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Research Article

EFFECT OF SUBLETHAL DOSE OF PENICILLIC ACID TOXICITY ON HAEMATOBIOCHEMICAL CHANGES IN BROILER CHICKENS

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ABSTRACT
Penicillic acid (PA), a mycotoxin, was originally isolated from the cultures of *Penicillium puberulum.* The present study was undertaken to find out the sublethal effect of penicillic acid mycotoxicosis on haematobiochemical, liver antioxidant status and pathological changes of broiler chicken. Forty eight day-old broiler chicks were randomly allotted to four groups of 12 birds each and fed with 0, 7.5, 15 and 30 ppm of penicillic acid from 0 to 21 days of age respectively. On 21st day of trial, the birds were sacrificed to study the haematobiochemical changes. Significant ($P<0.05$) decrease in the PCV in all toxin fed groups and Hb value in the 15 and 30 ppm groups were observed. The TEC value decreased significantly ($P<0.05$) in the 30 ppm group when compared to the other groups. Anaemia, hyperglycaemia, significant increase in the serum ALT ($P<0.05$), amylase ($P<0.01$), lipase ($P<0.05$), BUN ($P<0.01$) and uric acid ($P<0.05$), hypercholesterolaemia, hypertriglyceridaemia, increased LDL ($P<0.01$), VLDL ($P<0.05$) and hyponatraemia were observed in the penicillic acid fed birds. This research indicated that haematobiochemical alterations were seen even at sublethal doses of 7.5 ppm level.

Keywords: *Broiler Chicken, Penicillic Acid Toxicity, Haematology, Biochemistry*

INTRODUCTION
Penicillic acid occurred in high concentrations in corn (Kurtzman and Ciegler, 1970) and was also produced concomitantly with other mycotoxins in poultry feed (Bacon *et al*., 1973). Natural occurrence of penicillic acid has been detected in the poultry feed, corn, dried beans, cheese, salami and tobacco products (Kurtzman and Ciegler, 1970). The penicillic acid toxins interfered with protein formation by the action on nucleic acids. The increased glycogen level observed during toxicosis was attributed to the interaction of penicillic acid with the enzymes of carbohydrate metabolism. It also affected the lipid metabolism leading to lowered levels of total lipids (Pandiyan *et al*., 1987). Hence, the present study was undertaken to find out the sublethal effect of penicillic acid mycotoxicosis on haematobiochemical changes in broiler chicken.

MATERIALS AND METHODS
Penicillic Acid Production
The *Penicillium cyclopium* NRRL 1888 culture was obtained from National Center for Agricultural Utilization Research, Microbial Genomics and Bioprocessing Research Unit, 1815 N University Street, Peoria, Illinois 61604, USA. The penicillic acid toxin was produced on maize (LeBars, 1980). The maize samples were pre-tested for the presence of mycotoxins. The penicillic acid from ground maize culture samples were quantified by using thin layer chromatography at the Central Animal Feed and Food Residue Laboratory, Directorate of Centre for Animal Health Studies, Tamil Nadu Veterinary and Animal Sciences University, Chennai–600 051.
Experimental Design
Forty eight day-old broiler chicks were randomly allotted to four groups of 12 birds each. They were fed with 0, 7.5, 15 and 30 ppm of penicillic acid mixed diets from 0 to 21 days of age respectively. On 21st day of trial, the birds were sacrificed.

Haematology

Blood samples were collected by intracardiac puncture in Heller and Paul double oxalate anticoagulant mixture. Haematological studies included the estimation of PCV by microhaematocrit method, Hb by acid haematin method and TEC using Hayem's fluid (Coles, 1986).

Serum Biochemistry

Another set of blood samples collected were allowed to clot and centrifuged at 1500 rpm for 20 min 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, total cholesterol (TC) by cholesterol dehydrogenase peroxidase method, AST, ALT and ALP by IFCC (International Federation of Clinical Chemistry) method, BUN by glutamyl dehydrogenase method, creatinine by Jaffe's kinetic method, uric acid by enzymatic photometric test by IFCC method (Burtis and Ashwood, 1996), calcium by O-crespophthalein complexone method, phosphorus by modified metol method, sodium and potassium by colorimetric method, amylase by colorimetric method (Coles, 1986), serum lipase by turbidimetric UV method (Burtis and Ashwood, 1996), HDL-Cholesterol by precipitation method and triglycerides (TG) by colorimetric enzymatic method using semi-automatic analyser (Mispa excel, Agappe Diagnostic, India). VLDL was arrived by using the formula TG/5 and LDL using the formula TC-HDL-VLDL and TC/HDL was also found out.

Statistical Analysis

The data generated from different experimental trials were subjected to one-way analysis of variance (ANOVA) using SPSS version 10 software for windows

RESULTS AND DISCUSSION

Fungal Culture

The *P. cyclopium* NRRL 1888 subcultured on potato dextrose agar and the culture material yielded 20–80 ppm penicillic acid. The toxigenic fungus *Penicillium cyclopium* NRRL 1888 growth characteristics in the subculture and solid substrates in the present study correlated with the earlier reports (Birkinshaw *et al*., 1936; Ciegler *et al*., 1972; LeBars, 1980; Sarmadha, 2003).

Haematology

Mean ± SE haematological values of broiler chicken fed penicillic acid are presented in Table 1. No significant differences were observed among the toxin fed birds for PCV. The Hb values of 15 and 30 ppm groups differed significantly ($P<0.05$) from that of control group. No significant difference was observed between the control and 7.5 ppm groups for Hb values. The TEC value of 30 ppm penicillic acid fed group significantly ($P<0.05$) differed from that of control and 7.5 ppm penicillic acid fed groups. There was a significant ($P<0.05$) decrease in the PCV value in all toxin fed groups when compared to the control. There was a significant ($P<0.05$) decrease in the Hb value in the 15 and 30 ppm groups when compared to the control group. The TEC value decreased significantly ($P<0.05$) in the 30 ppm group when compared to the other groups except 15 ppm group.

Significant reduction in the PCV values of all penicillic acid fed groups, Hb in the 15 and 30 ppm groups and TEC values in the 30 ppm penicillic acid fed group indicated anaemic changes. The anaemic changes could be ascribed to reduced feed intake and malabsorption due to alimentary tract pathology observed in this study, suppressive effect of toxins on the bone marrow (Coles, 1986) and increased fragility of erythrocytes (Pandiyan and Shanmugasundaram, 1987). Sarmadha *et al.,* (2008b) also observed anaemia in the penicillic acid fed broiler chicken but found at higher dose levels of 240-480 ppm. While Huff *et al.,* (1980) observed no significant changes in the Hb and PCV values when broiler chicken were fed with penicillic acid up to 400 µg/g of diet from 0 to 3 weeks of age.

Serum Biochemistry

Serum Glucose

Highly significant ($P<0.01$) increase in the glucose value was observed in the 15 ppm penicillic acid fed group when compared to the other groups except 30 ppm group(Table 2). Highly significant increase in the glucose value was observed in the 15 ppm penicillic acid fed group when compared to the other

groups. Hyperglycaemia could probably be due to starvation (Kaneko *et al*., 1997), stress (Coles, 1986) inducing adrenocortical stimulation and hepatic damage observed in the toxin fed birds. Near normoglycaemia in the 30 ppm group probably indicated an exhaustion phase.

Serum Total Protein, Albumin, Globulin and Albumin to Globulin ratio Values

There was no significant difference between the control and treatment groups in total protein, albumin, globulin, albumin to globulin ratio. No significant changes were observed between the control and toxin fed birds for serum total protein, albumin, globulin and albumin to globulin ratio values which could be due to the lower levels of penicillic acid toxin fed to birds in this study. Similarly, Sarmadha *et al.,* (2008) did not find any appreciable changes in the protein value even at higher level of feeding penicillic acid toxin to broiler chicken (50-150 ppm).

Serum Enzymes

No significant differences were observed between the control and toxin fed birds for serum AST and ALP (Table 3). There was a significant ($P<0.05$) increase in the serum ALT levels in the toxin fed groups when compared to the control group. The amylase value increased in the 15 and 30 ppm toxin fed groups highly significantly ($P<0.01$) when compared to the control group (Table 3). The lipase value showed a significant ($P<0.05$) increase in the 7.5 ppm group when compared to the other groups. Significant increase in the serum ALT value in all penicillic acid toxin fed groups and lipase value in the 7.5 ppm group and highly significant increase in the amylase values in the 15 and 30 ppm fed groups were observed when compared to the control group.

Elevation of ALT levels could be attributed to the liver damage observed in this study. Sarmadha *et al.,* (2006) also observed elevation of ALT and AST in the 50-150 ppm penicillic acid fed broiler chicken. However, in the present study, the AST values were not affected in the toxin fed birds which might probably be due to the incorporation of lower level of toxin in the feed. Elevated levels of amylase and lipase could be due to the pancreatic damage which was evidenced by the presence of degenerative changes in the acinar cells and these findings are in accordance with the report of Sarmadha *et al.,* (2006).

Kidney Function Test

No significant differences were observed among the toxin fed groups for BUN values (Table 4). The 15 ppm group differed significantly ($P<0.05$) from other groups except 7.5 ppm group for uric acid. No significant differences were observed between the control and penicillic acid fed birds for creatinine. There was highly significant ($P<0.01$) increase in the BUN levels in the toxin treated groups and uric acid value ($P<0.05$) in the 15 ppm toxin treated group when compared to the other groups.

Highly significant increase in the BUN values were observed in all penicillic acid toxin treated groups, while uric acid values increased significantly in the 15 ppm toxin treated group when compared to the control group. Elevated BUN levels observed in the toxin treated birds could be due to the renal injury evident in this study which is in agreement with the finding of Sarmadha (2003). Hyperuricaemia observed in the 15 ppm group could be attributed to renal damage and normouricaemia might be due to hepatic pathology resulting in reduced uric acid synthesis which is in agreement with the observation of Sarmadha (2003).

Lipid Profile

Significant ($P<0.05$/$P<0.01$) differences were observed between the control and penicillic acid fed birds for serum lipid values except for HDL-cholesterol (Table 5). Comparison of means revealed that the 30 ppm penicillic acid group differed highly significantly ($P<0.01$) from that of the control group for serum total cholesterol and LDL. The 7.5 and 15 ppm did not differ significantly from that of control and 30 ppm groups for serum total cholesterol and LDL. No significant differences were observed among the penicillic acid fed groups for triglycerides and VLDL values. TC/HDL ratio did not differ among the control, 7.5 and 15 ppm fed groups and between 7.5 and 30 ppm fed groups. There was highly significant ($P<0.01$) increase in the serum total cholesterol and LDL values in the 30 ppm group when compared to other groups. The triglycerides and VLDL values of toxin treated birds showed significant increase ($P<0.05$) when compared to the control group. The TC/HDL ratio of 30 ppm group showed a significant ($P<0.05$) increase when compared to the other groups except 15 ppm group.

Highly significant increase in the serum total cholesterol and LDL values were observed in the 30 ppm penicillic acid fed group when compared to the other groups. Significant increase in the triglycerides and VLDL values were observed in the 15 and 30 ppm toxin fed birds when compared to the control group. The TC/HDL ratio of 30 ppm group showed a significant increase when compared to the control and other groups.

Table 1: Mean (± SE) haematological values in penicillic acid fed broiler chicks (n=6)

Penicillic acid toxin levels (ppm)	PCV (%)	Hb (g/dL)	TEC (millions/cmm)
0	$31.33^{a} \pm 0.49$	$11.00^{a} \pm 0.37$	$3.01^{ab} \pm 0.26$
7.5	$27.83^{b} \pm 1.22$	$9.67^{ab} \pm 0.67$	$3.12^{a} \pm 0.12$
15	$27.00^{b} \pm 1.65$	$9.33^{b} \pm 0.76$	$2.57^{bc} \pm 0.21$
30	$25.33^{b} \pm 0.88$	$8.50^{b} \pm 0.43$	$2.34^{c} \pm 0.15$

Means with same superscripts within a column do not differ from each other (P>0.05)

Table 2: Mean (± SE) serum glucose values (mg/dL) in penicillic acid fed broiler chicks (n=6)

Penicillic acid toxin levels (ppm)	Glucose (mg/dL)
0	$217.99^{y} \pm 11.08$
7.5	$204.57^{y} \pm 10.27$
15	$274.55^{x} \pm 21.51$
30	$241.98^{xy} \pm 7.15$

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

Table 3: Mean (± SE) serum enzyme values (U/L) in penicillic acid fed broiler chicks (n=6)

Penicillic acid levels (ppm)	ALT (U/L)	AST (U/L)	ALP (U/L)	Amylase (U/L)	Lipase (U/L)
0	$5.38^{b} \pm 0.35$	224.53 ± 14.25	4529.13 ± 1170.37	$398.89^{z} \pm 41.08$	$22.31^{b} \pm 2.23$
7.5	$11.27^{a} \pm 1.82$	253.28 ± 6.26	5142.05 ± 656.72	$694.86^{yz} \pm 69.07$	$25.92^{a} \pm 0.66$
15	$10.90^{a} \pm 1.32$	251.16 ± 10.42	9851.32 ± 193.11	$933.93^{y} \pm 2.23$	$23.83^{b} \pm 1.04$
30	$10.11^{a} \pm 1.38$	237.97 ± 11.23	8497.93 ± 318.97	$1199.89^{x} \pm 34.17$	$19.32^{b} \pm 1.76$

Means with same superscripts within a column (a,b/x,y,z) do not differ from each other (P>0.05/P>0.01)

Table 4: Mean (± SE) BUN and serum creatinine and uric acid values (mg/dL) in penicillic acid fed broiler chicks (n=6)

Penicillic acid toxin levels (ppm)	BUN (mg/dL)	Creatinine (mg/dL)	Uric acid (mg/dL)
0	$4.08^{y} \pm 0.26$	0.27 ± 0.02	$3.99^{b} \pm 0.35$
7.5	$16.90^{x} \pm 0.06$	0.38 ± 0.06	$5.15^{ab} \pm 0.97$
15	$16.94^{x} \pm 0.13$	0.29 ± 0.02	$6.45^{a} \pm 0.84$
30	$16.64^{x} \pm 0.20$	0.34 ± 0.03	$3.70^{b} \pm 0.20$

Means with same superscripts within a column (a,b/x,y) do not differ from each other (P>0.05/P>0.01)

Hypercholesterolaemia observed in the 30 ppm penicillic acid toxin fed birds agreed with earlier reports of Pandiyan and Shanmugasundaram (1987) and Sarmadha (2003) which might be attributed to the interaction of penicillic acid with the cell membrane resulting in the shedding of membrane components such as cholesterol into the plasma there by enhancing levels of these components in the plasma. Further, it corroborated with the hepatic damage observed in the present study, as liver being the organ for cholesterol metabolism (Kaneko *et al*., 1997). Significant increase in the triglycerides, LDL and VLDL might be attributed to the alteration in the lipid metabolism primarily as a result of impairment of the lipid transport (Kubena *et al*., 1988) and concurred with the hepatocellular damage observed in this study.

Table 5: Mean (± SE) serum lipid profile values (mg/dL) in penicillic acid fed broiler chicks (n=6)

Penicillic acid toxin levels (ppm)	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	TC/ HDL ratio
0	135.112^{y} ± 3.29	71.13^{b} ± 2.41	39.88 ± 2.26	81.56^{y} ± 3.93	14.20^{b} ± 0.50	3.50^{b} ± 0.23
7.5	171.37^{xy} ± 7.30	87.83^{a} ± 4.15	40.43 ± 1.46	111.59^{xy} ± 7.95	17.57^{a} ± 0.83	4.12^{ab} ± 0.30
15	166.51^{xy} ± 8.34	83.45^{a} ± 1.02	41.80 ± 0.96	108.07^{xy} ± 8.88	16.69^{a} ± 0.20	4.01^{b} ± 0.25
30	199.68^{x} ± 16.39	81.33^{a} ± 4.42	39.34 ± 1.32	142.42^{x} ± 16.19	16.27^{a} ± 0.88	4.88^{a} ± 0.38

Means with same superscripts within a column (a,b/x,y) do not differ from each other (P>0.05/P>0.01)

Table 6: Mean (± SE) serum electrolyte (mEq/L) and mineral values (mg/dL) in penicillic acid fed broiler chicks (n=6)

Penicillic acid toxin levels (ppm)	Sodium (mEq/L)	Potassium (mEq/L)	Calcium (mg/dL)	Phosphorus (mg/dL)	Ca:P
0	126.91^{a} ± 4.63	4.67 ± 0.32	13.17 ± 0.49	7.13 ± 0.24	1.86 ± 0.10
7.5	102.68^{b} ± 3.32	5.08 ± 0.38	14.55 ± 0.99	7.53 ± 0.81	2.29 ± 0.20
15	107.31^{b} ± 12.23	4.95 ± 0.41	15.18 ± 0.90	8.61 ± 0.59	1.82 ± 0.21
30	89.41^{b} ± 3.98	4.59 ± 0.18	13.89 ± 0.87	7.11 ± 0.45	1.97 ± 0.14

Means with same superscripts within a column do not differ from each other (P>0.05)

Serum Electrolytes and Minerals

No significant differences were observed between the control and penicillic acid treated birds for serum potassium, calcium, phosphorus and Ca: P values (Table 6). Significant (P<0.05) decrease in the serum sodium values were observed in the penicillic acid treated birds when compared to the control group. Significant decrease in the serum sodium levels was observed in the 7.5 to 30 ppm penicillic acid toxin treated birds. Hyponatraemia in the penicillic acid fed birds might be attributed to loss of sodium in the intestine and urine due to enteritis and degenerative changes in the kidney observed in this study

Conclusion

The above study indicated that Haematobiochemical alterations were observed even at 7.5 ppm level of penicillic acid.

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