Comparison of Bioavailability of 1α-Hydroxycholecalciferol and Cholecalciferol in Broiler Chicken Diets

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The present study aims to compare the relative bioavailability (RBV) of 1α-hydroxycholecalciferol (1α-OH-D3) to cholecalciferol (D3) in 1- to 21-d-old broiler chicks fed with calcium (Ca)- and phosphorus (P)-deficient diets. A total of 400 male of 1-d-old Ross 308 broilers were randomly assigned to 8 treatments with 5 replicates each. Five levels of D3 (0, 2.5, 5, 10, and 20 μg/kg) and three levels of 1α-OH-D3 (1.25, 2.5, and 5 μg/kg) were added to a basal diet. The basal diet contained 0.50% Ca and 0.25% non-phytate phosphorus (NPP), without D3. The RBV of 1α-OH-D3 to D3 was determined by the slope ratio method. Using bodyweight gain, feed intake, feed efficiency, and plasma Ca as criteria, the RBV of 1α-OH-D3 to D3 were 4.78, 4.75, 4.50, and 4.21, respectively. Using tibia breaking-strength, weight, length, width, ash weight and content, and Ca and P content as criteria, the RBV of 1α-OH-D3 to D3 were 5.58, 5.16, 4.42, 4.70, 5.03, 4.46, 4.70, and 4.79. Using femur weight, length, width, weight and content, and Ca and P content as criteria, the RBV of 1α-OH-D3 to D3 were 5.00, 4.05, 5.94, 4.73, 5.33, 5.64, and 4.28. These data indicate that the RBV of 1α-OH-D3 to D3 is 4.84 in promoting growth performance and bone mineralization in broilers from 1 to 21 d of age.

Key words: broiler chicken, cholecalciferol, 1α-hydroxycholecalciferol, relative bioavailability

Introduction

Cholecalciferol (D3) is widely used as feed additive in the feed industry for the vitamin D requirements of poultry. The compound 1α-hydroxycholecalciferol (1α-OH-D3) is the derivative of D3. Research has shown that D3 is originally hydroxylated to 25-hydroxycholecalciferol (25-OH-D3) in the liver and subsequently to its active hormonal form 1α,25-dihydroxycholecalciferol (1α, 25-(OH)2-D3) in the kidney (Norman et al., 1982). By contrast, 1α-OH-D3 is rapidly hydroxylated in the liver to 1α,25-(OH)2-D3 without hydroxylation of the kidney (Paaren et al., 1978).

Previous research has shown that D3 linearly increased body weight gain (BWG) and feed intake (FI) when broilers were fed calcium (Ca)- and phosphorus (P)-deficient diets; by contrast, the growth of birds quadratically responded to D3 when fed with Ca- and P-adequate diets (Baker et al., 1998). These results suggest that the biological activity of vitamin D should be evaluated at low levels of Ca and P. However, Edwards et al. (2002a) evaluated the relative bioavailability (RBV) of 1α-OH-D3 to D3 in Ca- and P-adequate diets, using only the tibia as criteria. Our unpublished data indicated differences in growth and mineralization among the tibia, femur, and metatarsus of broiler chickens. Thus, the femur and metatarsus should be used in evaluating the RBV of vitamin D derivatives.

This study aims to evaluate the RBV of 1α-OH-D3 to D3 in Ca- and P-deficient broiler chicken diets, using growth performance and mineralization of the femur, tibia, and metatarsus as criteria.

Materials and Methods

Birds, Diets and Management

All procedures used in this study were approved by the Animal Care Committee of Henan Agricultural University and Shangqiu Normal University. On the day of hatch, 400 male Ross 308 broilers were weighed and randomly allotted to 8 treatments with 5 cages having 10 birds each. Broilers from 1 to 12 d of age and 13
to 21 d of age were reared in starting cages (70 cm × 70 cm × 30 cm) and growing cages (190 cm × 50 cm × 35 cm), respectively. A basal diet was formulated to contain suboptimal concentrations of Ca (0.50%) and NPP (0.25%), without D3. Five levels of D3 (0, 2.5, 5, 10 and 20 μg/kg) and three levels of 1α-OH-D3 (1.25, 2.5, and 5 μg/kg) were added to the basal diet. The birds were provided ad libitum access to mash feed and water during the 21 d of experiment, with 20 h of light and 4 h of darkness. Room temperature was controlled at 34°C from d 1 to 3, and then gradually decreased by 3°C per week to a final temperature of 25°C.

1α-OH-D3 and D3

The crystalline 1α-OH-D3 and D3 were supplied by Taizhou Healtech Chemical Co. Ltd. (Taizhou, China) and Tongxiang Tianhecheng Food Technology Co. Ltd. (Tongxiang, China), respectively. The 1α-OH-D3 and D3 solution were prepared using the method of Biehl and Baker (1997). Briefly, the crystalline was dissolved in ethanol, and then diluted to a final concentration of 10 mg/L of 1α-OH-D3 or 50 mg/L of D3 in a solution with 5% ethanol and 95% propylene glycol. The vitamin D stock solutions were used to mix the diets.

Sample Collection

All broilers were weighed on d 1 and 21. A chicken weighing close to the average weight of the replicate was selected for collection of blood and bones. Plasma samples (5 mL) were collected through cardiac puncture, and then immediately centrifuged for 10 min at 3000 × g and 20°C. The left and right femur, tibia, and metatarsus of individual birds were excised and frozen at −20°C for further analysis (breaking-strength, weight, length, width, ash weight, and percentage content of ash, Ca, and P).

Chemical Analysis

Plasma Ca, inorganic phosphorus (Pi), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatinine (CRE), and total protein (TP) were determined using a Shimadzu CL-8000 analyzer (Shimadzu Corp., Kyoto, Japan) following the instructions of the manufacturer.

The left femur, tibia, and metatarsus were boiled for 5 min to loosen the muscle tissues using the method by Hall et al. (2003). The meat, connective tissue, and fibula bone were completely removed using scissors and forceps. The femur, tibia, and metatarsus were placed in a container with ethanol for 48 h (removing water and polar lipids) after cleaning. Afterward, the bones were further extracted in anhydrous ether for 48 h (removing non-polar lipids). The bones were dried at 105°C for 24 h, and then weighed. Bone width was determined at the medial point. Bone ash content was determined by ashing the bone in a muffle furnace for 18 h at 600°C.

The right tibia was used to analyze the breaking-strength, which was determined using an all-digital electronic universal testing machine (Shenzhen Hengen Instrument Co. Ltd., Shenzhen, China). Tibias were cradled on two support points measuring 4 cm apart. Force was applied to the midpoint of the same face of each tibia using a 50 kg load cell with a crosshead speed of 10 mm/min (Jendral et al., 2008).

Statistical Analysis

Replicate means are the experimental units in the statistical analysis. The data were analyzed by using the GLM procedure of the SAS software (SAS Institute, 2002). Means were compared using the Tukey’s test when the probability values were significant (P < 0.05). The relative bioavailability (RBV) of 1α-OH-D3 to D3 was determined by the slope ratio method (Littell et al., 1997). The regression equation $y=a+b_1x_1+b_2x_2$ was used, where $y$, $x_1$, and $x_2$ represent the response (growth performance and bone mineralization), $D_3$, and 1α-OH-D3, respectively. The ratio is the relative bioavailability value (RBV = $b_2/b_1$). Orthogonal contrasts were used to determine the linear and quadratic effects of 1α-OH-D3 or D3 on growth performance and bone mineralization.

Results

Growth Performance

The body weight gain (BWG), feed intake (FI), feed efficiency (FE) and mortality were affected by the levels of $D_3$ or 1α-OH-D3 in 1- to 21-d-old broiler chickens (P < 0.05) (Table 2). BWG, FI, and FE responded linearly to $D_3$ ranging from 0 to 20 μg/kg and 1α-OH-D3 from 0 to 5 μg/kg. Mortality decreased by the levels of $D_3$ or 1α-OH-D3 (P < 0.05).

### Table 1. Ingredients and nutrient composition of the basal diet

| Ingredient (%) | Day 1–21 |
|----------------|---------|
| Corn           | 60.61   |
| Soybean meal   | 32.00   |
| Soybean oil    | 1.60    |
| Soy protein concentrate | 3.47 |
| Limestone      | 0.67    |
| Dicalcium phosphate | 0.71 |
| L-Lysine·HCl   | 0.14    |
| DL-Methionine  | 0.14    |
| Trace mineral premix 1 | 0.10 |
| Vitamin premix 2 | 0.03 |
| Choline chloride (50%) | 0.20 |
| Sodium chloride | 0.30    |

1 The trace mineral premix contained the following (per kilogram of diet): 100 mg iron; 100 mg zinc; 8 mg copper; 120 mg manganese; 0.7 mg iodine; 0.3 mg selenium.

2 The vitamin premix contained the following (per kilogram of diet): 8,000 IU vitamin A; 20 IU vitamin E; 0.5 mg menadione; 2.0 mg thiamine; 8.0 mg riboflavin; 35 mg niacin; 3.5 mg pyridoxine; 0.01 mg vitamin B12; 10.0 mg pantothenic acid; 0.55 mg folic acid; 0.18 mg biotin.
Plasma Biochemical Parameters

The addition of D₃ or 1α-OH-D₃ increased plasma Ca and Pi, but decreased plasma ALT, AST, LDH, CRE, and TP (Table 3).

Bone Mineralization

Bone (tibia, femur, and metatarsus) quality was significantly influenced by the levels of D₃ or 1α-OH-D₃ in 1- to 21-d-old broilers (Tables 4, 5, and 6). The bone weight, length, width, bone ash weight and content, and Ca and P content responded linearly to D₃ ranging from 0 to 20 μg/kg and 1α-OH-D₃ from 0 to 5 μg/kg. The addition D₃ or 1α-OH-D₃ resulted in greater values of bone mineralization.
Table 4. Effects of D$_3$ and 1α-OH-D$_3$ on tibia mineralization of 1- to 21-d-old broilers fed with 0.50% calcium (Ca) and 0.25% non-phytate phosphorus (NPP)$^1$

| D$_3$ (μg/kg) | 1α-OH-D$_3$ (μg/kg) | Breaking-strength (N) | Weight (g) | Length (cm) | Width (cm) | Ash (g) | Ash (%) | Ca (%) | P (%) |
|--------------|---------------------|-----------------------|------------|-------------|-----------|---------|---------|--------|-------|
| 0            | 0                   | 23.84$^d$             | 0.83$^c$   | 4.51$^f$   | 0.45$^c$  | 0.22$^e$ | 26.24$^d$ | 9.03$^d$ | 5.17$^d$|
| 2.5          | 28.52$^c$           | 1.05$^{bc}$           | 5.12$^c$   | 0.51$^{bc}$ | 0.31$^f$  | 29.04$^d$ | 9.66$^d$  | 5.66$^d$|
| 5            | 58.11$^b$           | 1.21$^{cd}$           | 5.59$^a$   | 0.58$^{ab}$ | 0.45$^d$  | 36.81$^a$ | 12.42$^b$ | 7.08$^c$|
| 10           | 76.08$^a$           | 1.44$^{ab}$           | 6.07$^{bc}$| 0.59$^{ab}$ | 0.61$^{bc}$| 42.07$^{ab}$| 14.71$^{ab}$| 7.92$^{ab}$|
| 20           | 79.62$^{bc}$        | 1.57$^{ab}$           | 6.32$^{ab}$| 0.60$^a$   | 0.67$^{ab}$| 42.57$^{ab}$| 14.93$^{ab}$| 7.99$^{ab}$|
|              | 1.25                | 64.58$^{bc}$          | 1.36$^{bc}$| 5.73$^{d}$ | 0.58$^{ab}$| 52.52$^{bc}$| 38.56$^{bc}$| 7.15$^{bc}$|
|              | 2.5                 | 66.18$^{bc}$          | 1.50$^{ab}$| 6.11$^{ab}$| 0.59$^{ab}$| 62.82$^{bc}$| 41.35$^{ab}$| 14.77$^{ab}$| 7.99$^{ab}$|
|              | 5                   | 109.45$^a$            | 1.73$^a$   | 6.45$^a$   | 0.62$^a$  | 77$^a$    | 44.12$^a$ | 15.73$^a$ | 8.49$^a$|
| SEM$^3$      |                     | 4.33                  | 0.05       | 0.10       | 0.01      | 0.03     | 1.04     | 0.41    | 0.19     |
| $P$          |                     | <0.001                | <0.001     | <0.001     | <0.001    | <0.001   | <0.001   | <0.001   | <0.001   |

Source of variance

- $D_3$: Linear <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001
- Quadratic 0.198 0.490 0.052 0.036 0.064 0.023 0.032 0.001 0.001

- 1α-OH-D$_3$: Linear <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001
- Quadratic 0.786 0.017 0.001 0.003 0.022 0.000 0.002 0.001 0.001

$^1$Means in the same column without a common superscript significantly differ ($P<0.05$).

$^2$Data are the means of 5 replicate cages consisting of 1 chick per replicate cage.

$^3$SEM = pooled standard error of the mean.

Table 5. Effects of D$_3$ and 1α-OH-D$_3$ on femur mineralization of 1- to 21-d-old broilers fed with 0.50% calcium (Ca) and 0.25% non-phytate phosphorus (NPP)$^1$

| D$_3$ (μg/kg) | 1α-OH-D$_3$ (μg/kg) | Weight (g) | Length (cm) | Width (cm) | Ash (g) | Ash (%) | Ca (%) | P (%) |
|--------------|---------------------|------------|-------------|-----------|---------|---------|--------|-------|
| 0            | 0                   | 0.63$^d$   | 3.19$^e$   | 0.53$^b$  | 0.15$^f$ | 24.69$^e$ | 7.91$^d$ | 4.79$^d$|
| 2.5          | 28.52$^c$           | 3.65$^d$  | 0.63$^{ab}$ | 0.23$^e$  | 30.03$^d$ | 10.51$^b$ | 5.60$^cd$|
| 5            | 58.11$^b$           | 4.14$^e$  | 0.69$^a$   | 0.37$^d$  | 36.61$^c$ | 11.81$bc$ | 6.45$bc$|
| 10           | 1.11$^{bc}$         | 4.55$^{bc}$| 0.65$^a$   | 0.44$^{bc}$| 40.23$^{bc}$| 13.55$^{ab}$| 7.40$^{ab}$|
| 20           | 1.20$^{ab}$         | 4.80$^a$  | 0.67$^a$   | 0.49$^b$  | 41.32$^{ab}$| 14.56$^e$ | 7.59$^ab$|
|              | 1.25                | 1.02$^e$  | 4.32$^{bc}$| 0.60$^{ab}$| 40.09$^{bc}$| 13.53$^{ab}$| 7.30$^{ab}$|
|              | 2.5                 | 1.15$^{bc}$| 4.65$^{ab}$| 0.62$^{ab}$| 42.21$^{ab}$| 15.15$^e$ | 7.59$^{ab}$|
|              | 5                   | 1.33$^a$  | 4.86$^{ab}$| 0.69$^{a}$ | 59$^{a}$ | 44.09$^a$ | 15.23$^{a}$| 8.16$^{a}$|
| SEM$^3$      |                     | 0.04       | 0.09       | 0.01      | 0.02     | 1.04     | 0.40    | 0.19     |
| $P$          |                     | <0.001     | <0.001     | <0.001    | <0.001   | <0.001   | <0.001   | <0.001   |

Source of variance

- $D_3$: Linear <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001
- Quadratic 0.069 0.215 0.022 0.067 0.002 0.215 0.038 0.001 0.238

- 1α-OH-D$_3$: Linear <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001 <0.001
- Quadratic 0.010 0.001 0.920 0.001 0.001 0.001 0.001 0.001 0.003

$^1$Means in the same column without a common superscript significantly differ ($P<0.05$).

$^2$Data are the means of 5 replicate cages consisting of 1 chick per replicate cage.

$^3$SEM = pooled standard error of the mean.

compared with the birds fed with basal diets ($P<0.05$). Tibia breaking-strength was also enhanced linearly to $D_3$ or 1α-OH-D$_3.$

The RBV Value

Using BWG, FI, FE, and plasma Ca as criteria, the RBV of 1α-OH-D$_3$ to $D_3$ were 5.58, 5.16, 4.42, 4.70, 5.03, 4.46, 4.70, and 4.79. Using femur weight, length, width, ash weight and content, and Ca and P content as criteria, the RBV of 1α-OH-D$_3$ to $D_3$ were 5.09, 4.43, 3.19, 5.83, 5.21, 5.27, and 5.31. Using metatarsus weight, length, width, ash weight and content, and Ca and P content...
Table 6. Effects of D₃ and 1α-OH-D₃ on metatarsus mineralization of 1- to 21-d-old broilers fed with 0.50% calcium (Ca) and 0.25% non-phytate phosphorus (NPP)¹

| D₃ (µg/kg) | 1α-OH-D₃ (µg/kg) | Weight (g) | Length (cm) | Width (cm) | Ash (g) | Ash (%) | Ca (%) | P (%) |
|------------|------------------|------------|-------------|------------|---------|---------|--------|------|
| 0          | 0                | 0.44b      | 3.30a       | 0.49a      | 0.10b   | 23.09b  | 6.66b  | 4.35b|
| 2.5        | 0.69b            | 3.76b      | 0.59b       | 0.16ab     | 26.88b  | 9.21b   | 5.32c  |
| 5          | 0.75cd           | 4.07cd     | 0.66bc      | 0.26d      | 34.04a  | 11.66bc | 6.68ab |
| 10         | 0.92abc          | 4.53ab     | 0.68b       | 0.34bc     | 37.12a  | 12.83a  | 7.48a  |
| 20         | 0.99abc          | 4.70a      | 0.72ab      | 0.40bc     | 36.84a  | 12.95a  | 7.02a  |
| 1.25       | 0.89bc           | 4.24bc     | 0.70b       | 0.32cd     | 36.30a  | 12.71a  | 6.42ab |
| 2.5        | 0.93ab           | 4.46ab     | 0.73ab      | 0.36bc     | 38.79a  | 14.14a  | 7.14a  |
| 5          | 1.09a            | 4.70a      | 0.79a       | 0.43a      | 39.39a  | 14.20a  | 7.37a  |

SEM³

| D₃ | Linear | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
|----|--------|--------|--------|--------|--------|--------|--------|--------|
| 1α-OH-D₃ | Linear | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

Source of variance

¹α=Means in the same column without a common superscript significantly differ (P<0.05).
²D₃=cholecalciferol, 1α-OH-D₃=1α-hydroxycholecalciferol, P=phosphorus.
³SEM=pooled standard error of the mean.

Table 7. Relative bioavailability (RBV) of 1α-OH-D₃ to D₃ in 1- to 21-d-old broilers fed with 0.50% calcium (Ca) and 0.25% non-phytate phosphorus (NPP)¹

| Item          | Criteria | Formula                      | P    | R²     | RBV |
|---------------|----------|------------------------------|------|--------|-----|
| Growth       | Body weight gain | y=404.0918+16.5774x₁+79.993x₂ | <0.001 | 0.71  | 4.78 |
|              | Feed intake | y=712.1154+19.0561x₁+90.4751x₂ | <0.001 | 0.72  | 4.75 |
|              | Feed efficiency | y=0.5590+0.0064x₁+0.0288x₂ | <0.001 | 0.39  | 4.50 |
|              | Plasma Ca | y=7.1910+0.1109x₁+0.4672x₂ | <0.001 | 0.51  | 4.21 |
| Tibia        | Breaking-strength | y=34.1606+2.6993x₁+15.0712x₂ | <0.001 | 0.77  | 5.58 |
|              | Weight    | y=1.0024+0.0305x₁+0.1574x₂ | <0.001 | 0.74  | 5.16 |
|              | Length    | y=5.0449+0.0728x₁+0.3216x₂ | <0.001 | 0.73  | 4.42 |
|              | Width     | y=0.5118+0.0053x₁+0.0249x₂ | <0.001 | 0.41  | 4.70 |
|              | Ash weight | y=0.3188+0.0197x₁+0.0990x₂ | <0.001 | 0.78  | 5.03 |
|              | Ash content | y=30.9996+0.6894x₁+3.0759x₂ | <0.001 | 0.64  | 4.46 |
|              | Ca content | y=10.4936+0.2609x₁+1.2272x₂ | <0.001 | 0.63  | 4.70 |
|              | P content  | y=5.9826+0.1210x₁+0.5800x₂ | <0.001 | 0.65  | 4.79 |
| Femur        | Weight    | y=0.7842+0.0236x₁+0.1201x₂ | <0.001 | 0.76  | 5.09 |
|              | Length    | y=3.6572+0.0643x₁+0.2848x₂ | <0.001 | 0.69  | 4.43 |
|              | Width     | y=0.5925+0.0052x₁+0.0166x₂ | 0.007 | 0.24  | 3.19 |
|              | Ash weight | y=0.2470+0.0141x₁+0.0759x₂ | <0.001 | 0.78  | 5.38 |
|              | Ash content | y=31.0489+0.6078x₁+3.1665x₂ | <0.001 | 0.61  | 5.21 |
|              | Ca content | y=10.3304+0.2345x₁+1.2355x₂ | <0.001 | 0.62  | 5.27 |
|              | P content  | y=5.7303+0.1075x₁+0.5713x₂ | <0.001 | 0.58  | 5.31 |
| Metatarsus   | Weight    | y=0.6035+0.0219x₁+0.1094x₂ | <0.001 | 0.68  | 5.00 |
|              | Length    | y=3.7008+0.0568x₁+0.2303x₂ | <0.001 | 0.70  | 4.05 |
|              | Width     | y=0.5796+0.0081x₁+0.0481x₂ | <0.001 | 0.62  | 5.94 |
|              | Ash weight | y=0.1752+0.0123x₁+0.0582x₂ | <0.001 | 0.74  | 4.73 |
|              | Ash content | y=28.8116+0.4988x₁+2.6572x₂ | <0.001 | 0.49  | 5.33 |
|              | Ca content | y=9.4990+0.2115x₁+1.1921x₂ | <0.001 | 0.49  | 5.64 |
|              | P content  | y=5.4692+0.1058x₁+0.4533x₂ | <0.001 | 0.42  | 4.28 |

¹x₁=D₃, x₂=1α-OH-D₃, P=phosphorus.
as criteria, the RBV of 1α-OH-D₃ to D₃ were 5.00, 4.05, 5.94, 4.73, 5.33, 5.64, and 4.28. The results showed that the RBV of 1α-OH-D₃ to D₃ ranged from 4.21 to 4.78 in terms of growth performance and 3.19 to 5.94 with respect to bone mineralization.

**Discussion**

**Growth Performance**

Addition of D₃ (Qian et al., 1997) or 1α-OH-D₃ (Han et al., 2012a) significantly increased BWG and FI in birds fed with Ca- and P-deficient diet. In the present study, the increase in D₃ or 1α-OH-D₃ improved the BWG, FI, and FE of broilers fed with 0.50% Ca and 0.25% NPP. Broilers fed with Ca- and P-deficient diet supplemented with D₃ exhibited equal growth performance compared with birds fed with diet having adequate Ca and P (Rao et al., 2006).

Vitamin D deficiency caused severe tibial dyschondroplasia (Rennie and Whitehead, 1996; Roberson and Edwards, 1996; Fritts and Waldroup, 2003) or mortality (Han et al., 2012b) of broiler chicks. In this study, the mortality of broilers was significantly reduced by the addition of D₃ or 1α-OH-D₃.

**Plasma Biochemical Parameters**

Vitamin D deficiency decreased the serum Ca in broilers (Aslam et al., 1998), or slightly depressed plasma P in rats (Bronner and Freund, 1975). The levels of D₃ or 1α-OH-D₃ increased serum Ca and Pi concentrations in the current study, in agreement with the studies conducted by Edwards et al. (2002a) and Rao et al. (2006).

The insufficiency of vitamin D resulted in an abnormal increase in plasma total protein (TP) (39.6 g/L) in this study, in agreement with the findings of Han et al. (2009a). Addition of D₃ or 1α-OH-D₃ decreased the plasma TP to a normal level. Bowes et al. (1989) and Silva et al. (2007) measured the normal TP level (26.5 to 30.6 g/L) of 3-week-old broilers.

ALT and AST are the biochemical indicators of hepatocyte damage. Previous studies have shown that plasma ALT (Hsu et al., 2011; Price et al., 2012) and AST (Price et al., 2012) increased because of liver diseases. LDH and CRE were used as indicators for evaluating kidney function. Serum LDH isozyme activity was elevated because of kidney damage induced by high doses of drugs (Chan et al., 1995). According to Myers et al. (2006) and Prowle et al. (2014), kidney disease also contributes to the increase in serum CRE. In this study, deficiency of vitamin D increased plasma ALT, AST, LDH and CRE, which decreased after addition of D₃ or 1α-OH-D₃.

**Bone Mineralization**

Bone ash weight and content increased with the increase in D₃ (Fritts and Waldroup, 2003; Rao et al., 2007) or 1α-OH-D₃ (Edwards, 2002b). Similar results were obtained in this study. The weight, length, width, ash weight and content, and Ca and P content of the femur, tibia, and metatarsus increased with the increase in D₃ or 1α-OH-D₃. Tibia breaking-strength was also enhanced by D₃ or 1α-OH-D₃ in this study, which was consistent with our previous results (Han et al., 2009a, b, 2012b). These results suggest that D₃ and 1α-OH-D₃ promote bone growth and mineralization by increasing the absorption and retention of Ca and P.

**The RBV Value**

The biological activity of 1α-OH-D₃ was higher than that of D₃ in this study. This result agrees with the findings of Edwards et al. (2002a), who found that 1α-OH-D₃ was approximately 8 times as active as D₃ in increasing growth. However, the RBV of 1α-OH-D₃ to D₃ in the present study were lower than that reported by Edwards et al. (2002a).

The RBV values of 1α-OH-D₃ to D₃ were 10.08, 9.50, 11.26, and 4.48 when BWG, plasma Ca, and tibia ash weight and percentage were used as criteria in 1- to 16-d-old mixed-sex broilers fed with diets containing 1.00% Ca and 0.70% tP (Edwards et al., 2002a). According to Liem (2009), the RBV values of 1α-OH-D₃ to D₃ were 6.92, 5.97, 4.56, 6.40, and 5.68 when BWG, FI, plasma Ca, tibia ash weight and content were used as criteria in 10- to 26-d-old mixed-sex broilers fed with diets containing 1.00% Ca and 0.73% tP. However, 1- to 21-d-old male birds fed with diet containing 0.50% Ca and 0.25% NPP was used in the current study. The RBV of 1α-OH-D₃ to D₃ in the present study were lower than that of other studies, which maybe due to the sex and age of the birds, the product of D₃ and 1α-OH-D₃, and the levels of Ca and NPP. Furthermore, femur and metatarsus parameters have not yet been used as criteria to evaluate the RBV of 1α-OH-D₃ to D₃ in previous research.

In conclusion, the increasing levels of D₃ or 1α-OH-D₃ improved growth performance, plasma Ca and Pi, and bone quality of the broilers. These data indicate that the RBV of 1α-OH-D₃ to D₃ is 4.84 in promoting growth performance and bone mineralization in 1- to 21-d-old broiler chicks fed with Ca- and P-deficient diets.

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