Biochemical Alterations in Hypervitaminosis D$_3$ in Broiler Chicks Concomitantly Challenged with Endotoxin

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ABSTRACT

Vitamin D$_3$ is ten times more biologically active than vitamin D$_2$, over supplementation of vitamin D$_3$ causes hypercalcemia with deposition of calcium and phosphate as crystals in the visceral organs. Birds are considered more resistant to endotoxin shock and information on inflammation and homeostasis in birds supplemented with higher dose of vitamin D$_3$ when suffer endotoxic shock is lacking. The present study was conducted to compare the effect on hemoglobin concentration and biochemical parameters of broiler chicks by administering toxic dose of vitamin D$_3$ for 21 days concomitantly challenged with endotoxin. The chicks were randomly divided into four groups viz. A, B, C and D. Hemoglobin concentrations of control groups (A and B) and treatment groups (C and D) did not differ significantly (P<0.05). Hypercalcemia and hyperphosphatemia was observed in both treatment groups in comparison to the control group. No significant (P<0.05) change was observed in the concentrations of total protein and albumin and in the activity of plasma Alanine Aminotransferase, Aspartate Aminotransferase and Alkaline Phosphatase on day 28 of control (A and B) and treatment (C and D) groups. Vitamin D$_3$ supplementation causes immunomodulation; hence acute endotoxic shock does not incite inflammatory response and disturb the homeostasis in broiler chicks.

Key words: Broiler chicks, Hypercalcaemia, Hypervitaminosis D$_3$, Hyperphosphatemia,

INTRODUCTION

Vitamin D$_3$ is a fat-soluble vitamin, which originates from pro-vitamins ergosterol and 7-dehydrocholesterol based on activity of sun radiation. Vitamin D is added to diets in its crystal form as cholecalciferol (vitamin D$_3$) which is converted to 25-hydroxycholecalciferol (calcidiol, 25-OH D$_3$) in the liver and is further converted to the active metabolite 1,25-dihydroxycholecalciferol [1,25-(OH)$_2$ D$_3$] in the kidneys (Beasley, 1999; Adams, 2010; Kelly et al., 2016). 1,25-dihydroxycholecalciferol (calcitriol) supports calcium and phosphorus absorption in the intestine, affects bone calcification and co-participates in calcium and phosphorus metabolism in the organism (Price et al., 2001; Cheng et al., 2016). In poultry, vitamin D$_3$ is ten times more biologically activity than vitamin D$_2$ (Soares et al., 1995; Fritts and Waldroup, 2003; Wideman et al., 2015). Vitamin D$_3$ is supplemented in the diets of poultry and because the optimum levels of dietary vitamin D$_3$ are rarely known, there is always a risk of over-supplementation (Nain et al., 2007), which causes hypercalcemia and promotes deposition of calcium and phosphate as crystals in the kidneys, heart and major blood vessels (Cheng et al., 2016; Armstrong et al., 2018).

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). It is considered that birds might be relatively more resistant to endotoxins than mammals (Roeder et al., 1989) and Vitamin D$_3$ supplementation provide immunomodulation (Bikle, 2010; Schwarz et al., 2012; Shojadoost et al., 2015; Rodriguez-Lecompte et al., 2016; Kelly et al., 2016).

There is a paucity of information on the effects of LPS on cytokines and the acute phase response and their...
relationship with inflammation and homeostasis in birds supplemented with higher dose of vitamin D₃, hence, the study is framed to find out the effect of higher dose of vitamin D₃ concomitantly challenged with endotoxin on biochemical parameters of broiler birds.

MATERIALS AND METHODS

The broiler chicks of strain IBL-80 procured from the hatchery, department of animal genetics and breeding (AGB), college of veterinary science, GADVASU, Ludhiana, India were kept for 7 days to acclimatize in laboratory conditions prior to start of sampling protocols. On day 7, the chicks were randomly divided in 2 broad groups (n=16 chicks) viz. treatment group and control group. Treatment group was administered with vitamin D₃ @ 2.5mg/kg body weight (BW) in groundnut oil daily by oral route. On day 28, 8 chicks from the control group were instilled with Normal saline solution (NSS) @ 0.5ml/chick (group A) and other 8 chicks were challenged with Lipopolysaccharide (LPS) @ 0.5ml/chick by intranasal route (group B) and then sacrificed after 12 hours of challenge with endotoxin. Similarly, 8 chicks from the treatment group were challenged with NSS @ 0.5 ml/chick (group C) and other 8 chicks were challenged with LPS @ 0.5 ml/chick by intranasal route (group D) and then sacrificed after 12 hours of challenge with endotoxin. The effect of oral administration of vitamin D₃ and intranasal LPS on biochemical parameters in chicks on day 28 has been summarized and presented in table 1. The level of hemoglobin in treated chicks did not differ significantly (P<0.05) from control chicks. The mean plasma calcium and phosphorous concentration, Hb, ALT, AST, ALP in chicks of treatment groups did not differ significantly (P<0.05) from control groups (A and B).

Table 1. Effect of oral administration of vitamin D₃ (2.5mgkg⁻¹day⁻¹) and intranasal lipopolysaccharide (0.5 mg bird⁻¹) on biochemical parameters in broiler chicks on day 28 (Mean±SE)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control groups</th>
<th>Treatment groups</th>
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<tbody>
<tr>
<td></td>
<td>Group A (n=8)</td>
<td>Group B (n=8)</td>
</tr>
<tr>
<td></td>
<td>(Mean±SE)</td>
<td>(Mean±SE)</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>12.19±0.33</td>
<td>12.06±0.22</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>10.05±0.56ᵃ</td>
<td>9.48±0.31ᵃ</td>
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<tr>
<td>Phosphorous (mg/dL)</td>
<td>4.97±0.28ᵃ</td>
<td>4.65±0.16ᵃ</td>
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<tr>
<td>Total protein (g/dL)</td>
<td>5.72±0.12</td>
<td>5.56±0.21</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.38±0.18</td>
<td>3.38±0.18</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>12.23±0.96</td>
<td>12.55±1.15</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>226.00±8.44</td>
<td>211.75±10.40</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>1278.12±79.18</td>
<td>1216.38±99.13</td>
</tr>
</tbody>
</table>

Group A: control + Normal Saline Solution; Group B: control + LPS; Group C: Vitamin D₃ @ 2.5mg/kg BW + NSS; Group D: Vitamin D₃ @ 2.5mg/kg Body Weight + Lipopolysaccharide. ᵃᵇ values within a row lacking a common superscript differ significantly at P<0.05.; Hb: haemoglobin, ALT: Alanine transaminase; AST: Aspartate transaminase; ALP: Alkaline phosphatase

Statistical analysis

The statistical analysis was performed using One-Way Analysis of Variance (ANOVA) and all data were analyzed by using SPSS software (SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, SPSS Inc.). The results were presented as mean ± SEM (standard error of mean). Results were considered significant if P<0.05.

Ethical approval

The study was conducted without affecting the birds’ general wellbeing. Approval was taken from concern authority.

RESULTS

The mean plasma total protein, albumin concentration, ALT, AST and ALP in chicks of treatment groups did not differ significantly (P<0.05) from control groups.
DISCUSSION

Administration of vitamin D₃ @ 2.5mg/kg BW for 21 days did not influence the mean hemoglobin concentration. The chicks of the group D which were administered vitamin D₃ @ 2.5mg/kg BW orally daily and challenged with intranasal LPS 12 hours before sacrifice caused no alteration in hemoglobin concentration as compared to control chicks that only challenged with intranasal LPS 12 hours before sacrifice. These results are in agreement with Borissov and Andonova (2000) and Kumar and Mallik (2001) who reported no change in hemoglobin concentration in piglets and calves respectively on endotoxin exposure. However, the results of the present study are in contrast with the findings of Roberson et al. (2000) who conducted vitamin D₃ toxicity in lambs and reported increased hemoglobin and hematocrit values.

Bahman et al. (2011) reported normal plasma/serum calcium in the range of 9-10.5 mg/dL in broiler chicks. In the present study treated groups showed hypercalcemia. The toxic effects of vitamin D₃ are primarily related to the role of vitamin D₃ in the regulation of plasma calcium (Davies and Adams 1978; Reichel and Norman, 1989). The active metabolites of cholecalciferol have been reported to increase the blood calcium level by increased resorption/mobilization of calcium from bone, increased absorption of calcium from intestine and decreased calcium excretion by kidney (Vieth, 1990; Lumeij, 1994; Norman, 1996; Pettifor et al., 1995). Taylor et al., (1968) reported that in chicks given the toxic level of vitamin D₃ there was an increase in plasma calcium, similar results were reported by authors in their studies (Mazumdar et al., 2017; Cheng et al., 2016) whereas no increase in serum calcium level was reported by Cavia et al. (2015) in guinea pigs. Armstrong et al. (2018) reported increased plasma calcium level in dogs prophylactically given calcitriol. Vitamin D₃ toxicity results to higher blood calcium level leading to renal and cardiac failure culminating to death (Beasley, 1999; Radostits et al., 2000; Price et al., 2001) in different species.

Phosphate flux through the gastrointestinal epithelium is enhanced by vitamin D₃. This results from a direct effect of 1,25-dihydroxycholecalciferol, this hormone's action on calcium absorption, the calcium in turn acting as a transport mediator for the phosphate. Vitamin D₃ also increases calcium and phosphate reabsorption by the epithelial cells of the renal tubules, thereby tending to decrease excretion of these substances in the urine (Guyton and Hall, 2001). Similar results were reported by Cavia et al. (2015) in guinea pigs, on the contrary Linda and Aaron (2016) reported decrease in phosphorous concentration as a result of vitamin D₃ supplementation in laying hen. The normal levels of plasma/serum phosphorous in the range of 4-6 mg/dL in apparently healthy broiler chicks (Bahman et al., 2011).

The normal plasma total protein and plasma albumin level in vitamin D₃ toxicity in present study was dissimilar with low total protein level in vitamin D₃ toxicity reported by Roberson et al. (2000) in lambs. The findings in the present study are in contrast with Beasley (1999) who reported clinical signs like proteinuria and hence decrease in plasma total protein concentration in vitamin D₃ toxicity. The non-significant (P<0.05) change in the plasma total protein and albumin values may be due to dehydration because of polyuria and hence no change in relative protein concentration of plasma. The pathological changes in the kidneys (mineralization) might have prevented the excretion of plasma proteins and albumin via urine hence, maintaining the concentration of these proteins in the plasma. Bosch et al. (1988) and Al-Dughaym (2004) reported decreased total protein and albumin concentration in endotoxin treated animals. However, in the present study plasma total protein and albumin concentration did not show any significant (P<0.05) alteration between the chicks of group B and D exposed to LPS.

The serum transaminases (ALT and AST) are liver specific enzymes and thus their values help in detection of hepatocellular injury (Tenant, 1997). In the present study there was no alteration in their values which might be attributed to normal histoarchitecture of liver of the treatment groups. However, significant (P<0.05) increases in serum transaminases have been reported in cross bred calves (Kumar and Mallik, 2001), in rabbits (Yajar et al., 2004) and in pigs (Borissov and Andonova, 2000) challenged with endotoxin. Moreover, it has been opined that damage to any particular organ cannot be cited as cause of increased level of serum transaminases (Kaneko et al., 2008).

There was no significant decrease (P>0.05) in the plasma ALP activity in treatment group C and group D. This may be due to decreased osteoblast cell activity and increased osteoclast cell activity in bones because of high dose of vitamin D₃. Acute doses of vitamin D₃ (>100 times than required level) can result in negative calcium balance because of bone resorption is accelerated as in Solanum malacoxylon, Cestrum diurnum and Trisetum flavescenti toxicity (Kaneko et al., 2008). Cavia et al. (2015) reported increase in ALP activity in
hypervitaminosis D in guinea pigs. ALP activity of LPS challenged chicks (groups B and D) did not reveal any significant (P<0.05) difference from NSS challenged chicks (groups A and C). Similar results were reported was comparable to control group chicks (group A) in crossbred calves (Kumar and Mallik, 2001) and in rats (Bosch et al., 1988) reported similar findings in endotoxemia. This non-significant decrease (P<0.05) in ALP activity in treated groups (group C and D) might be due to mineralization causing damage to kidney.

There is no significant change in the hematological and biochemical parameters of broiler chick, this may be due to the immunomodulation provided by Vitamin D3 fed to the broiler chicks. Different authors in their studies confirmed similar immunomodulatory effects of Vitamin D3 (Bikle, 2010; Schwarz et al., 2012; Shojadoost et al., 2015; Rodriguez-Lecompte et al., 2016).

CONCLUSION

The Vitamin D3 is an immunomodulator; broiler diet supplemented with Vitamin D3 causes immunomodulation in broiler birds, hence acute endotoxic shock does not incite inflammatory response and disturb the homeostasis in broiler chicks.

DECLARATIONS

Consent to publish

All authors gave their informed consent prior to their inclusion in the study.

Competing interests

The authors declare that they have no competing interests.

Author’s contribution

All authors have equally contributed to this work.

REFERENCES


Rodrique-Lecompte JC, Yitbarek A, Cuperus T, Echevery H and Van Dijk A (2016). The immunomodulatory effect of vitamin D in chickens is dose-dependent and influenced by calcium and phosphorus levels Poultry Science, 95(11); 2547–2556. Doi: doi.org/10.3382/ps/pew186


