chlorpyriphos alone caused hypoglycaemia and hypocholesterolaemia. Less than additive effect was observed between these two toxins in broiler chicken. However, further studies are needed to find out the minimal individual and combined toxin levels causing changes in the haemato-biochemical parameters in the broiler chicken.

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