Original Research Article

Effects of dietary 1 alpha-hydroxycholecalciferol in calcium and phosphorous-deficient diets on growth performance, tibia related indices and immune responses in broiler chickens

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Abstract

This experiment was conducted to investigate the effect of dietary 1 alpha-hydroxycholecalciferol (1a-OH-D3) in calcium (Ca)- and phosphorous (P)-deficient diets on growth performance, carcass characteristics, tibia related parameters, and immune responses of broiler chickens. A total of 280 one-day-old broiler chickens (Ross 308) were assigned to 20 floor pens and 4 dietary treatments with 5 replicates. Dietary treatments consisted of starter diets (starter diet of treatment A: 1% Ca, 0.73% total phosphorus [tP]; starter diet of treatment B: 0.85% Ca, 0.64% tP + 5 μg/kg of 1a-OH-D3; starter diet of treatment C: 0.85% Ca, 0.59% tP + 5 μg/kg of 1a-OH-D3; starter diet of treatment D: 0.85% Ca, 0.54% tP + 5 μg/kg of 1a-OH-D3), grower diets (grower diet of treatment A: 0.86% Ca, 0.68% tP; grower diet of treatment B: 0.73% Ca, 0.59% tP + 5 μg/kg of 1a-OH-D3; grower diet of treatment C: 0.73% Ca, 0.55% tP + 5 μg/kg of 1a-OH-D3; grower diet of treatment D: 0.73% Ca, 0.50% tP + 5 μg/kg of 1a-OH-D3) and finisher diets (finisher diet of treatment A: 0.81% Ca, 0.64% tP; finisher diet of treatment B: 0.68% Ca, 0.56% tP + 5 μg/kg of 1a-OH-D3; finisher diet of treatment C: 0.68% Ca, 0.52% tP + 5 μg/kg of 1a-OH-D3; finisher diet of treatment D: 0.68% Ca, 0.48% tP + 5 μg/kg of 1a-OH-D3). Results showed that body weight gain (BWG) and feed intake (FI) of broilers in treatment B were similar to those of broilers in treatment A at the end of the trial (P < 0.05). Broilers in treatments C and D had lower BWG and FI than those in treatment A during the whole trial (P < 0.05). Feed conversion ratio, carcass traits and relative weight of lymphoid organs were not affected by dietary treatments (P > 0.05). Dietary treatments had no significant effect on antibody titers against Newcastle and Influenza disease viruses as well as sheep red blood cells. Dietary treatments had no significant effects on tibia ash and tibial dyschondroplasia score. Broilers fed Ca-P deficient diets had lower tibia Ca and P than those in treatment A (P < 0.05). In conclusion, results indicated that broilers fed Ca-P deficient diets supplemented with 5 μg/kg 1α-OH-D3 failed to achieve the same tibia Ca and P values as broilers fed nonphytate phosphorous adequate diets.

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1. Introduction

A major issue facing the poultry industry is maintaining bone quality while decreasing feed cost and phosphorus (P) excretion to the environment. In recent years, public pressure on poultry producers has increased to reduce excessive P wastage in the manure, which stimulated researchers into ways to increase the availability of dietary phytate phosphorus (PP) content. Several methods have been investigated to improve available PP utilization. Supplementation of low P diets with phytase has been shown to improve dietary P digestibility in sows (Torrallardona et al., 2012; Sands...
et al., 2001) and broilers (jiang et al., 2013; Pieniazek et al., 2017; Woyengo et al., 2010; Ravindran et al., 2006). Consistently, Pillai et al. (2006) reported that inclusion of *Escherichia coli* phytase to P-deficient diets could improve growth performance, bone quality, and carcass yield in broiler chickens. Mitchell and Edwards (1996) reported the ability of 1,25-dihydroxycholecalciferol to enhance performance parameters and tibia related indices of young chickens by increasing dietary P absorption and retention.

Several trials have indicated affirmative efficacy of 25-OH-D₃ supplementation in broiler’s diets on performance criteria (Fritts and Waldroup, 2003) and P utilization (Zhang et al., 1997) which thereby makes it suitable to be included in poultry feed. It could be possible to use 1α-hydroxycholecalciferol (1α-OH-D₃) as an active vitamin D analog to be substituted for cholecalciferol in broiler feed. Edwards et al. (2002) reported that the 1α-OH-D₃ is approximately 8 times more effective than cholecalciferol. Landy and Toghyani (2014) indicated the ability of 1α-OH-D₃ to be replaced for cholecalciferol in broiler chickens. Han et al. (2009) reported that interaction between phytase and 1α-OH-D₃ in diets containing 2.9 g/kg nonphytate phosphorus (NPP) could improve tibia related parameters in broiler chicks, while it could not improve performance parameters. Han et al. (2015) reported that supplementation of 5 μg/kg 1α-OH-D₃ in diets containing 0.30% of NPP could improve growth performance and tibia mineralization of broiler chickens. Landy et al. (2015) reported that supplementation of broiler diets with 5 μg/kg 1α-OH-D₃ and 500 FTU/kg of phytase could not maximize growth performance and tibia parameters.

Kolb et al. (2000) and Van der Srede et al. (2000) reported that cholecalciferol and 1,25-dihydroxycholecalciferol have immunomodulatory effects. Bouillon et al. (2000) compared the efficacy of cholecalciferol and 1,25-(OH)₂-D₃ to treat cancer and skin disorders in mice. They reported that 1,25-(OH)₂-D₃ helped mice to treat cancer, skin, and immune related disorders. Vazquez et al. (2018) suggested that supplementation of 25-OH-D₃ to diet of broilers containing cholecalciferol could improve cellular immune responses.

Most of the studies on 1α-OH-D₃ only focused on the starter rearing period, and few experiments conducted on growing and finishing phases. Moreover, no study has evaluated the effect of 1α-OH-D₃ in P-deficient diets on the immunity of broiler chickens. Therefore, this experiment was conducted to investigate the effect of dietary 1α-OH-D₃ supplementation in Ca-P deficient diets on growth performance, carcass characteristics, immunity, and tibia related parameters in broiler chickens.

## 2. Materials and methods

### 2.1. Ethical matters

Broilers were raised in accordance with the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. All procedures used in this study were approved by the Ethical Committee of Islamic Azad University, Isfahan branch, Iran (approval ref no. 2016-003).

### 2.2. Birds, diets, feeding, and management

Two hundred and eighty as-hatched chicks (Ross 308) were purchased from a local hatchery. They were weighed and randomly allocated to 20 pens (120 cm × 120 cm × 80 cm) with 14 chicks per pen. Dietary treatments were as follows: starter diets (starter diet of treatment A: 1% Ca, 0.73% total phosphorus [tP]; starter diet of treatment B: 0.85% Ca, 0.64% tP + 5 μg/kg of 1α-OH-D₃ [Vitamin Derivatives Inc., Georgia, USA]; starter diet of treatment C: 0.85% Ca, 0.59% tP + 5 μg/kg of 1α-OH-D₃; starter diet of treatment D: 0.85% Ca, 0.54% tP + 5 μg/kg of 1α-OH-D₃), grower diets (grower diet of treatment A: 0.86% Ca, 0.68% tP; grower diet of treatment B: 0.73% Ca, 0.59% tP + 5 μg/kg of 1α-OH-D₃; grower diet of treatment C: 0.73% Ca, 0.55% tP + 5 μg/kg of 1α-OH-D₃; grower diet of treatment D: 0.73% Ca, 0.50% tP + 5 μg/kg of 1α-OH-D₃) and finisher diets (finisher diet of treatment A: 0.81% Ca, 0.64% tP; finisher diet of treatment B: 0.68% Ca, 0.56% tP + 5 μg/kg of 1α-OH-D₃; finisher diet of treatment C: 0.68% Ca, 0.52% tP + 5 μg/kg of 1α-OH-D₃; finisher diet of treatment D: 0.68% Ca, 0.48% tP + 5 μg/kg of 1α-OH-D₃) (Table 1). Broilers were fed the starter diets from 0 to 14 d (Table 2), grower diets from 15 to 28 d (Table 3), and finisher diets from 29 to 42 d (Table 4) according to Aviagen nutritional recommendation except for Ca and P. Broiler chickens had free access to mash feed and water throughout 6 weeks of trial. The lighting system consisted of 23 h of light from d 0 to 3, 20 h of light from d 4 to 14, and 18 h of light from d 15 to 42. The room temperature was controlled at 33 °C for the first week, and then gradually reduced by 3 °C per week to a final temperature of 23 °C.

### 2.3. Feed analyses

Feed samples were dried by oven at 100 °C for 16 h. Dry matter, tP, and Ca contents of each feed sample from the 4 experimental diets were measured. Calcium and tP contents of the feed were analyzed by the ICPOES method 2011.14 (AOAC, 1990).

### 2.4. Performance and carcass characteristics

At the end of trial, body weight and feed intake (FI) were recorded on a pen basis, for the determination of body weight gain (BWG) and average daily feed intake. Feed conversion ratio (FCR) was calculated accordingly. Mortality was recorded daily. On d 42 of experiment, 2 chickens per replicate were chosen based on the BWG and average daily feed intake. Carcass yield was calculated by dividing eviscerated weight by live weight. Abdominal fat pad was removed, weighed, and calculated as a percentage of live weight.

### Table 1

| Treatments | 1α-OH-D₃, μg/kg | Starter period (0 to 14 d) | Grower period (15 to 28 d) | Finisher period (29 to 42 d) |
|------------|----------------|--------------------------|--------------------------|--------------------------|
|            | Ca, %          | tP, %                    | Ca, %                    | tP, %                    | Ca, %                    | tP, %                    |
| A²         | –              | 1.00                     | 0.73                     | 0.86                     | 0.68                     | 0.81                     | 0.64                     |
| B²         | 5              | 0.85                     | 0.64                     | 0.73                     | 0.59                     | 0.68                     | 0.56                     |
| C²         | 5              | 0.85                     | 0.59                     | 0.73                     | 0.55                     | 0.68                     | 0.52                     |
| D²         | 5              | 0.85                     | 0.54                     | 0.73                     | 0.50                     | 0.68                     | 0.48                     |

tP = total phosphorus.

1 Ca and tP adequate diets without 1α-OH-D₃.

2 Ca-P deficient diets with 1α-OH-D₃.
Table 2
Ingredients, calculated and analyzed nutrient content of starter diets (g/kg, as fed basis).

| Item | Treatments | A | B | C | D |
|------|------------|---|---|---|---|
| Ingredients | | 542.3 | 556.2 | 558.0 | 561.3 |
| Corn, 8% CP | | 390.0 | 387.0 | 387.0 | 386.0 |
| Soybean meal, 44% CP | | 22.4 | 18.3 | 17.6 | 16.8 |
| Soybean oil | | 5.0 | 10.5 | 8.7 | 6.4 |
| Monocalcium phosphate | | 17.3 | 15.0 | 15.7 | 16.6 |
| CaCO₃ | | 3.0 | 3.0 | 3.0 | 3.0 |
| NaCl | | 2.5 | 2.5 | 2.5 | 2.5 |
| Trace mineral premix² | | 2.5 | 2.5 | 2.5 | 2.5 |
| Vitamin premix¹ | | 3.1 | 3.1 | 3.0 | 3.0 |
| DL-methionine | | 1.9 | 1.9 | 1.9 | 1.9 |
| L-lysine | | 2.900 | 2.900 | 2.900 | 2.900 |
| Calcium | | 215 | 215 | 215 | 215 |
| Crude protein | | 10.0 | 8.5 | 8.5 | 8.5 |
| Nonphytate phosphorus | | 4.8 | 3.8 | 3.3 | 2.8 |
| Total phosphorus (tP) | | 7.3 | 6.4 | 5.9 | 5.4 |
| Digestible methionine + cysteine | | 9.0 | 9.0 | 9.0 | 9.0 |
| Digestible lysine | | 12.2 | 12.2 | 12.2 | 12.2 |
| Analyzed nutrient content | | 9.4 | 8.8 | 8.0 | 9.0 |
| tP | | 7.0 | 6.6 | 5.7 | 5.3 |

1 Treatment A was Ca and tP adequate diets without 1α-OH-D₃, and treatments B, C, D were Ca-P deficient diets with 5 μg/kg of 1α-OH-D₃.
2 Provided the following per kg of diet: Mg, 120 mg; Fe, 40 mg; Cu, 16 mg; Zn, 100 mg; Se, 0.3 mg; I, 1.25 mg.
3 Provided the following per kg of diet: vitamin A, 11,000 IU; vitamin D₃, 5,000 IU; vitamin E, 7.5 IU; vitamin K, 3 mg; vitamin B₁, 3 mg; riboflavin, 8 mg; nicotinic acid, 60 mg; pantothenic acid, 4 mg; pyridoxine, 2 mg; biotin, 0.1 mg; folic acid, 1.5 mg; vitamin B₁₂, 0.016 mg; choline, 3 mg.

Table 3
Ingredients, calculated and analyzed nutrient content of grower diets (g/kg, as fed basis).

| Item | Treatments | A | B | C | D |
|------|------------|---|---|---|---|
| Ingredients | | 564.0 | 575.0 | 577.5 | 579.0 |
| Corn, 8% CP | | 368.0 | 366.0 | 365.0 | 365.0 |
| Soybean meal, 44% CP | | 30.0 | 27.2 | 26.5 | 26.0 |
| Soybean oil | | 12.9 | 9.1 | 7.1 | 5.2 |
| Monocalcium phosphate | | 14.4 | 12.5 | 13.3 | 14.1 |
| CaCO₃ | | 3.0 | 3.0 | 3.0 | 3.0 |
| NaCl | | 2.5 | 2.5 | 2.5 | 2.5 |
| Trace mineral premix² | | 2.5 | 2.5 | 2.5 | 2.5 |
| Vitamin premix¹ | | 2.2 | 2.2 | 2.2 | 2.2 |
| DL-methionine | | 0.4 | 0.4 | 0.4 | 0.4 |
| L-lysine | | 3,000 | 3,000 | 3,000 | 3,000 |
| tP | | 207 | 207 | 207 | 207 |
| Calcium | | 8.6 | 7.3 | 7.3 | 7.3 |
| Nonphytate phosphorus | | 6.8 | 5.9 | 5.5 | 5.0 |
| Total phosphorus (tP) | | 8.0 | 8.0 | 8.0 | 8.0 |
| Digestible methionine + cysteine | | 10.5 | 10.5 | 10.5 | 10.5 |
| Digestible lysine | | 8.1 | 7.8 | 7.8 | 7.8 |
| Analyzed nutrient content | | 7.0 | 5.7 | 5.6 | 5.2 |

1 Treatment A was Ca and tP adequate diets without 1α-OH-D₃, and treatments B, C, D were Ca-P deficient diets with 5 μg/kg of 1α-OH-D₃.
2 Provided the following per kg of diet: Mg, 120 mg; Fe, 40 mg; Cu, 16 mg; Zn, 100 mg; Se, 0.3 mg; I, 1.25 mg.
3 Provided the following per kg of diet: vitamin A, 9,000 IU; vitamin D₃, 5,000 IU; vitamin E, 5 IU; vitamin K, 2 mg; vitamin B₁, 2 mg; riboflavin, 5 mg; nicotinic acid, 40 mg; pantothenic acid, 15 mg; pyridoxine, 2 mg; biotin, 0.1 mg; folic acid, 1.5 mg; vitamin B₁₂, 0.016 mg; choline, 3 mg.

Table 4
Ingredients, calculated and analyzed nutrient content of finisher diets (g/kg, as fed basis).

| Item | Treatments | A | B | C | D |
|------|------------|---|---|---|---|
| Ingredients | | 611.7 | 624.0 | 627.2 | 628.8 |
| Corn, 8% CP | | 322 | 320 | 319 | 319 |
| Soybean meal, 44% CP | | 29.0 | 26.0 | 25.0 | 24.5 |
| Soybean oil | | 12.0 | 8.4 | 6.5 | 4.7 |
| Monocalcium phosphate | | 13.8 | 12.1 | 12.8 | 13.5 |
| CaCO₃ | | 3 | 3 | 3 | 3 |
| NaCl | | 2.5 | 2.5 | 2.5 | 2.5 |
| Trace mineral premix² | | 2.5 | 2.5 | 2.5 | 2.5 |
| Vitamin premix¹ | | 1.5 | 1.5 | 1.5 | 1.5 |
| Calcium | | 8.6 | 6.5 | 6.9 | 6.3 |
| tP | | 6.6 | 5.4 | 5.1 | 5.0 |

1 Treatment A was Ca and tP adequate diets without 1α-OH-D₃, and treatments B, C, D were Ca-P deficient diets with 5 μg/kg of 1α-OH-D₃.
2 Provided the following per kg of diet: Mg, 120 mg; Fe, 40 mg; Cu, 16 mg; Zn, 100 mg; Se, 0.3 mg; I, 1.25 mg.
3 Provided the following per kg of diet: vitamin A, 9,000 IU; vitamin D₃, 5,000 IU; vitamin E, 5 IU; vitamin K, 2 mg; vitamin B₁, 2 mg; riboflavin, 5 mg; nicotinic acid, 40 mg; pantothenic acid, 15 mg; pyridoxine, 2 mg; biotin, 0.1 mg; folic acid, 1.5 mg; vitamin B₁₂, 0.016 mg; choline, 3 mg.

2.5. Immune responses

On d 9 of the experiment, broiler chickens from each pen (n = 14) were injected with a single dose (0.2 mL) of commercially vaccine against Newcastle (NDV) and avian influenza disease viruses (AIV; serotype H9N2) subcutaneously. Two male broilers from each pen were bled by a puncture of the brachial vein on d 19 of post-vaccination to collect serum. Serum samples were applied to hemagglutination inhibition in order to measure antibody titers against NDV and AIV and expressed as log₂. On d 24 of trial, 1 mL of 1% sheep red blood cells (SRBC) was injected intravascularly to 2 broilers per pen. After 6 d, blood samples were taken and individual sera were tested for antibody production. Antibody titers were expressed as the log₂ (Wegmann and Smithies, 1966). Lymphoid organs were sampled on d 42 of trial. In this respect 2 male broilers were randomly selected from each pen, slaughtered, and lymphoid organs (bursa of Fabricius and spleen) were removed, weighted, and calculated as a percentage of live body weight.

2.6. Tibia parameters

At the end of trial, 2 chickens per pen were selected based on the average weight of the pen and sacrificed by exsanguinations, and the left and right tibias were excised. The right tibia was evaluated for tibial dyschondroplasia (TD) as described by Edwards and Veltmann (1983). Tibia ash content was determined by removing organs (bursa of Fabricius and spleen) were removed, weighted, and calculated as a percentage of live body weight.

2.7. Statistical analysis

Performance, tibia quality, and immune related parameters were analyzed via Analysis of Variance (ANOVA) using the General Linear...
3. Results

3.1. Growth performance and carcass yield

Data on the growth performance indices of broilers in starter, grower and finisher periods are summarized in Table 5. During the starter phase (0 to 14 d), broilers in treatment D had lower (P < 0.05) FI than those in treatments A, B, and C. Furthermore, broilers in treatment D had higher (P < 0.05) FCR than those in treatments A, B, and C. In the grower period, body weight gain was higher in treatment A than treatments B, C, and D. Broilers in treatment D had lower (P < 0.05) BWG than those received diets of treatments A, B, and C. Moreover, dietary treatments failed to induce any significant effect on FCR, though broilers in treatment A had better FCR values than those in treatments B, C, and D. Broilers in treatment A had significantly higher (P < 0.05) FI than those in treatments C, D, but did not significantly differ from those in treatment B.

During the finisher phase, broilers in treatments A and B had significantly higher (P < 0.05) BWG than those in treatment D, but did not differ from treatment C. There was no significant difference in FCR among treatments in this period. Broilers in treatment D had significantly lower (P < 0.05) FI than those in treatments A and B, but did not differ from those in treatment C.

For broilers in treatment B, BWG, FI, and FCR were similar to those fed Ca-P adequate diets added with 1α-OH-D3 (Table 6). At 42 d of age, broilers in treatment D, had significantly (P < 0.05) lower FI than those in treatments A, B, and C. Significant differences among treatments were observed in BWG of broilers during the entire trial. Broilers in treatments D and C had lower BWG than those in treatments A and B. Overall FCR values were better for treatments A, B, and C than for treatment D whereas the results were not significantly different. There were no significant differences in the carcass yield and abdominal fat of broilers among treatments (Table 7).

3.2. Immune responses

There were no significant differences in the weight of lymphoid organs among treatments (Table 7). Dietary treatments had no significant effects on the antibody titers against AIV, NDV, and SRBC (Table 8).

3.3. Parameters of tibia

Effects of experimental diets on tibia parameters in broiler chickens are presented in Table 9. Tibia ash of broilers did not significantly differ among experimental treatments, whereas it
4.1. Performance and carcass yield

Previous studies on 1α-OH-D3 in broilers have focused on the starter period, in which 1α-OH-D3 improved performance related parameters (Biehl and Baker, 1997; Edwards, 2002). The supplementation of 1α-OH-D3 could not maximize BWG, FI, and FCR of broilers. Driver et al. (2005) reported that broilers fed diets containing 1α-OH-D3 and phytase had lower tibia ash compared with those fed phytase alone, considering the interaction between 1α-OH-D3 and phytase had lower tibia ash than those fed normal P diet. However, Snow et al. (2004) reported that interaction between phytase and 1α-OH-D3 had an affirmative influence on PP release in broilers from 1 to 21 d of age. In this experiment, we evaluated the efficacy of 1α-OH-D3 alone, considering the interaction between 1α-OH-D3 and phytase, and the efficiency of 1α-OH-D3 either alone or in combination with phytase is worthy to be investigated in broiler chickens further.

In the current trial, the tibia ash of broilers did not differ among treatments, whereas it tended to decrease in birds fed dietary treatments C and D. Broilers in 1α-OH-D3 diets had significantly (P < 0.05) lower tibia P than those in treatments A and B but did not differ from treatment C. Dietary treatments failed to induce any marked effect on TD score, whereas it tended to enhance TD in treatments C and D (P > 0.05).

4.2. Immune responses

Dietary 1,25-dihydroxycholecalciferol and cholecalciferol have been proposed to exhibit immunomodulatory effects (Kolb et al., 2000; Van der Stede et al., 2000), so enhanced antibody titers were expected. However, in the current trial, dietary treatments did not induce any marked influence on the relative weights of lymphoid organs and homoral immune responses. Results of a trial conducted by Chou et al. (2009) indicated that a supplementation of 25-OH-D3 could enhance humoral immune responses in challenged broilers. Gomez-Verduzco et al. (2013) indicated that the supplementation of dietary high levels of cholecalciferol (2,000 IU/kg) in comparison with the levels recommended by NRC (1994) enhanced the antibody titer against NDV, and the supplementation of 25-OH-D3 enhanced the cellular immunity of broiler chickens. Vazquez et al. (2018) reported that the supplementation of 25-OH-D3 to diets containing cholecalciferol improved cellular immune responses in broiler chickens. There has been a dearth of information on the effect of 1α-OH-D3 on immune responses in broiler chickens, thus further investigations are warranted.

4.3. Tibial parameters

In the present trial, the tibia ash of broilers did not differ among treatments, whereas it tended to decrease in birds fed dietary treatments C and D. Broilers in 1α-OH-D3 deficient diets had significantly lower tibia Ca than those fed normal P diet. Driver et al. (2005) reported that broilers fed diets containing 1α-OH-D3 and phytase had lower tibia ash than those fed normal P diet. However, Snow et al. (2004) reported that interaction between phytase and 1α-OH-D3 had an affirmative influence on PP release in broilers from 1 to 21 d of age. In this experiment, we evaluated the efficacy of 1α-OH-D3 alone, considering the interaction between 1α-OH-D3 and phytase, and the efficiency of 1α-OH-D3 either alone or in combination with phytase is worthy to be investigated in broiler chickens further.

In the current trial, we evaluated the efficacy of 1α-OH-D3 in Ca-P deficient diets containing 5,000 IU cholecalciferol/kg of diets. Biehl and Baker (1997) reported that the supplementation of 1α-OH-D3 in purified diets could improve the tibia ash only in broilers fed diets without cholecalciferol. Landy et al. (2015) reported that in Ca-P deficient diets and without vitamin D3 supplementation, 1α-OH-D3 improved tibia parameters in broiler chickens. However, when vitamin D3 was enough, the tibia quality of broilers was not improved by dietary 1α-OH-D3 supplementation. Atencio et al. (2005) reported that the supplementation of 25-OH-D3 increased the hen-day egg production in broiler breeders but only at very low levels of dietary vitamin D3 supplementation. Similarly, Edwards (2002) indicated that an interaction between cholecalciferol and
calcitriol exists in tibia ash. It seemed that 1x-OH-D3 could not improve tibia parameters via high levels of cholecalciferol inclusion in the diet in our experiment.

Ledwaba and Roberson (1976) reported that dietary 25-OH-D3 enhanced PP digestion at low levels of dietary Ca compared with diets containing high levels of Ca. Han et al. (2012) investigated the relationship between dietary Ca levels (0.40%, 0.60%, 0.80%, 1.00%, and 1.20% Ca) and 1x-OH-D3 in P-deficient diets. Results indicated that 1x-OH-D3 had the highest activity at a lower concentration of dietary Ca. It seemed that 1x-OH-D3 could not improve tibia parameters in our experiment due to the applied dietary Ca levels.

In our study, dietary treatment failed to induce any marked effect on TD score, whereas it tended to enhance in birds in treatments C and D. In another trial, the supplementation of 1x-OH-D3 as a replacement for cholecalciferol in broiler diets increased TD score (Landy and Toghyani, 2014). Edwards (1990) investigated effects of vitamin D analogs in order to inhibit TD in broiler chickens and reported that the supplementation of vitamin D analogs except 24R,25-(OH)2D3 could influence favorable increases in incidence and severity of TD compared with the control group. Edwards and Veltmann (1983) reported that diets containing high levels of Ca might prevent TD and the incidence of TD in 2-week-old chicks was only 13% when received with a commercial diet containing 1.1% Ca and 0.55% available P, but was 39% in diets containing 0.8% Ca. The present study was in agreement with the results reported by Edwards and Veltmann (1983), when dietary Ca and P levels were decreased and 5μg/kg of 1x-OH-D3 was supplemented (treatments C and D), the average TD score was increased as a result of lower tibia Ca and P contents.

5. Conclusion

In conclusion, results indicated that broilers fed Ca-P deficient diets supplemented with 5 μg/kg of 1x-OH-D3 were unable to achieve the same tibia Ca and P content as broilers fed Ca-P adequate diets without 5 μg/kg of 1x-OH-D3. Considering the possibility of interaction between 1x-OH-D3 and cholecalciferol, the efficiency of 1x-OH-D3 in diets containing different levels of cholecalciferol should be investigated in broilers chickens. Furthermore, considering the interaction between 1x-OH-D3 and phytase, the efficiency of 1x-OH-D3 alone or in combination with phytase should be investigated in broilers chickens.

Conflict of interest

The authors declare that they have no competing interests.

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References

AOAC. Official methods of analysis. 15th ed. 1990. Washington, DC.
AOAC. Official methods of analysis. 16th ed. 1995. Washington, DC.
Atencio A, Pesti G, Edwards Jr H. Twenty-five hydroxycholecalciferol as a cholecalciferol substitute in broiler breeder hen diets and its effect on the performance and general health of the progeny. Poult Sci 2005;84:1277–85.
Biel RR, Baker DH. Utilization of phytate and nonphytate phosphates in chicks as affected by source and amount of vitamin D3. J Anim Sci 1997;75:2986–93.
Bouillon R, Verstuyf A, Segesta S, Verlinden L, Mathieu C. Recent developments in the use of vitamin D analogues. Expert Opin Invest Drug. 2000;9:443–55.
Chou SH, Chung TK, Yu B. Effects of supplemental 25-hydroxycholecalciferol on growth performance, small intestinal morphology, and immune response of broiler chickens. Poult Sci 2009;88:2333–41.
Driver JP, Pesti GM, Bakalli RI, Edwards Jr HM. Phytase and 1 alpha hydroxycholecalciferol supplementation of broiler chickens during the starting and growing/finishing phases. Poult Sci 2005;84:1616–28.
Edwards Jr HM. Efficacy of several vitamin D compounds in the prevention of tibial dyschondroplasia in broiler chickens. J Nutr 1990;120:1054–61.
Edwards Jr HM. Studies on the efficacy of cholecalciferol and derivatives for stimulating phytate utilization in broilers. Poult Sci 2002;81:1026–31.
Edwards Jr HM, Veltmann JR. The role of calcium and phosphorus in the etiology of tibial dyschondroplasia in young chicks. J Nutr 1983;113:1568–75.
Edwards Jr HM, Shirley RB, Escoie WB, Pesti GM. Quantitative evaluation of 1x-hydroxycholecalciferol as a cholecalciferol substitute for broilers. Poult Sci 2002;81:664–9.
Fritts CA, Waldroup PW. Effect of source and level of vitamin D on live performance and bone development in growing broilers. J Appl Poult Res 2003;12:45–52.
Gomez-Orozco G, Morenza-Diez R, Avila-Gonzalez E. Use of 25-hydroxycholecalciferol in diets of broiler chickens: effects on growth performance, immunity and bone calcification. J Poult Sci 2013;50:60–4.
Han JC, Yang XD, Zhang LM, Li WL, Zhang T, Zhang ZY, et al. Effects of 1α-hydroxycholecalciferol and phytase on growth performance, tibia parameter and meat quality of 1- to 21- d-old broilers. Asian Australas J Anim Sci 2009;22:857–64.
Han JC, Liu Y, Yao J, Wang J, Qi H, Yan Y, et al. Dietary calcium levels reduce the efficacy of one alpha-hydroxycholecalciferol in phosphorus-deficient diets of broilers. J Poult Sci 2012;49:34–8.
Han JC, Ma K, Wang JG, Chen GH, Zhang JL, Qi HX, et al. Effects of Non-phytate phosphorus and 1α-Hydroxycalciferol on growth performance, bone mineralization, and carcass traits of broiler chickens. Braz J Poult Sci 2015;17:509–18.
Jiang XR, Luo FH, Qu MR, Bontempo V, Wu SG, Zhang HJ, et al. Effect of non-phytate phosphorus levels and phytase sources on the growth performance, serum biochemical and tibial parameters of broiler chickens. Ital J Anim Sci 2013;12:375–80.
Kolb E, Kaskous S, Seehawer J. The influence of stress on the secretion of various hormones, on the metabolism of vitamins, and on the immune system of sheep. Tierarztl Umschau 2000;55:614–21.
Landy N, Toghyani M. Evaluation the effects of dietary cholecalciferol substitution with 1 alpha-hydroxycholecalciferol on performance and tibia parameters in broiler chickens. Int J Poult Sci 2014;13:515–7.
Landy N, Toghyani M. Evaluation of 1αOH13 alone or in combination with cholecalciferol in Ca-P deficiency diets on development of tibial dyschondroplasia in broiler chickens. Anim Nutr 2018;4:109–12.
Landy N, Toghyani M, Bahadoran R, Eghbali-Shaie A. The effects of 1α,25hydroxycalciferol supplementation on performance and tibia parameter of broiler chickens. Res Opin Anim Vet Sci 2015;5:342–7.
Ledwaba MF, Roberson KD. Effectiveness of twenty five hydroxycholecalciferol in the prevention of tibial dyschondroplasia in Ross cockerels depends on dietary calcium level. Poult Sci 1969;58:1769–77.
Mitchell RD, Edwards Jr HM. Effects of phytase and 1,25-dihydroxycholecalciferol on phytate utilization and the quantitative requirement for calcium and phosphorus in young broiler chickens. Poult Sci 1996;75:95–110.
NRC. Nutritional requirements of poultry. 9th ed. Washington: Acad Press; 1994.
Pieniazek J, Smith KA, Williams MP, Manangi MK, Vázquez-Aron M, Solbak A, et al. Evaluation of increasing levels of a microbial phytase in phytase deficient broiler diets via live broiler performance, tibia ash, apparent metabolizability, and amino acid digestibility. Poult Sci 2017;96:170–82.
Pillai PB, O’Connor-Denne T, Owens CM, Emmert JL. Efficacy of an Escherichia coli phytase in broilers fed adequate or reduced phosphorus diets and its effect on carcass characteristics. Poult Sci 2006;85:1737–45.
Ravindran V, Morel PC, Partridge GC, Hruby M, Sands JS. Influence of an Escherichia coli-derived phytase on nutrient utilization in broiler starters fed diets containing varying concentrations of phytic acid. Poult Sci 2006;85:82–9.
Sands JS, Ragland D, Baxter C, Joern BC, Sauber TE, Adeola O. Phosphorus bioavailability, growth performance, and nutrient balance in pigs fed high available phosphorus corn and phytase. J Anim Sci 2001;79:2134–42.
Snow JL, Baker DH, Parsons CM, Phytase, citric acid, and 1α-hydroxycholecalciferol improve phytate phosphorus utilization in chicks fed a corn-soybean meal diet. Poult Sci 2004;83:1187–92.
Torrallardona D, Llaudrado L, Broz J. The supplementation of low-P diets with microbial 6-phytase expressed in Aspergillus oryzae improves P digestibility in sows. J Anim Sci 2012;90(Suppl):104–6.
Van der Steede Y, Cox E, Goddeers IM. 1,25 dihydroxyvitamin D3 [cholecalciferol], Part 2. Role in the immune system. Vlaams Diergen Tijds 2000;69:229–34.
Vazquez JR, Gómez GV, López-C, Cortés AC, Díaz AC, Fernández SRT, et al. Effects of 25-hydroxycholecalciferol with two DJ 2 vitamin levels on production and immuno parameters in broiler chickens. J Anim Physiol Anim Nutr 2018;102:493–7.
Wegmann TG, Smithies O. A simple hemagglutination system requiring small amounts of red blood cells and antibodies. Transfusion 1966;6:87–73.
Woyengo TA, Slominski BA, Jones RO. Growth performance and nutrient utilization of broiler chickens fed diets supplemented with phytase alone or in combination with citric acid and multicarehdy, Phytase. Poult Res 2010;9:8221–9.
Zhang X, Liu C, McDaniel GA, Fritts CA, Baker DH. Response of broiler lines selected for tibial dyschondroplasia incidence to supplementary 25 hydroxycholecalciferol. J Appl Poult Res 1997;6:410–6.
