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## A Study on Immunopathologic Effect of Thiram Toxicosis in Broiler Chicken

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**Abstract:** An investigation was undertaken to study the body weight gain and feed conversion efficiency in broiler chicken fed with thiram a fungicide used for treating corn and storing food grains. Forty eight birds were randomly distributed into four groups of twelve each and thiram was incorporated at 15, 30 and 60 ppm into the toxin free diet for four weeks from the day of hatch. Blood samples were collected, sera separated and HI titres were measured in the control and treated sera. Birds were sacrificed at the end of second and fourth week for detailed examination of lymphoid organs. There was a significant reduction in the antibody titre in the thiram fed birds. Lymphocyte proliferation assay conducted from the lymphocytes prepared from the spleen showed significant reduction in the stimulation index. Histopathology of the lymphoid organs including bursa, spleen thymus and caecal tonsils showed lymphoid depletion to lymphocytolysis.

**Key words:** Antibody titre, broiler chicken, lymphocyte proliferation assay

### Introduction

Thiram, tetramethyl thiuram disulphide an organic sulphur compound is used a fungicide to prevent crop damage in the field and to protect the harvested crops and seeds from deterioration in storage or transport. The treated grains occasionally find their way into the feed of birds causing tibial dyschondroplasia in them. Tibial dyschondroplasia is a disorder of endochondral ossification characterized by an abnormal mass of cartilage, representing persistent prehypertrophic cartilage that has not been calcified and has not been invaded by blood vessels from the metaphysis (Leach and Nesheim, 1965).

Signs of dyschondroplasia were apparent when the birds were fourteen days of age (Leach and Nesheim, 1965). The histopathological changes in thiram toxicity were dose dependent (Nageswara *et al.*, 1996). Histologically, tibial dyschondroplasia was due to apparently transitional chondrocytes that have been unable to differentiate to hypertrophic chondrocytes (Webster *et al.*, 2003). The accumulated cells were the consequence of a failure of transitional (prehypertrophic) chondrocytes to hypertrophy fully (Thorp *et al.*, 1995). The accumulated chondrocytes eventually became necrotic due to energy depletion with increasing distance from the vascular supply (Hargest *et al.*, 1985). The incidence of the lesion could be affected by a number of other factors including diet, surgical interference, environmental factors, selective breeding (Lawler *et al.*, 1988) and a mycotoxin produced by *Fusarium equiseti* (Walser *et al.*, 1982).

### Materials and Methods

A total of forty eight day-old broiler chicks were obtained from a commercial hatchery and divided into four groups of 12 each. The control and thiram mixed diets were fed

to the different groups in the concentration of 0, 15, 30 and 60 ppm for 28 days from the day of hatch. The birds were sacrificed at the end of second and fourth week.

**Haemagglutination Inhibition (HI) titres against NDV:** Haemagglutinating Inhibition (HI) titres against NDV were measured in both the control and treated sera (Alexander, 1988).

**Histopathology:** After exsanguination, a detailed necropsy was conducted on each sacrificed bird. Gross lesions observed were recorded. Representative pieces of tissues from the lymphoid organs like bursa of fabricius, spleen, thymus and caecal tonsils were collected in 10 percent formal saline and processed for histopathological examination (Bancroft and Stevens, 1996).

**Lymphocyte proliferation assay:** Lymphocytes were prepared from spleen and the MTT calorimetric assay for the proliferation of splenocytes against concavalin A (Con A) was essentially that described by Bounous *et al.* (1992). The lymphocytes collected from spleen were carefully pipetted out and transferred to another sterile centrifuge tube. The cells were washed twice with cold RPMI-1640 media. The cells were resuspended in the media.

The viable cells were counted by trypan blue dye exclusion technique (Hudson and Hay, 1980) and were suspended at a concentration of  $1 \times 10^6$  lymphocytes/mL of culture medium. Foetal calf serum was added to the medium, at the rate of 100  $\mu$ L/mL. The cell suspension was distributed in 96 well flat bottom tissue culture plate and Con A mitogen was added. The cultures were incubated at 37°C for 72 h at 5 percent carbon dioxide level in a water-jacketed carbon dioxide incubator.

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**Table 1:** Mean ( $\pm$ SE) values of haemagglutination inhibition (HI) titres against Newcastle disease virus in thiram fed broiler chicken

Thiram (ppm)	2 <sup>nd</sup> week (n=6)	4 <sup>th</sup> week (n=6)	Overall mean
0	28.00 $\pm$ 3.66	58.66 $\pm$ 4.88	43.33 $\pm$ 7.62
15	29.33 $\pm$ 5.96	13.33 $\pm$ 1.54	20.50 $\pm$ 9.20
30	29.33 $\pm$ 5.96	10.66 $\pm$ 1.54	20.66 $\pm$ 4.11
60	32.00 $\pm$ 0.00	14.66 $\pm$ 1.22	23.33 $\pm$ 3.65

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

**Table 2:** Mean ( $\pm$ SE) values of lymphocyte proliferation assay in thiram fed broiler chicken

Thiram (ppm)	S.I. (Stimulation Index) (n=6)
0	1.48 $\pm$ 0.10
15	0.91 $\pm$ 0.04
30	0.85 $\pm$ 0.04
60	0.86 $\pm$ 0.03

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

Twenty  $\mu$ L of MTT (5 mg/mL) was added to each of the wells and the plate was incubated at 37°C for 4-6 h. One hundred  $\mu$ L of lysis buffer was added to each well and the plate was incubated at 37°C overnight. The optical density was measured in a microspectrophotometer at 570/690 nm.

The mean Optical Density (OD) was read on a ELISA reader. Blastogenic responses for the MTT assay were expressed as a mean Stimulation Index (SI) by dividing OD values of stimulated cells ( $C_s$ ) minus relative cell numbers of unstimulated cells ( $C_u$ ) by relative OD values of unstimulated cells.

$$SI_{MTT} = (C_s - C_u) / C_u$$

The data generated were subjected to statistical analysis (Snedecor and Cochran, 1989). The results of the study were subjected to one or two way analysis of variance (ANOVA) test.

**Results and Discussion**

**Haemagglutination Inhibition (HI) titres against NDV:**

The respective means $\pm$ SE for 0, 15, 30 and 60 ppm thiram were 43.33 $\pm$ 7.62, 20.5 $\pm$ 9.2, 20.66 $\pm$ 4.11 and 23.33 $\pm$ 3.65 as shown in Table 1. Comparison of means revealed highly significant ( $p<0.01$ ) difference between the control and thiram treated groups. There was a highly significant decrease in the HI titre against NDV in the thiram treated groups. No significant differences were observed among the thiram treated groups. The reduction in the HI titre against NDV observed in this study correlated with lymphoid cell depletion and lymphocytolysis observed histopathologically in the lymphoid organs affecting the humoral immunity.

**Histopathology**

**Bursa:** Mild lymphoid depletion which was prominent in the medulla in the 15 ppm group and moderate to severe lymphoid depletion with focal plical epithelial

proliferation were seen in the 30 and 60 ppm groups in the fourth week.

**Spleen:** During the second week congestion, lymphocytolysis in the subcapsular area and germinal center, apoptotic bodies, mitotic figure in germinal center and reticulum cell hyperplasia were observed in the 15 ppm thiram fed group. Multifocal lymphocytolysis including the germinal centers and reticulum cell hyperplasia were observed in the 30 ppm group. The 60 ppm thiram fed birds showed moderate lymphoid depletion and heterophilic infiltration. There were a few germinal centers which showed lymphoid depletion. In the fourth week moderate lymphoid depletion was noticed in the 15 ppm group and severe lymphoid depletion with reticulum cell hyperplasia were seen in the 30 and 60 ppm thiram fed groups.

**Thymus:** The 15 ppm thiram fed birds showed severe medullary congestion, cortical haemorrhage, medullary haemorrhage and heterophilic infiltration in the second week. Medullary congestion was observed in all the thiram fed birds in the fourth week .

**Caecal tonsils:** Mild to moderate depletion of nodular and diffuse lymphoid tissue and proliferation of crypt epithelium were seen in all thiram fed birds during the second week. Mild lymphoid depletion with an increase in lymphoid follicles were observed in the 30 ppm group and lymphoid depletion with lymphocytolysis were observed in the 60 ppm group during the fourth week. Thus lymphoid depletion and lymphocytolysis were the consistent lesions observed in the bursa, spleen and caecal tonsils of the thiram fed birds, the effect being dose and time dependent. Congestion and haemorrhage were observed in the thymus. These changes indicated the immunosuppressive potential of the toxin.

**Lymphocyte proliferation assay:** The respective means $\pm$ SE, stimulation index S.I. for 0, 15, 30 and 60 ppm thiram were 1.48 $\pm$ 0.10, 0.91 $\pm$ 0.04, 0.85 $\pm$ 0.04 and 0.86 $\pm$ 0.03 as shown in Table 2. Comparison of means revealed highly significant ( $p<0.01$ ) difference between the control and thiram treated groups. There was a highly significant decrease in the stimulation index in the thiram treated groups in the fourth week. Feeding 15, 30 and 60 ppm thiram in broiler chicken for 28 days resulted in significant ( $p<0.01$ ) reduction in mitogenic response of splenocytes to concavalin A. This correlated well with the damage to the lymphoid organs as shown by histopathology. No comparative studies were found for thiram induced changes in blastogenic response of splenocytes to concavalin A.

The findings testified the effect of feeding lower levels of thiram to broiler chicken affecting the health and

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performance. The toxin also has the potential to affect the immune organs leading to reduced humoral and cell mediated immunity which was indicated by low level of HI titre against NDV and lowered stimulation index in the thiram fed groups. These findings corroborated with the histopathological findings. The immunosuppression might also predispose to other diseases causing economic losses to the poultry industry.

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