

EFFECT OF SUBLETHAL DOSE OF PENICILLIC ACID TOXICITY ON GROWTH, IMMUNITY AND LYMPHOID ORGANS 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 growth rate and immune status 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. Two birds from each group were sacrificed on 7th, 14th and 21st day of age to study the cell mediated immunity (CMI) of birds. On 21st day of trial, remaining birds were sacrificed to study the pathological changes in lymphoid organs and immune status. There were highly significant reduction ($P<0.01$) in the body weight gain of broiler chicken at 15 and 30 ppm levels. Highly significant reduction ($P<0.01$) in the NDV antibody titres were observed in the 7.5 and 15 ppm penicillic acid fed birds. Highly significant decrease ($P<0.01$) in the splenocyte stimulation index value was found in the 30 ppm group when compared to the control and other groups. Microscopically, Bursa of Fabricius showed hyperplastic lining epithelium with multiple cystic changes in the 7.5 ppm group. Splenic reticulum cell hyperplasia, cystic changes in the bursa of Fabricius, starry-sky appearance in the thymus, mild lymphoid depletion in the caecal tonsils and mild plasma cell depletion in the Harderian gland were also observed in 15 ppm group. This research indicated that the PA toxin caused a reduction in growth rate at 15 in broiler chicken and immune suppression even at sublethal dose of 7.5 ppm

Key words: Broiler chicken, Penicillic acid toxicity, growth, cell mediated immunity and humoral immunity

INTRODUCTION

Penicillic acid (PA), a mycotoxin, was originally isolated from the cultures of *Penicillium puberulum* (Alsberg CL and Black OF, 1913). Later it was found that *P. cyclopium* Westling produced relatively larger amounts of penicillic acid (Bentley R and Keil JG, 1962). Penicillic acid occurred in high concentrations in corn (Kurtzman CP and Ciegler A, 1970) and was also produced concomitantly with other mycotoxins in poultry

feed (Bacon CW et al., 1973). Natural occurrence of penicillic acid has been detected in the poultry feed, corn, dried beans, cheese, salami and tobacco products. Penicillic acid, a mycotoxin produced by *Penicillium* and *Aspergillus* species, was isolated from blue-eye diseased corn, poultry feed, commercial corn, dried beans, cheese and tobacco products (LeBars J. 1980). Penicillic acid toxicity produced the effects of depression, inappetence,

poor growth and poor and abnormal feathering of the wings in broiler chicken fed 50 to 480 ppm of penicillic acid toxin for 28 days (Sarmadha MK. 2003). The present study was undertaken to find out the sublethal effect of penicillic acid mycotoxicosis on growth rate and immune status of broiler chicken.

MATERIALS AND METHODS

1. PENICILLIC ACID PRODUCTION

The *Penicillium cyclopium* NRRL 1888 culture was obtained from the 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 J. 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. The *P. cyclopium* NRRL 1888 subcultured on potato dextrose agar and the culture material yielded 20–80 ppm penicillic acid.

2. 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. Body weights (g) were recorded at weekly intervals.

$$\text{Feed consumption} = \frac{\text{Total feed consumed during the week (g)}}{\text{Number of birds fed during the week}}$$

$$\text{Feed conversion} = \frac{\text{Average feed consumption per bird during the week (g)}}{\text{Average weight gain per bird during the week (g)}}$$

Two birds from each group were sacrificed on 7th, 14th and 21st day of age to study the cell mediated immunity. On 21st day of trial, remaining birds were sacrificed to study the pathological changes in different immune related organs and immune status.

3. HUMORAL IMMUNITY

The D58 live thermostable Newcastle disease (ND) vaccine was obtained from the Department of Veterinary Microbiology, Madras Veterinary College, Chennai, for immunizing birds against ND through oronasal route on 7th day. The ND antibody titre was determined after 28 days by using indirect ELISA developed by the Department of Veterinary Microbiology, Madras Veterinary College, Chennai. The antibody titre against NDV was determined by indirect ELISA method at 28th day as per the procedure described by John Kirubakaran J et al. (2008).

4. CELL MEDIATED IMMUNITY

Cell mediated immunity was assessed by colorimetric blastogenesis assay as described by Reynolds DL and Maraqa AD, (2000). The organs were collected in 10 per cent formalin for histopathological examination. The paraffin

embedded tissue sections were cut into 4 to 6 μm thickness and stained with haematoxylin and eosin as per the standard procedures described by Bancroft JD and Gamble G, (2008).

5. 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

GROWTH STUDY

BODY WEIGHT

Penicillic acid treated groups and control group showed significant ($P<0.05/P<0.01$) differences from first week onwards as shown in Table 1. Among the toxin treated groups, 15 and 30 ppm groups differed highly significantly ($P<0.01$) from 7.5 ppm group in the third week. Comparison of means revealed no significant differences between the control and 7.5 ppm groups throughout the experimental period. The 15 and 30 ppm groups differed significantly ($P<0.05$) from that of control in the first week. In the second week, no

significant differences were observed between the 0, 7.5 and 15 ppm groups.

TABLE. 1
Mean (\pm SE) weekly body weights (g) of penicillic acid fed broiler chicks

Penicillic acid toxin levels (ppm)	Hatch weight (g)	Age in weeks					
		1		2		3	
		BW (n=10)	RBW	BW (n=8)	RBW	BW (n=6)	RBW
0	47.85 \pm 1.09	122.00 ^a \pm 6.51	100	305.00 ^x \pm 20.42	100	605.83 ^x \pm 21.03	100
7.5	47.35 \pm 1.13	109.00 ^{ab} \pm 4.46	89	315.63 ^x \pm 18.36	103	593.33 ^x \pm 18.56	98
15	48.14 \pm 1.03	97.50 ^b \pm 3.96	80	258.13 ^{xy} \pm 15.32	85	488.33 ^y \pm 19.39	81
30	48.36 \pm 1.00	104.00 ^b \pm 4.93	85	233.75 ^y \pm 14.84	77	417.50 ^y \pm 24.52	69

BW - Body weight; *RBW* - Relative body weight Means with same superscripts within a column (a,b/x,y) do not differ from each other ($P>0.05/P>0.01$)

The 30 ppm group differed highly significantly ($P<0.01$) from that of other groups barring the 15 ppm group in the second week. In the third week, body weight of 15 and 30 ppm group differed highly significant ($P<0.01$) from that of control and 7.5 ppm groups. In the first week, 15 and 30 ppm groups showed significant ($P<0.05$) decrease in the body weight gain when compared to the control and 7.5 ppm groups. During the second week, 30 ppm group showed highly significant ($P<0.01$)

decrease in the body weight gain when compared to the other groups except 15 ppm group. The 15 and 30 ppm groups showed highly significant ($P<0.01$) decrease in the body weight gain when compared to the control and 7.5 ppm groups in the third week. There was no significant difference in the feed consumption and feed conversion between the control and toxin treated groups as shown in Table 2, 3.

TABLE. 2
Mean weekly feed consumption (g) of penicillic acid fed broiler chicks

Penicillic acid toxin levels (ppm)	Weeks			Overall feed consumption	Relative feed consumption
	1 (n=10)	2 (n=8)	3 (n=6)		
0	112.30	302.73	553.33	968.36	100
7.5	105.38	313.64	575.00	994.02	103
15	80.00	268.00	422.50	770.50	80
30	72.00	160.00	283.13	515.13	53

TABLE. 3
Mean weekly feed conversion (feed/gain) of penicillic acid fed broiler chicks

Penicillic acid toxin levels (ppm)	Weeks			Overall means
	1 (n=10)	2 (n=8)	3 (n=6)	
0	1.66	1.73	1.89	1.76
7.5	1.80	1.57	2.24	1.87
15	1.76	1.71	2.08	1.85
30	1.32	1.23	1.76	1.43

HUMORAL IMMUNITY

There was highly significant ($P<0.01$) difference in the antibody titres of NDV between the control and toxin treated birds except 30 ppm group as shown in Table 4. No significant differences were observed between the 7.5 ppm and 15 ppm, 15 ppm and 30 ppm and control and 30 ppm groups. There was highly significant ($P<0.01$) decrease in the NDV antibody titre values in the 7.5 ppm and 15 ppm penicillic acid fed birds when compared to the control and 30 ppm groups

TABLE 4
Mean (\pm SE) antibody titre of NDV in penicillic acid fed broiler chicks (n=6)

Penicillic acid toxin levels (ppm)	NDV (\log^2)
0	2.700 ^x \pm 0.063
7.5	1.491 ^z \pm 0.164
15	1.685 ^{yz} \pm 0.248
30	2.149 ^{xy} \pm 0.180

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

CELL MEDIATED IMMUNITY

The 30 ppm penicillic acid fed group differed highly significantly (P<0.01) from that of the other groups (Table 5). There was a highly significant (P<0.05) decrease in the stimulation index values of splenic lymphocytes in the 30 ppm penicillic acid fed birds when compared to the other groups.

TABLE 5
Mean (\pm SE) splenic lymphocyte stimulation index in penicillic acid fed broiler chicks (n=6)

Groups	I week	II week	III week	Overall means
Vaccine + No toxin	0.786 \pm 0.006	1.458 \pm 1.118	0.888 \pm 0.004	1.044 ^x \pm 0.550
7.5 ppm	0.006 \pm 0.002	1.392 \pm 0.032	0.727 \pm 0.113	0.708 ^x \pm 0.442
15 ppm	0.958 \pm 0.338	0.367 \pm 0.021	0.150 \pm 0.088	0.492 ^x \pm 0.307
30 ppm	-0.115 \pm 0.065	-0.573 \pm 0.075	-0.309 \pm 0.007	-0.332 ^y \pm 0.152

Overall means with same superscript within a column do not differ from each other (P>0.01)

PATHOLOGY OF IMMUNE ORGANS

GROSS PATHOLOGY

Grossly, no changes were observed in lymphoid organs

HISTOPATHOLOGY

The 7.5 ppm penicillic acid toxin fed birds showed hyperplastic plical epithelium with multiple cystic changes as shown in Figure 1 in the Bursa of Fabricius. Two cases showed mild medullary lymphoid cell depletion as shown in Figure 2.

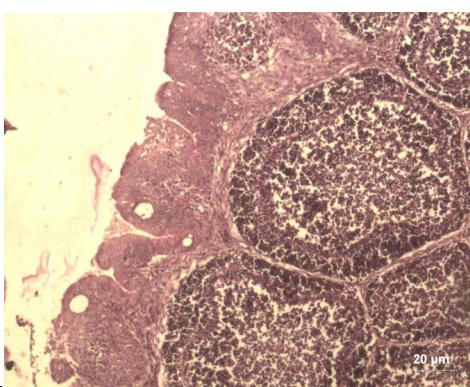
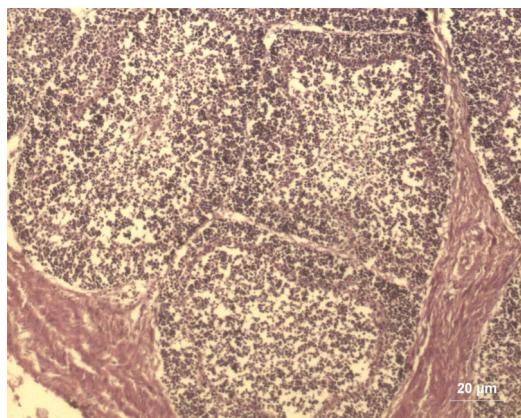


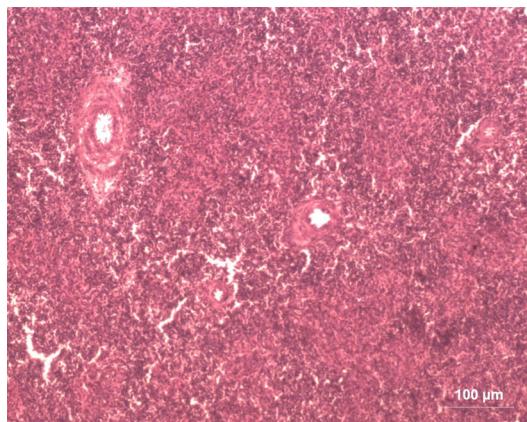
Figure 1

Bursa of Fabricius-7.5 ppm PA-Multiple cystic changes in the plical epithelium H&E Scale Bar=20 μ m

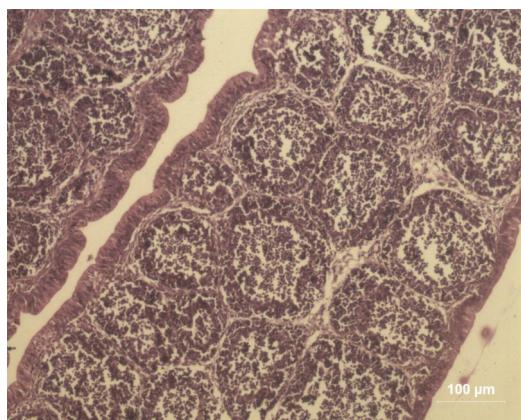
**Figure 2**

Bursa of Fabricius-7.5 ppm PA- mild medullary lymphoid cell depletion H&E Scale Bar=20 μm

The 15 ppm penicillic acid fed birds showed lymphoid cell depletion and reticulum cell hyperplasia as shown in Figure 3 in the spleen. Bursa of Fabricius showed mild to moderate lymphoid cell depletion in the cortex and medulla as shown in Figure 4. Cystic changes were seen involving single to multiple follicles. Thymus showed starry-sky appearance in two cases as shown in Figure 5. Caecal tonsils showed mild lymphoid depletion as shown in Figure 6. Mild plasma cell depletion was found in the Harderian gland as shown in Figure 7.

**Figure 3**

Spleen-15 ppm PA- lymphoid cell depletion and reticulum cell hyperplasia H&E Scale Bar=100 μm

**Figure 4**

Bursa of Fabricius-15 ppm PA- Mild to moderate lymphoid cell depletion H&E Scale Bar=100 μm

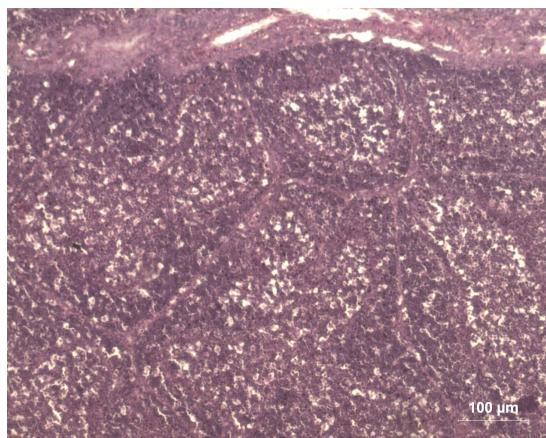


Figure 5
Thymus-15 ppm- Starry sky appearance H&E Scale Bar=100 μ m

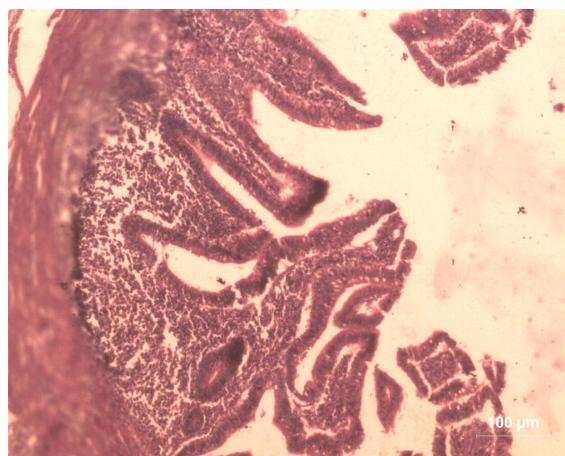


Figure 6
Caecal tonsils-15 ppm PA -Mild lymphoid cell depletion H&E Scale Bar=100 μ m

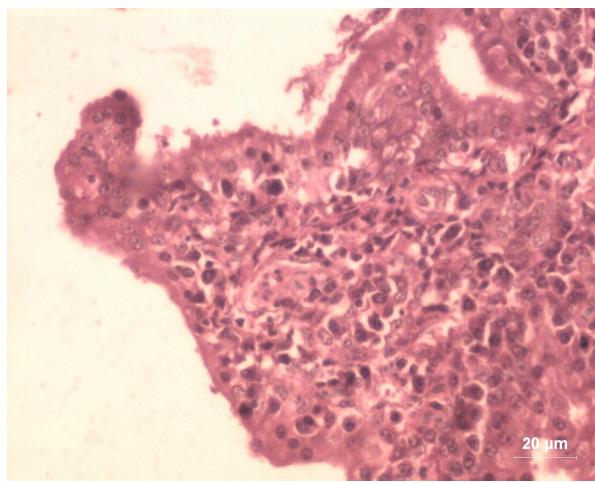


Figure 7
Harderian gland-15 ppm PA- Mild plasma cell depletion H&E Scale Bar=20 μ m

The 30 ppm penicillic acid fed birds showed moderate lymphoid cell depletion as shown in Figure 8 and reticulum cell hyperplasia in the spleen. Bursa of Fabricius showed moderate lymphoid cell depletion mainly involving medullary areas as shown in Figure 9. Cystic changes were also noticed in the lymphoid

follicles containing homogenous eosinophilic substances. Caecal tonsils showed mild to moderate lymphoid cell depletion in the nodular and diffuse lymphoid tissues as shown in Figure 10.

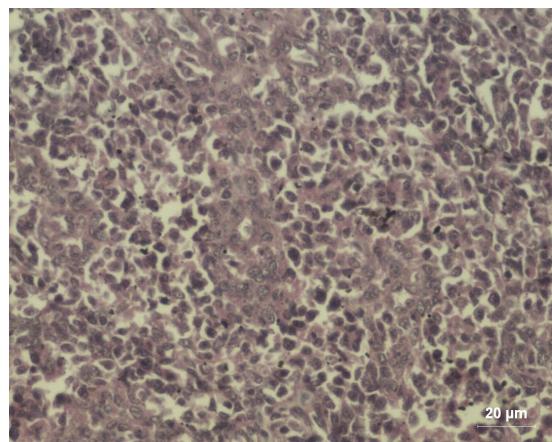


Figure 8
Spleen-30 ppm PA- lymphoid cell depletion Scale Bar=20μm

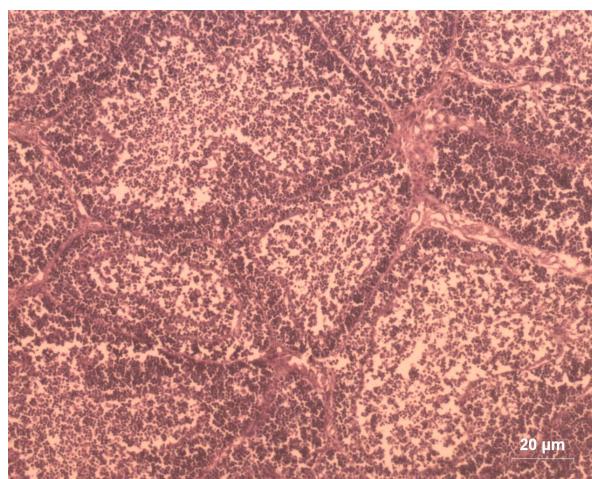


Figure 9
Bursa of Fabricius-30 ppm PA- Moderate lymphoid cell depletion Scale Bar=20μm

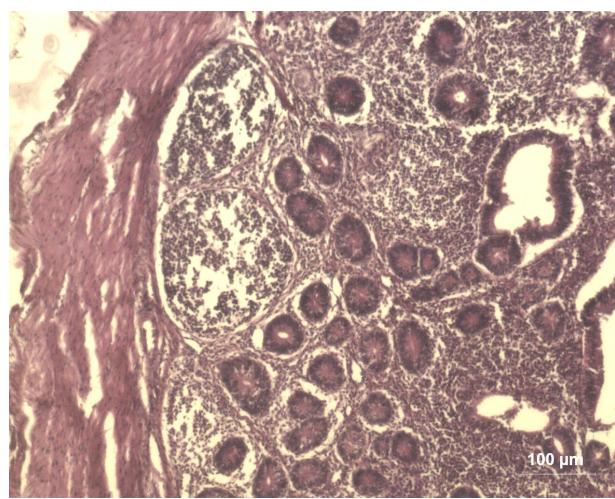


Figure 10
Caecal tonsils-30 ppm PA -Moderate lymphoid cell depletion H&E Scale Bar=100μm

DISCUSSION

GROWTH STUDY

BODY WEIGHT

Feeding 7.5, 15 and 30 ppm penicillic acid toxin from 0 to 3 weeks of age resulted in a highly significant reduction in the body weight gain of broiler chicken at 15 and 30 ppm levels. The reduction in the body weight gain was observed from first week onwards when compared to the control group. The differences were highly significant from the second week. In the 15 ppm group, the body weight did not differ from that of control group in the second week, however, decreased highly significant in the third week. Though, the 15 ppm fed birds showed marginal improvement during the second week, but it could not sustain in the subsequent week. The respective relative body weights of control, 7.5, 15 and 30 ppm groups were 100, 98, 81 and 69 at the end of 21 days experimental trial. The 15 ppm group gained 19 per cent less weight than the control and the 30 ppm group gained 31 per cent less than that of control at the end of experimental trial. Sarmadha MK *et al.* (2006) also reported a significant reduction in body weight gain of broiler chicken fed penicillic acid, but at higher levels of 50-480 ppm. While Huff WE *et al.* (1980) reported no significant effects of penicillic acid on the body weight gain of broiler chicks fed with 0, 100, 200 and 400 μg penicillic acid/g diet from 0 to 3 weeks of age. Reduction in the body weight gain in the 15 and 30 ppm groups could be attributed to inappetence and reduced feed consumption observed in this study. Besides, the pathological changes observed from crop to intestine, liver and pancreas must have affected the digestion, absorption and metabolism leading to reduce body weight gain.

FEED CONSUMPTION AND FEED CONVERSION

There was no significant difference between the control and penicillic acid treated groups for feed consumption and feed conversion which could be due to the low level of toxin used in the present study. Though there was no statistically significant difference in the feed consumption between the control and penicillic acid treated birds, there was a gradual decrease in the overall feed consumption in the penicillic acid treated birds when compared to the control birds. The 15 and 30 ppm group of

birds consumed 20 and 47 per cent less than that of the control group respectively. Similarly, no significant difference was reported in the feed consumption of birds fed up to 480 μg penicillic acid/g of diet to the broiler chicken when compared to the control group (Huff WE *et al.* 1980; Sarmadha MK, 2003).

HUMORAL IMMUNITY

Highly significant reduction in the NDV antibody titres in the 7.5 and 15 ppm penicillic acid fed birds correlated with the lymphoid cell depletion in the lymphoid organs which might have affected the humoral immunity. Though there were histological changes in the 30 ppm level, there was no appreciable decrease in the humoral immunity against NDV. The variation could be attributed to the differences in the biological susceptibility of individual birds. Sarmadha MK, (2003) also observed a reduction in the humoral immunity at the levels of 50-480 ppm penicillic acid fed broiler chicken.

CELL MEDIATED IMMUNITY

Highly significant decrease in the splenocyte stimulation index value was found in the 30 ppm group when compared to the control and other toxin treated groups. The result indicated that the toxin at the level of 30 ppm could impair the cell mediated immunity. No comparable literature is available on the penicillic acid toxicity affecting CMI.

PATHOLOGY

The 7.5 ppm penicillic acid toxin fed birds showed hyperplastic lining epithelium with multiple cystic changes in the lymphoid follicles in the bursa of Fabricius. The 15 ppm penicillic acid fed birds showed splenic reticulum cell hyperplasia, cystic changes in the bursa of Fabricius, starry-sky appearance in the thymus, mild lymphoid depletion in the caecal tonsils and mild plasma cell depletion in the Harderian gland were also observed. In addition to the changes observed in the 15 ppm group, the 30 ppm penicillic acid fed birds also showed focal cystic dilatation and mild to moderate lymphoid cell depletion in the bursa of Fabricius, spleen and caecal tonsils. The above changes are in accordance with the findings of Sarmadha MK, (2003) but were reported at higher levels of penicillic acid toxicity (50-480 ppm) in broiler chicken. The above changes observed in the

immune organs correlated with reduction in humoral and cell mediated immunity. PA toxin

affected the growth parameters even at 15 ppm level.

CONCLUSION

This research indicated that the PA toxin had immune suppression even at sublethal dose of 7.5 ppm

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