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Hypervitaminosis D_3 in broiler chicks: histopathological, immunomodulatory and immunohistochemical approach

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Summary

The present study was undertaken to investigate the toxic effects of higher doses (1,00,000 IU i.e. 2.5 mg/kg body weight (BW)) of vitamin D_3, concomitantly with bacterial endotoxins (lipopolysaccharides: LPS) to study the immunomodulatory potential of vitamin D_3 in IBL-80 broiler chicks. The chicks were divided into four groups [group I (NSS), group II (LPS), group III (Vit. D_3 + NSS), and group IV (Vit. D_3 + LPS)] containing eight chicks in each group, treated accordingly for 21 days. Birds were kept under close observation for apparent clinical signs and symptoms. Clinically, vitamin D_3 treated chicks were dull, off feed, showed polydipsia, polyuria, watery faeces, rigidity of limbs, severe dehydration, weakness and significant progressive emaciation. Grossly, the bones were soft whereas most organs revealed congestion and hemorrhages in visceral organs. Histopathologically, renal tubular epithelium showed coagulative necrosis and metastatic calcification. The lung parenchyma and bronchi showed hemorrhages, congestion with diffuse heterophilic cell infiltration in inter-alveolar septa and infiltration of heterophils in alveoli along with proteinacious fluid in LPS treated chicks of treatment group, suggesting immunomodulatory action of vitamin D_3. Vitamin D_3 treated chicks showed strong expression of Calbindin D28k in intestine and kidney but weak expression in lung, which can be linked to nephrocalcinosis seen in kidney and from its prospective role in cellular calcium homeostasis.

Key words: Broiler chick, Calbindin D28K, Cholecalciferol, Immunomodulation, LPS

Introduction

A nutritionally balanced diet helps the broiler birds remain healthy. One such nutrient is vitamin D_3, which is very beneficial for the bird. However, if it is found in excess in the body, it will result in vitamin D_3 toxicity. Vitamin D_3 is a fat-soluble vitamin that regulates the concentration of calcium and phosphorus in the body. To use vitamin D_3 following ingestion, the body metabolizes it to 25-hydroxycholecalciferol (25(OH) D_3) in the liver and successively into its active metabolite 1,25-dihydroxycholecalciferol (1,25$(OH)_2D_3$) in the kidneys. It is involved in the physiological processes like absorption of calcium and phosphorus, bone mineralization and mobilization (Rennie and Whitehead, 1996; Driver *et al*., 2005; Korver, 2005; Kasim *et al*., 2006). When plasma calcium level falls, vitamin D_3 stimulates release of calcium from bone and limits calcium excretion by the kidneys. Furthermore, in the body vitamin D regulates the secretion of parathyroid hormone (PTH) and stimulates several tissues with vitamin D receptors (Norman, 1985).

Broiler diets are frequently fortified with vitamin D_3 to prevent commonly occurring bone problems. Since the basal levels of dietary vitamin D_3 are rarely known, there is always a risk of over-supplementation (Nain *et al*., 2007), which causes toxicity and promotes deposition of calcium and phosphate as crystals in the kidneys, heart and major blood vessels. Young broiler chicks have a tolerance for excess vitamin D_3 as high as 50,000 IU per kg with no apparent negative effects on bone mineralization and growth (Baker *et al*., 1998). Based on renal calcification and body weight (BW), 25-(OH) D_3 was found to be 5 to 10 times more toxic than vitamin D_3 (Yarger *et al*., 1995a). Hypervitaminosis D in the chicken and turkey has been associated with renal tubular and, less commonly, arterial mineralization (Scott *et al*., 1978). Hypercalcemia, hyperphosphatemia, soft tissue mineralization and bone resorption are consistent findings with hypervitaminosis D in mammalian species (Hass *et al*., 1958; Jubb *et al*., 1970; Kirui *et al*., 1981). In poultry, vitamin D_3 toxicity causes anorexia, reduced eggshell quality, reduced egg production, reduced egg weight, renal tubular calcification, muscular atrophy and emaciation (NRC, 1987).

Lipopolysaccharides (LPS) are cell wall components of Gram-negative bacteria, which cause release of cytokines that regulate different metabolic responses and cause fever, inflammation and cachexia (Abbas *et al*., 1997). Studies on the effects of LPS on poultry are limited compared with studies in mammals because it was generally considered that birds might be relatively more resistant to endotoxins than mammals (Roeder *et al*., 1989). There is a paucity of information on the effects of LPS on cytokines and the acute phase response in birds and their relationship with inflammation and homeostasis.

A number of clinico-pathological studies have been carried out on vitamin D_3 toxicity in animal models. However, the combined effects of toxicity of vitamin D_3 and bacterial endotoxins in broiler chicks have not been tested. The present study has been designed keeping in mind the effect of excess vitamin D_3 and LPS in broiler chicks.

Materials and Methods

Animal source, housing and management

Day old broiler chicks of strain IBL-80 were obtained from the hatchery, Department of Animal and Genetics Breeding (AGB), College of Veterinary Science, GADVASU, Ludhiana. The chicks were housed in cages at room temperature with 12:12 h light dark cycle. The chicks were provided *ad libitum* feed (standard broiler feed supplied by Godrej Agrovet Limited, Khanna) and drinking water.

Chemicals

Vitamin D_3 was purchased from Sigma Life Science, St. Louis, USA. Stock solution of Vitamin D_3 in groundnut oil as per the dose regimen was prepared and kept in refrigerator. The solution was brought to room temperature before use. Anti-Calbindin D28k was purchased from Sigma Aldrich Chemicals Pvt. Ltd.

Experimental protocol

The experiment was conducted after approval by Institutional Animal Ethics Committee (IAEC), GADVASU, Ludhiana. The chicks were kept for 7 days to acclimatize tolaboratory conditions prior to start of sampling protocols. On day 7, the chicks were randomly divided in 2 groups (control and treatment) (n=16 chicks) and further subdivided into four groups as shown in Table 1.

Treatment group was administered vitamin D_3 in groundnut oil[@] 2.5 mg/kg BW daily. On day 28, 8 chicks from the control group were challenged with NSS[@] 0.5 ml/chick (group A) and other 8 chicks were challenged with LPS[@] 0.5 ml/chick by intranasal route (group B) and then sacrificed after 12 h. Similarly, 8 chicks from the treatment group were challenged with NSS[@] 0.5 ml/chick (group C) and the other 8 chicks were challenged with LPS[@] 0.5 ml/chick by intranasal route (group D) on day 28 of the study. The chicks in the control group and treated group were sacrificed after 12 h of LPS challenge by cervical dislocation.

Collection, processing and staining of tissue samples for histopathology

The necropsy of dead chicks sacrificed on day 28 was performed immediately and the tissue samples of various organs were collected in 10% neutral buffered formalin for histopathological examination. The formalin fixed tissues were washed overnight in running tap water, dehydrated in ascending grades of alcohol and cleared in benzene. The 4-5 μm thick tissue sections were cut from the paraffin embedded tissues and were stained with haematoxylin and eosin (H&E) stain for routine histopathology. Von Kossa stain (Luna, 1968) was used to demonstrate calcium in tissue sections.

Immunohistochemistry

The necropsy of sacrificed chicks was performed and tissue samples were collected in 10% neutral buffered formalin. The formalin fixed tissues were washed overnight in running tap water, dehydrated in ascending grades of alcohol and cleared in benzene. The 4-5 μm thick tissue sections were obtained on poly-L-lysine coated clean glass slides. Following dewaxing, antigen retrieval was performed by citrate buffer by using EZ-Retriever™ System (Biogenex Laboratories Inc., SanRamon, California, USA). After unmasking antigen, tissue sections were washed thrice with PBS (pH = 7.2-7.4) for 5 min each time. The endogenous peroxidase was quenched with a solution of 3% H_2O_2 in methanol for 15 min at room temperature in a humid chamber, followed by 3 washings with PBS (pH = 7.2-7.4) for 5 min each time. After blocking nonspecific sites with 2% normal serum in PBS buffer for 30 min, the sections were treated with primary antibody (anti-Calbindin D28k Sigma, USA) overnight in a humid chamber at 4°C. The sections were then washed three times in PBS for 5 min each, followed by incubation in biotinylated secondary antibody, tissue sections were incubated with Vectastain ABC reagent for 30 min in a humid chamber followed by 3 washings with PBS for 3 min each. The sections were incubated in 3,3'-Diaminobenzidine (DAB) solution for 1-3 min for color development. The sections were counter stained with hematoxylin. In negative control, tissue sections were processed without application of primary antibody.

Table 1: Experimental design showing treatment and description about the groups

Group	Treatment	On day 28
Control (n=16)	Control group was given only oil with *ad lib* feed and water	**Group A:** 8 chicks were challenged with NSS[@] 0.5 ml/chick by intranasal route and sacrificed after 12 h **Group B:** 8 chicks were challenged with LPS[@] 0.5 mg/chick by intranasal instillation and sacrificed after 12 h
Treatment (n=16)	Treatment group was given vitamin D_3 (in oil) @ 2.5 mg/kg BW daily for 21 days by oral route	**Group C:** 8 chicks were challenged with NSS[@] 0.5 ml/chick by intranasal route and sacrificed after 12 h **Group D:** 8 chicks were challenged with LPS[@] 0.5 mg/chick by intranasal instillation and sacrificed after 12 h

Statistical analysis

The results were expressed as mean±SE. To assess the significance of the differences between the groups of broiler chicks in experiment, statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey, Duncan and LSD with significance assessed at 5% confidence level. Calcification score was statistically determined by using Mann-Whitney U test.

Results

Clinical signs

The broiler chicks were acclimatized for 7 days. Clinical signs were observed in control chicks (n=16) challenged with NSS (group A) or LPS (group B) 12 h before sacrifice and chicks were treated with vitamin D_3[@] 2.5 mg/kg BW orally daily and challenged with NSS (group C) or LPS (group D) 12 h before sacrifice. Prior to vitamin D_3 administration chicks showed normal feed and water intake and were alert. The chicks of treatment group (groups C and D) were given vitamin D_3[@] 2.5 mg/kg BW orally dissolved in groundnut oil, single dose daily on day 8 onwards till day 28. On day 14, chicks of treatment group (C and D) showed watery faeces and polyuria. The broiler chicks were dull, showed decrease in feed intake and increased water intake. These signs became more severe with course of treatment. On day 22 and onwards the vents of broiler chicks (treatment group) were soiled. The limbs showed rigidity and chicks showed difficulty in movement. The chicks were resting on the hocks. The body coat was ruffled, soiled and the birds showed severe progressive emaciation, dehydration and weakness.

Body weight

The mean BW (g) in control chicks (n=16) (groups A+B) and chicks (n=16) treated with vitamin D_3[@] 2.5 mg/kg BW orally daily (groups C+D) on day 7 and day 28 are given in Table 2.

Table 2: Effect of oral administration of vitamin D_3 (2.5 $mgkg^{-1}day^{-1}$) on body weight (mean±SE)

Day	Body weight (g)	
	Control group (n=16)	Treatment group (n=16)
Day 7	86.25 ± 2.86	88.12 ± 2.45
Day 28	708.75 ± 14.88*	366.88 ± 34.87*

Control group: control feed, and treatment group: control feed + vitamin D_3[@] 2.5 mg/kg BW. * Indicates values differ significantly within a row at 5% level of significance by independent sample t-test

The mean BWs of control group and treatment group chicks were 86.25 ± 2.86 and 88.12 ± 2.45 g, respectively on day 7 (Table 2). The mean BWs of chicks from control and treatment group were 708.75 ± 14.88 g and 366.88 ± 34.87 g, respectively on day 28 (Table 2). There was a significant decrease in the BW of chicks treated with vitamin D_3[@] 2.5 mg/kg BW orally daily as compared to control chicks.

The mean BW of chicks of treatment group decreased by 51.69% as compared to mean BW of chicks of control group on day 28.

Gross post-mortem changes

Control group chicks did not show any appreciable gross lesions. The chicks from treatment group (vitamin D_3 @ 2.5 mg/kg BW) sacrificed on day 28 though showed decrease in BW, emaciation and ruffled body coat. The bones broke easily. The lungs of chicks of treatment group revealed focal hemorrhages and congestion. The kidneys of chicks of treatment group were yellowish white and focally showed mild hemorrhages and congestion. Brain and pancreas of chicks of treatment group (groups C+D) were slightly congested. Other organs did not show any significant gross lesions.

Histopathological findings

The following histopathological changes were observed in the tissues collected from sacrificed broiler chicks showing clinical signs of toxicity on day 28. The histopathological changes are presented below according to severity of calcification and lesions:

1) Lungs: In-group A; no pathological changes were observed.

In-group B; the lung parenchyma showed hemorrhages and congestion with marked heterophilic cell infiltration. The bronchi showed hemorrhages and congestion with heterophilic cell infiltration.

In-group C; lung sections showed congestion and massive hemorrhages beneath bronchiolar epithelium and no calcification was observed (Fig. 1A).

In-group D; histopathologically, the lung parenchyma showed hemorrhages and congestion with diffuse and strong heterophilic cellular infiltration in inter-alveolar septas besides presence of heterophils in alveoli along with proteinacious fluid (Fig. 1D). Bronchi showed hemorrhages and mucous in parabronchiolar area (Fig. 1G) and marked heterophilic infiltration but there was no calcification in the lungs and bronchi.

2) Kidney: No pathological lesions were observed in-group A and B.

In-group C; coagulative necrosis of renal tubular epithelium was observed along with congestion of glomerulus (Fig. 1B). Focal areas of slight to mild metastatic calcification were observed in renal tubules and renal parenchyma. Fibrosis was also observed (Fig. 1C).

In-group D; coagulative necrosis of renal tubular epithelium was observed along with hemorrhages. Slight to mild metastatic calcification was observed in renal tubules (Fig. 1E).

3) Ureter: No pathological lesions were observed in ureter of broiler chicks of group A and B.

In-group C and D; slight to mild focal calcification was observed in wall of ureter.

4) Proventriculus: No pathological lesions were observed in proventriculus of broiler chicks from group A and B.

In-group C; slight calcification was observed in

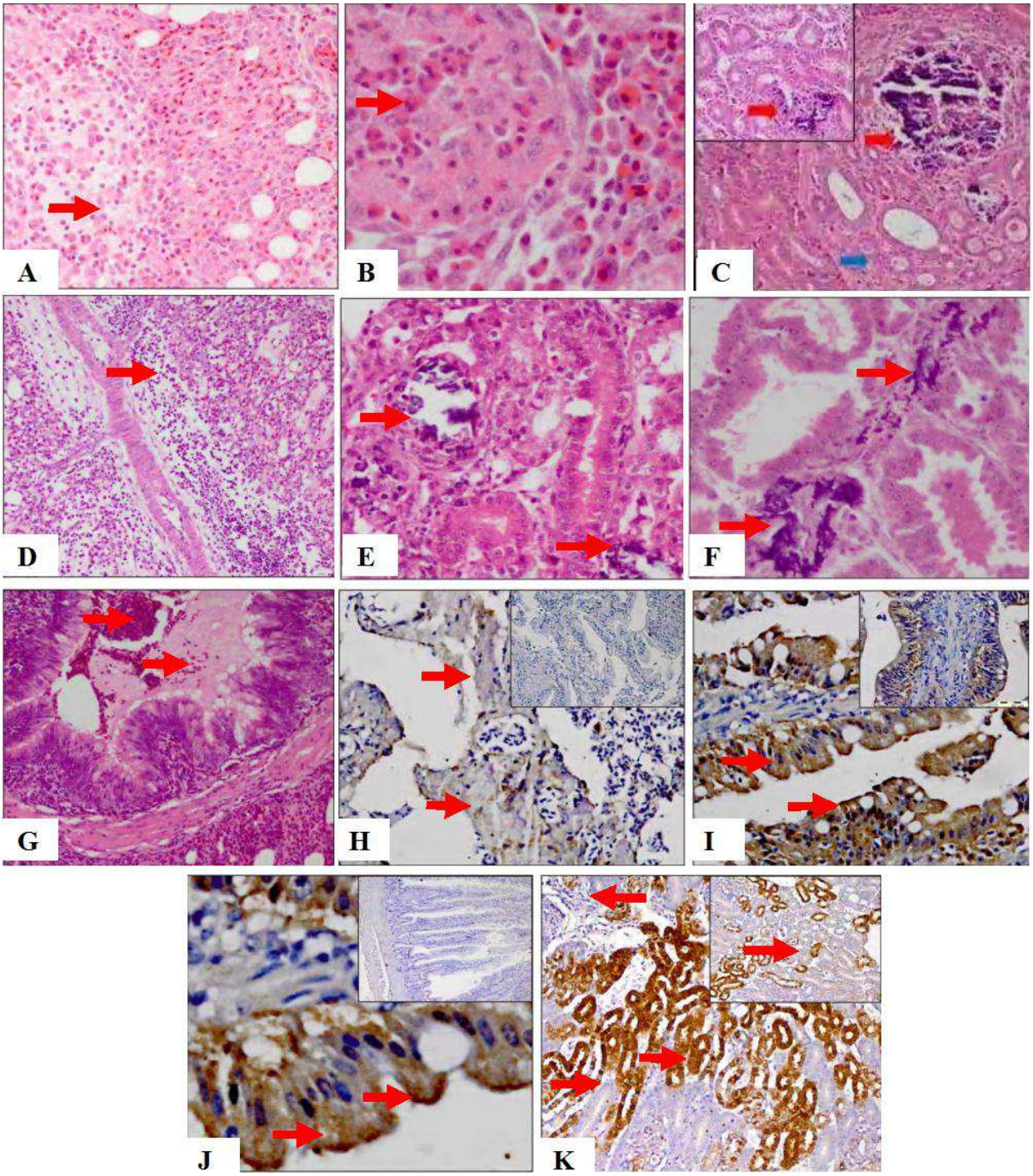

Fig. 1: Histological sections of various organs from chicks in the experiment showing histopathological, immunomodulatory and immunohistochemical changes in different organs. **A**) Section of lung from chicks of group C showing congestion and hemorrhages with proteinaceous fluid (red arrow), (H&E, ×4), **B**) Section of kidney from chicks of group C showing congestion of glomerulus (red arrow), (H&E, ×20), **C**) Section of kidney from chicks of group C showing focal areas of calcification (red arrow) with fibroplasia (blue arrow) in renal parenchyma (inset: calcification (red arrow) in renal tubules), (H&E, ×20), **D**) Section of lung from chicks of group D showing marked diffuse heterophilic cell infiltration in interlobular septas besides presence of heterophils in air spaces (red arrow) along with proteinecious transudate, (H&E, ×20), **E**) Section of kidney from chicks of group D showing calcification (red arrow) in renal tubules, (H&E, ×20), **F**) Section of proventriculus from chicks of group D showing calcification (red arrow) in tunica propria and connective tissue, (H&E, ×20), **G**) Section of lung from chick challenged with LPS (group D) showing parabronchiolar congestion and mucous secretion (red arrow), (H&E, ×20), **H**) Section of lung showing weak expression of Calbindin D28k (brown colour) (red arrow), (IHC, ×40). Inset negative control, **I**) Section of intestine showing strong expression of Calbindin D28k (red arrow) as shown by intense brown color in intestinal villi, (IHC, ×40). Inset showing weak expression of Calbindin D28k (arrow) in control group, **J**) Section of intestine showing strong expression of Calbindin D28k (red arrow) as shown by intense brown colour in intestinal villi, (IHC, ×100). Inset showing negative control, and **K**) Section of kidney showing strong expression of Calbindin D28k in renal tubules (red arrow), (IHC, ×20). Inset showing weak expression of Calbindin D28k (red arrow) in control group

Table 3: Comparative calcification of different organs in vitamin D toxicity in broiler chicks

Organs	Groups				P-value	
	Control (n=16)		Treatment (n=16)			
	A	B	C	D	A and C	B and D
Proventriculus	--	--	+ to ++	+ to ++	0.005	0.005
Kidney	--	--	+ to ++	+ to ++	0.005	0.005
Ureter	--	--	+ to ++	+ to ++	0.005	0.005

Group A: Control feed + NSS, Group B: Control feed + LPS, Group C: Control feed + vitamin D_3[@] 2.5 mg/kg BW, and Group D: Control feed + vitamin D_3[@] 2.5 mg/kg BW + LPS. Mean calcification score, -- : No change, + : Slight, ++ : Mild. Statistical difference between group A, B, C and D was determined by Mann-Whitney U test. Significance assumed at $P<0.05$

proventricular glands only and in tunica propria and connective tissue of proventriculus (Fig. 1F), of birds of group D.

Other organs viz. brain, esophagus, thymus, bursa of fabricius, ovary adrenal, liver, pancreas, spleen, aorta, heart, trachea, gizzard, small and large intestine, tongue, spinal cord, nerves, skin, skeletal muscles, eyes, parathyroid and thyroid did not show any histopathological alterations under light microscopy.

The calcification score was recorded and subjected to statistical analysis by Mann-Whitney U test. The p-values by comparision between the groups were estimated to check the significant difference between the calcification in different groups (Table 3).

The calcification score is given in Table 3. Various organs viz. proventriculus, kidneys and ureters as mentioned in Table 3 showed significant difference ($P=0.05$) between calcification scores in different groups.

Slight to mild calcification was observed in proventriculus, kidneys and ureters of chicks of treatment group (group C and D) and no calcification was observed in the above mentioned organs of chicks of control group (group A and B).

The mechanism of calcification/mineralization and degenerative changes observed in various organs in vitamin D_3 toxicity and also in present study is well explained by many authors.

In present study, calcification was found in proventriculus, kidneys and ureters only. No mineralization of tissues was observed in heart, aorta, intestines, tongue, trachea, spleen and brain. Thus, in chicks heart, aorta intestines, tongue, trachea, spleen and brain are resistant to vitamin D_3 toxicity as compared to rats (Chavhan *et al*., 2011).

Immonohistochemical expression of Calbindin D28k

The Calbindin D28k expression was studied in intestine, kidney and lung sections that showed appreciable metastatic calcification. In present study vitamin D_3 treated chicks showed strong expression of Calbindin D28k in intestine (Figs. 1I, J) and kidney (Fig. 1K) but weak expression in lungs (Fig. 1H) than in the control chicks.

Discussion

In the present study the clinical signs observed like anorexia, diarrhoea, progressive emaciation/weight loss, dehydration, weakness and difficulty in movement are in agreement with earlier findings in different species reported by Long (1984), Braun *et al*. (2000), Radostits *et al*. (2000), Roberson *et al*. (2000), and Sandhu and Brar (2009) in vitamin D_3 toxicity. Machlin (1984) also reported similar clinical signs like anorexia, polyuria, polydypsia, regurgitation, weight loss, depression and renomegaly. Polydypsia and polyuria may be explained as in hypercalcemic state, the ability of the renal tubules to respond to ADH decreases, thus inhibiting the absorption of water (Meric, 1995). Calcification in the kidney may also contribute to polyuria. Other clinical signs like muscle weakness, painful joints, demineralization of bone and disorientation may be present. These abnormalities eventually lead to death (Kaneko, 2008).

The decrease in BW in vitamin D_3 toxicity has been reported in poultry birds by Riddell (1975b), Morrissey *et al*. (1977), Poulos *et al*. (1978), Edwards and Sorensen (1987), Edwards *et al*. (1992), Rennie *et al*. (1993), and Thorp *et al*. (1993). This may be due to adverse effects of high doses of vitamin D_3 on growth and BW gain. Mulligan and Stricker (1948) conducted experiment on vitamin D_3 toxicity in dogs. They reported loss in BW of about 62% of their original BW in the vitamin D_3 treated dogs. However, Harrington and Page (1983) reported 29% BW loss due to vitamin D_3 toxicity in horses. The unregulated increase in plasma calcium and phosphorus in vitamin D_3 toxicity cause mineralization of tissues/organs like kidneys, GIT, cardiac muscles, skeletal muscles, blood vessels and ligaments when calcium × phosphorus rises above 130 and cause structural damage that leads to decreased functional capacity of these tissues and organs (Morrow, 2001). This loss of function further contributes to the development of severe clinical signs. In present study, hypercalcaemia along with mineralization of various organs was observed. Thus, it may be concluded that the clinical signs were the result of biochemical and histopathological alterations in various tissues/organs due to vitamin D_3 toxicity.

The histopathology of respiratory system and immunomodulatory findings of this study are in accordance with Lorenzoni *et al*. (2008) who reported that intra-tracheal administration of LPS elicits pulmonary hypertension. He determined the possible changes in the number or proportion of airway

leukocytes that could contribute to the magnitude of the pulmonary hypertensive responses elicited in broilers. Cells recovered from the lavage fluid from both groups were primarily heterophils. The concentration of leukocytes was greater in the lavage fluid of broilers from the LPS treated group compared with broilers from the control group, but the proportions among leukocytes were not different between the 2 groups. He proposed that the increased concentration of leukocytes present within the airways was one of the components that enabled broilers pre-treated with aerosolized LPS to exhibit PH responses to intra-tracheal LPS. In present study the heterophilic response was more in group D as compared to group B, suggesting immunostimulatory and immunostimulatory function of vitamin D_3 leading to more pronounced heterophilic cell reaction and infiltration.

The results of histopathology of excretory system in the present study goes with Morrissey *et al*. (1977) who found renal tubular calcification when vitamin D_3 was fed at the rate of 10.0 mg/kg of diet and when 25-OH vitamin D was fed at the rate of 0.1 mg/kg diet. It is suggested that in vitamin D intoxication, the factor responsible for the pathological changes in soft tissues is cholecalciferol itself. Metz *et al*. (1985) reported that large white male turkey poults when fed a diet containing a high level of vitamin D (9,00,000 ICU/kg), developed hypervitaminosis D as evidenced by mild growth depression and renal tubular mineralization. The lesions observed in present study in kidneys are in consonance with lesions reported by Hunt *et al*. (1972), Stevenson *et al*. (1976), Long (1984), and Morita *et al*. (1995) in cholecalciferol toxicity in different animal species. Hunt *et al*. (1972) studied a toxicity of cholecalciferol in Rhesus monkeys (*Macaca mulatta*). They reported the mineralization of cortical tubular epithelium, their basement membrane, within interstitium, in walls of renal blood vessels and within epithelium of collecting ducts in medulla.

Histopathologically, hypercalcemia induced extensive glomerular and renal tubular damage (nephrocalcinosis) were also reported in vitamin D_3 toxicity by Hunt *et al*. (1972), Stevenson *et al*. (1976), Long (1984), and Morita *et al*. (1995). Beasley (1999) reported the similar clinical signs like polyuria and proteinuria in vitamin D_3 toxicity.

Beasley (1999) and Price *et al*. (2001) reported the mechanisms of hypercalcemia in vitamin D_3 toxicity. In vitamin D_3 toxicity the active metabolites of cholecalciferol have been reported to increase the blood calcium level (hypercalcemia) by increased resorption/ mobilization of calcium from bone, increased absorption of calcium from intestine and decreased calcium excretion by kidney. The net result is high concentration of blood calcium level (hypercalcemia). The effect of hypercalcemia on cells includes altered cell membrane permeability, altered calcium pump activity, decreased cellular energy production and cellular necrosis. As the toxicity increases, clinical signs include hypertension, polyuria and extreme elevated serum calcium level may cause cardiac arrhythmias (Morrow, 2001) and death was reported due to renal and cardiac failure.

The mechanism of mineralization or calcification in vitamin D_3 toxicity is well explained by Morrow (2001). He reported that, with unregulated increase in plasma calcium and phosphorus in vitamin D_3 toxicity, their product (calcium × phosphorus) can rise above 130 which causes mineralization of tissues/organs like kidneys, GIT, cardiac muscles, skeletal muscles, blood vessels and ligaments and cause structural damage that leads to decreased functional capacity of these tissues and organs. The loss of function contributes to the development of ongoing end stage clinical signs as well as long-term signs in animals that survive. The cause of death reported in vitamin D_3 toxicity includes cardiac and renal failure.

The immunohistochemical findings in the present study are similar to Jande *et al*. (1981a, b), who localized Calcium-binding protein (CaBP) with the immuno-peroxidase method using antiserum against purified chick duodenal CaBP. Calbindin D28k was most evident in the distal convoluted tubule of kidney in chicken (Christakos *et al*., 1981; Roth *et al*., 1981; Taylor *et al*., 1982). In kidney it indicates that Calbindin D28k play a role in selective reabsorption of calcium and its regulation or modulation of a number of cellular processes in kidney. These results indicate that Calbindin immune-reactive sites were found to play a role in the regulation of intracellular calcium and it has a very basic and specific role in maintaining cellular calcium homeostasis.

The study concludes that Calbindin D28k play a vital role in calcium homeostasis, facilitating the absorption of calcium intensively from intestine and kidneys evidenced by calcification and feebly from lungs in hyper-vitaminosis D_3. Intense cellular reaction against LPS in lung section from chicks of vitamin D_3 treated group suggests immunomodulatory role of vitamin D_3.

Conflict of interest

No potential conflict of interest was reported by the authors.

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