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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

Pathomorphological Changes in Thiram Toxicosis in Broiler Chicken

S. Subapriya, S. Vairamuthu, B. Murali Manohar and C. Balachandran

Department of Veterinary Pathology, Madras Veterinary College, Chennai 600 007, India

Abstract: Thiram a fungicide used for treating corn and for storing food grains were incorporated at 15, 30 and 60 ppm into the toxin free diet of broiler chicken for four weeks from the day of hatch. The clinical signs were reduced weight gain, lameness, abnormal bending of the tibial bones, enlarged hock joints and sternal recumbency. Grossly tibial dyschondroplasia was seen as a white opaque unmineralized cartilage plug in the tibiotarsus. Histopathologically, tibiotarsus revealed thinning of the growth plate, irregular arrangement of the proliferating layer, abnormal thickening of the transitional layer and thickened hypertrophic layer in the thiram fed groups. Changes were also seen in the liver, bile duct, kidney, heart, crop, gizzard, intestine, lymphoid organs including bursa, spleen and caecal tonsils and brain. There was no mortality at any levels of thiram tested. Results of the present study thus revealed that thiram at a level of even 15 ppm affected the health and performance of broiler chicken.

Key words: Broiler chicken, histopathology, thiram, tibial dyschondroplasia

Introduction

Thiram, tetramethyl thiuram disulphide an organic sulphur compound is a fungicide commonly used for treating corn and other grains intended for seed purposes and also for storing food grains. Treated grains occasionally find their way into the market (Vargas *et al.*, 1983). The most dramatic effect produced by feeding diets containing thiram is tibial dyschondroplasia in birds. Grossly tibial dyschondroplasia is seen as a white, opaque cartilage plug in the growth plate region of the tibiotarsus. Histologically it is an accumulation of avascular cartilage consisting mainly of transitional chondrocytes characterized by smaller ovoid chondrons and more matrix than the normal hypertrophic cartilage (Leach and Nesheim, 1965).

Materials and Methods

Day old broiler chicks were obtained from a commercial hatchery. Thiram at graded levels were incorporated into the toxin free diet of the treatment groups so as to have a concentration of 15, 30 and 60 ppm. The birds were sacrificed at second and fourth week.

After exsanguination, a detailed post mortem was conducted on each sacrificed bird. Gross lesions observed were recorded. Representative pieces of tissues from liver, kidney, crop, proventriculus, gizzard, intestine, pancreas, bursa of fabricius, spleen, thymus, spinal cord, brain, heart and tibiotarsus were collected in 10 percent formal saline. The bones were decalcified in 5% nitric acid and paraffin embedded tissues were sectioned to get 5 μ thickness sections and stained by haematoxylin and eosin (H and E) for histopathological examination (Bancroft and Stevens, 1996).

Results and Discussion

Clinical signs: The thiram treated birds showed reduced weight gain, lameness and reluctance to move. The locomotory disturbances were observed 12 days after feeding 15-60 ppm thiram containing diets. After 21 days of feeding thiram, enlarged hock joints, shortened tibiotarsus, extended limbs, curled toes and straddles were observed. The severely affected birds were on sternal recumbency. The clinical signs of reduced weight gain, lameness and enlarged hock joints, shortened tibiotarsus, extended hindlimbs and sternal recumbency observed in this study were reported earlier (Veltmann *et al.*, 1985). Curled toes and straddles observed in the study were also reported earlier by Waibel *et al.* (1957).

Morbid pathology: Grossly the pathological changes were found in tibiotarsus. Abnormal bending of the tibiotarsus was observed in all the thiram fed groups at second and four weeks of age. Tibial dyschondroplasia was observed on longitudinally sectioning the bone as a mass of white, opaque cartilage, irregular in shape and size below the epiphyseal hyaline cartilage region and extending into the metaphysis. Liver showed congestion to paleness. Tibial dyschondroplasia was observed on slicing the bone as a mass of white opaque cartilage, irregular in shape and size below the epiphyseal hyaline cartilage. This correlated with the findings of Lakshman *et al.* (2002). Similar changes were also reported earlier (Riddell *et al.*, 1971; Veltmann *et al.*, 1985; Rosselot *et al.*, 1994).

Histopathology

Liver: The 15 ppm thiram fed birds showed venous and sinusoidal congestion in the second week. The

hepatocytes revealed vacuolar degeneration and focal necrosis. Micro granuloma and micro vesicular to macro vesicular fatty changes were also observed in the hepatocytes. Kupffer cells were hypertrophic. Focal mononuclear cell collection was observed. Portal triad showed congestion of portal vein, mild periportal fibrosis, mononuclear cell infiltration and biliary hyperplasia. Occasionally degenerative changes were observed in the bile duct epithelium. Catarrhal cholangitis was also observed. Periductular fibrosis was evident in a few cases.

The 30 ppm thiram fed birds showed focal necrosis of hepatocytes and marked biliary hyperplasia with periductular mononuclear cell infiltration besides the 15 ppm changes in the second week. The liver of 60 ppm thiram fed birds showed diffuse micro vesicular fatty changes, bile duct hyperplasia, lymphocyte infiltration hypertrophy of Kupffer cells, Micro granuloma, venous dilatation and perivenous fibrosis in the second week.

The liver of 15 ppm thiram fed birds in the fourth week showed diffuse vacuolar degenerative changes and hypertrophy of Kupffer cells. Micro granuloma and congestion were seen along with bile duct stasis, perivenous fibrosis and bile duct hyperplasia. The 60 ppm group of fourth week showed bile duct hyperplasia, focal mononuclear cell collection and micro vesicular fatty changes in the hepatocytes.

Vacuolar degenerative changes in the hepatocytes, mononuclear cell infiltration and bile duct hyperplasia were the consistent changes in all the thiram fed birds. These indicated the route of entry of the toxin by entero-hepatic circulation as the toxin was fed through the diet.

Kidney: Congestion and tubular epithelial cell degeneration and necrosis were seen in the 30 and 60 ppm thiram fed groups in the second week. These changes were observed even at 15 ppm level in the fourth week. Focal interstitial mononuclear cell infiltration accompanied these changes in 30 and 60 ppm groups.

Heart: Degeneration, loss of striations and granularity of cytoplasm in the cardiac myocytes were observed in the myocardium in the second and fourth week in all thiram fed groups. Further the 30 ppm group showed myocardial edema.

Degeneration, loss of striations and granularity of cytoplasm were seen in the cardiac myocytes in the second and fourth week in all thiram fed groups. Similar degenerative changes were also observed earlier by Nageswara *et al.* (1996) in layers.

Crop: The 60 ppm thiram fed birds showed mild mucosal keratinization of crop in the second week and hyperplastic crop mucosa with keratinization in the fourth week.

These changes could be attributed to the local irritant action of thiram on the crop mucosa.

Proventriculus: The proventriculus from 15 ppm thiram fed birds showed focal hyperplastic changes in the mucosal epithelium, partial mucosal epithelial necrosis a few dilated crypts, infiltration of lymphocytes into lamina propria and focal mucosal fibrosis. Some proventricular glands showed necrosis in the second week. The 30 and 60 ppm groups showed partial to full thickness necrosis of the mucosal epithelium, infiltration of lymphocytes into lamina propria, elongation of the villi and muscular degeneration in the second week.

In the fourth week, the 15 ppm thiram fed birds showed mucosal hyperplasia with necrosis, focal collection of mononuclear cells and heterophilic infiltration in the proventricular gland. The 30 to 60 ppm group revealed proventriculitis and elongation of the villi with multifocal mononuclear cell infiltration.

These findings indicated a dose dependent damage to the proventriculus. The lesion in the glandular stomach could affect the digestive process.

Gizzard: The 15 ppm thiram fed birds in second week showed dilatation of glands without secretion. The 30 ppm group showed dilatation of glands with focal necrosis and mononuclear cell infiltration in the lamina propria. The 60 ppm group showed distended gland with lumen containing the secretion and lamina propria infiltrated with mononuclear cells. The 15 ppm group in fourth week showed distended gland with secretion within the lumen. A mild increase in the fibrous tissue in the 30 ppm group and increased interstitial fibrosis in the glandular area of 60 ppm group were observed.

Dilatation of glands, focal necrosis, mononuclear cell infiltration and increased interstitial tissue fibrosis were the dose dependent changes in the gizzard. This could interfere with grinding of feed and affect the digestion and absorption in the intestine.

Intestine: Catarrhal changes and partial to full thickness necrosis of the villi leaving the crypts, catarrhal changes in the crypt epithelium and infiltration of mononuclear cells in the lamina propria were seen in all thiram fed birds in the second week. The 30 and 60 ppm thiram fed birds showed fusion of villi in the fourth week in addition to the changes seen in the second week. This could lead to reduced absorptive surface area of the intestine. These alimentary tract lesions affected the digestion and absorption of nutrients as was evident from the reduced weight gain of the birds despite increased feed consumption.

Pancreas: Degeneration of exocrine acinar cells, ductular hyperplasia with necrosis of acinar cells, distorted acini with mononuclear cell infiltration and

reduced zymogen granules in acinar cells were seen in all thiram fed birds in the second and fourth week which indicated the toxic effect of thiram on pancreas.

Lymphoid organs: 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.

Brain: Congestion and submeningeal focal gliosis in cerebrum and necrosis with satellitosis and apoptotic bodies in cerebellum were observed in 15 ppm thiram fed birds in the second week. The 30 ppm thiram fed birds showed cerebellum undergoing necrosis while 60 ppm group birds showed neuronal degeneration in Purkinje cells, congestion of vessels in the meninges and subependymal gliosis in the second week. The 60 ppm thiram fed birds showed congestion of meningeal vessels in the fourth week.

Necrotic and degenerative changes observed in the brain which were also observed earlier by Nageswara *et al.* (1996).

Bone

Epiphyseal hyaline cartilage: The epiphyseal hyaline cartilage was of normal thickness in both the thiram fed and control groups.

Growth plate: Thinning of the growth plate was observed in the thiram fed groups of second and fourth week.

Proliferating layer: Proliferating layer lacked parallel arrangement and was found in irregular columns. Flattened chondrocytes were seen. Reduced vascularity and focal areas showing clusters of osteoblasts at the terminal end were seen.

Transitional layer: An abnormal thickening of the transitional layer was seen. The chondrocytes were ovoid with more eosinophilic matrix.

Hypertrophic layer: The hypertrophic layer was thickened and invaded by a few blood vessels which appeared empty. Eosinophilic matrix, pyknotic nuclei and empty capsules were seen. The bone trabeculae were normal in size but were irregularly arranged. Defective cartilaginous mould, abrupt ossification, osteoblastic and osteoclastic activity and transition of cartilage to bone tissue were seen.

There was a dose dependent increase in the number of non nucleated chondrocytes within the treatment groups when compared to the control in both the second and fourth week. In the tibiotarsus, thinning of the growth

plate, thickening of the proliferating layer, pyknotic nuclei, increase in eosinophilic matrix and empty capsules were seen in the hypertrophic layer of the thiram fed birds which correlated with the earlier findings (Lakshman *et al.*, 2002).

Dyschondroplasia in thiram toxicity has been particularly confined to the tibiotarsus as it is a long bone developing by endochondral ossification with increased metabolic activity. Tibial dyschondroplasia has been suggested to occur by the alteration in the extracellular matrix and reduced vascularization. Defective extracellular matrix was due to the absence of type×collagen deposition. Reduced vascularity was due to the increase in anti invasion factor produced by the altered extracellular matrix which impairs the penetration of the metaphyseal vessels and due to reduced angiogenesis (Webster *et al.*, 2003). Thorp (1994) reported that tibial dyschondroplasia differed from rickets. In rickets the proliferating layer or the hypertrophic layer was thickened whereas in dyschondroplasia the transitional layer was found to be abnormally thickened. However no mortality was observed in the birds fed with different levels of thiram. This indicated that the dose levels of thiram employed in this study were sublethal. However, further studies are required to assess other harmful effects of this toxin at the minimum dose levels which would affect the health and performance of broiler chicken in order to advise the poultry industry about the probable subtle effect of thiram toxicosis.

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