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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-D_3) to cholecalciferol (D_3) 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 D_3 (0, 2.5, 5, 10, and 20 μg/kg) and three levels of 1α-OH-D_3 (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 D_3. The RBV of 1α-OH-D_3 to D_3 was determined by the slope ratio method. Using body weight gain, feed intake, feed efficiency, and plasma Ca as criteria, the RBV of 1α-OH-D_3 to D_3 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-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 as criteria, the RBV of 1α-OH-D_3 to D_3 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-D_3 to D_3 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

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Introduction

Cholecalciferol (D_3) is widely used as feed additive in the feed industry for the vitamin D requirements of poultry. The compound 1α-hydroxycholecalciferol (1α-OH-D_3) is the derivative of D_3. Research has shown that D_3 is originally hydroxylated to 25-hydroxycholecalciferol (25-OH-D_3) in the liver and subsequently to its active hormonal form 1α,25-hydroxycholecalciferol (1α, 25-$(OH)_2$ -D_3) in the kidney (Norman *et al.*, 1982). By contrast, 1α-OH-D_3 is rapidly hydroxylated in the liver to 1α,25-$(OH)_2$-D_3 without hydroxylation of the kidney (Paaren *et al.*, 1978).

Previous research has shown that D_3 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 D_3 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-D_3 to D_3 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-D_3 to D_3 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

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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 D_3. Five levels of D_3 (0, 2.5, 5, 10 and 20 μg/kg) and three levels of 1α-OH-D_3 (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℃ from d 1 to 3, and then gradually decreased by 3℃ per week to a final temperature of 25℃.

1α-OH-D₃ and D₃

The crystalline 1α-OH-D_3 and D_3 were supplied by Taizhou Healtech Chemical Co. Ltd. (Taizhou, China) and Tongxiang Tianhecheng Food Technology Co. Ltd. (Tongxiang, China), respectively. The 1α-OH-D_3 and D_3 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-D_3 or 50 mg/L of D_3 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℃. The left and right femur, tibia, and metatarsus of individual birds were excised and frozen at −20℃ 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℃ 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℃.

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).

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
Nutrient composition	
Metabolizable energy (MJ/kg)	12.45
Calculated crude protein (%)	21.24
Analyzed calcium (%)	0.49
Analyzed total phosphorus (%)	0.49
Calculated non-phytate phosphorus (%)	0.25

[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 B_{12}; 10.0 mg pantothenic acid; 0.55 mg folic acid; 0.18 mg biotin.

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-D_3 to D_3 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-D_3, 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-D_3 or D_3 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-D_3 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-D_3 from 0 to 5 μg/kg. Mortality decreased by the levels of D_3 or 1α-OH-D_3 ($P<$ 0.05).

Table 2. **Effects of D_3 and 1α-OH-D_3 on the growth performance of 1- to 21-d-old broilers fed with 0.50% calcium (Ca) and 0.25% non-phytate phosphorus (NPP)[1]**

D_3[2] (μg/kg)	1α-OH-D_3 (μg/kg)	Body weight gain (g/bird)	Feed intake (g/bird)	Feed efficiency (gain/feed)	Mortality (%)
0	0	267^{f}	588^{f}	0.45^{c}	46^{a}
2.5		382^{e}	685^{e}	0.56^{b}	24^{b}
5		546^{d}	826^{d}	0.67^{a}	12^{bc}
10		655^{b}	1012^{bc}	0.65^{a}	0^{c}
20		686^{b}	1043^{ab}	0.66^{a}	2^{c}
	1.25	596^{c}	942^{c}	0.63^{a}	2^{c}
	2.5	674^{b}	1004^{bc}	0.67^{a}	2^{c}
	5	741^{a}	1102^{a}	0.67^{a}	0^{c}
SEM[3]		25	28	0.01	3
P		<0.001	<0.001	<0.001	<0.001
Source of variance					
D_3	Linear	<0.001	<0.001	<0.001	<0.001
	Quadratic	<0.001	0.262	<0.001	0.017
1α-OH-D_3	Linear	<0.001	<0.001	<0.001	<0.001
	Quadratic	<0.001	<0.001	<0.001	<0.001

a–f Means in the same column without a common superscript significantly differ ($P<0.05$).
1 Data are the means of 5 replicate cages consisting of 10 chicks per cage.
2 D_3=cholecalciferol, 1α-OH-D_3=1α-hydroxycholecalciferol.
3 SEM=pooled standard error of the mean.

Table 3. **Effects of D_3 and 1α-OH-D_3 on plasma biochemical parameters of 1- to 21-d-old broilers fed with 0.50% calcium (Ca) and 0.25% non-phytate phosphorus (NPP)[1]**

D_3[2] (μg/kg)	1α-OH-D_3 (μg/kg)	Ca (mg/dL)	Pi (mg/dL)	ALT (U/L)	AST (U/L)	LDH (U/L)	CRE (μmol/L)	TP (g/L)
0	0	7.37^{c}	5.98^{b}	5.2^{a}	443.3^{a}	1496.6^{a}	6.5^{a}	39.6^{a}
2.5		7.57^{bc}	6.85^{ab}	3.8^{b}	379.1^{ab}	1376.6^{ab}	4.0^{b}	35.8^{ab}
5		6.88^{c}	6.76^{ab}	4.0^{b}	256.8^{cd}	791.2^{c}	3.4^{b}	30.0^{bc}
10		9.08^{a}	6.87^{ab}	3.6^{b}	230.5^{d}	718.6^{c}	3.8^{b}	33.6^{abc}
20		9.22^{a}	7.29^{a}	4.0^{b}	250.6^{cd}	772.6^{c}	3.4^{b}	29.7^{bc}
	1.25	7.40^{c}	6.49^{ab}	4.1^{ab}	340.8^{bc}	1505.6^{a}	3.1^{b}	32.1^{bc}
	2.5	8.91^{ab}	5.90^{b}	4.4^{ab}	293.8^{bcd}	1095.6^{b}	2.8^{b}	30.1^{bc}
	5	9.34^{a}	7.03^{a}	4.3^{ab}	240.0^{d}	759.4^{c}	3.8^{b}	28.3^{c}
SEM[3]		0.18	0.10	0.1	13.2	56.4	0.2	0.8
P		<0.001	<0.001	0.003	<0.001	<0.001	<0.001	<0.001
Source of variance								
D_3	Linear	<0.001	0.001	0.002	<0.001	<0.001	<0.001	0.001
	Quadratic	0.039	0.417	0.002	0.002	<0.001	0.001	0.180
1α-OH-D_3	Linear	<0.001	0.037	0.054	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.506	0.231	0.070	0.319	0.059	<0.001	0.069

a–d Means in the same column without a common superscript significantly differ ($P<0.05$).
1 Data are the means of 5 replicate cages consisting of 1 chick per replicate cage.
2 D_3=cholecalciferol, 1α-OH-D_3=1α-hydroxycholecalciferol, ALT=alanine aminotransferase, AST=aspartate aminotransferase, LDH=lactate dehydrogenase, CRE=creatinine, TP=total protein, Pi=inorganic phosphorus.
3 SEM=pooled standard error of the mean.

Plasma Biochemical Parameters

The addition of D_3 or 1α-OH-D_3 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 D_3 or 1α-OH-D_3 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 D_3 ranging from 0 to 20 μg/kg and 1α-OH-D_3 from 0 to 5 μg/kg. The addition D_3 or 1α-OH-D_3 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[2] (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[e]	4.51[f]	0.45[c]	0.22[e]	26.24[d]	9.03[d]	5.17[d]
2.5		28.52[d]	1.05[de]	5.12[e]	0.51[bc]	0.31[e]	29.04[d]	9.66[d]	5.66[d]
5		58.11[c]	1.21[cd]	5.59[d]	0.58[ab]	0.45[d]	36.81[c]	12.42[c]	7.08[c]
10		76.08[b]	1.44[bc]	6.07[bc]	0.59[ab]	0.61[bc]	42.07[ab]	14.71[ab]	7.92[abc]
20		79.62[b]	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[cd]	0.58[ab]	0.52[cd]	38.56[bc]	13.21[bc]	7.17[bc]
	2.5	66.18[bc]	1.50[ab]	6.11[ab]	0.59[ab]	0.62[bc]	41.35[ab]	14.77[ab]	7.99[ab]
	5	109.45[a]	1.73[a]	6.45[a]	0.62[a]	0.77[a]	44.12[a]	15.73[a]	8.49[a]
SEM3		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
	Quadratic	0.198	0.490	0.052	0.036	0.646	0.063	0.495	0.057
1α-OH-D_3	Linear	<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	<0.001	0.002	0.001

[a–f] Means in the same column without a common superscript significantly differ ($P<0.05$).
[1] Data are the means of 5 replicate cages consisting of 1 chick per replicate cage.
[2] D_3=cholecalciferol, 1α-OH-D_3=1α-hydroxycholecalciferol, P=phosphorus.
[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[2] (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		0.78[d]	3.65[d]	0.63[ab]	0.23[e]	30.03[d]	10.51[c]	5.60[cd]
5		1.00[c]	4.14[c]	0.69[a]	0.37[d]	36.61[c]	11.81[bc]	6.45[bc]
10		1.11[bc]	4.55[ab]	0.65[a]	0.44[bc]	40.27[abc]	13.55[ab]	7.40[ab]
20		1.20[ab]	4.80[a]	0.67[a]	0.49[b]	41.32[ab]	14.56[a]	7.59[ab]
	1.25	1.02[c]	4.32[bc]	0.60[ab]	0.40[cd]	39.67[bc]	13.53[ab]	7.30[ab]
	2.5	1.15[bc]	4.65[ab]	0.62[ab]	0.49[b]	42.21[ab]	15.15[a]	7.59[ab]
	5	1.33[a]	4.86[a]	0.69[a]	0.59[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	0.002	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.069	0.215	0.022	0.067	0.002	0.021	0.238
1α-OH-D_3	Linear	<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	0.003

[a–f] Means in the same column without a common superscript significantly differ ($P<0.05$).
[1] Data are the means of 5 replicate cages consisting of 1 chick per replicate cage.
[2] D_3=cholecalciferol, 1α-OH-D_3=1α-hydroxycholecalciferol, P=phosphorus.
[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 4.78, 4.75, 4.50, and 4.21, respectively (Table 7). Using tibia breaking-strength, 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 5.58, 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 D_3 and 1α-OH-D_3 on metatarsus mineralization of 1- to 21-d-old broilers fed with 0.50% calcium (Ca) and 0.25% non-phytate phosphorus (NPP)[1]**

D_3[2] (μg/kg)	1α-OH-D_3 (μg/kg)	Weight (g)	Length (cm)	Width (cm)	Ash (g)	Ash (%)	Ca (%)	P (%)
0	0	0.44^{e}	3.30^{e}	0.49^{d}	0.10^{e}	23.09^{b}	6.66^{c}	4.35^{c}
2.5		0.60^{de}	3.76^{d}	0.59^{c}	0.16^{e}	26.88^{b}	9.21^{bc}	5.32^{bc}
5		0.75^{cd}	4.07^{cd}	0.66^{bc}	0.26^{d}	34.04^{a}	11.66^{ab}	6.60^{ab}
10		0.92^{abc}	4.53^{ab}	0.68^{b}	0.34^{bc}	37.12^{a}	12.83^{a}	7.48^{a}
20		0.99^{ab}	4.70^{a}	0.72^{ab}	0.40^{ab}	36.84^{a}	12.95^{a}	7.01^{a}
	1.25	0.89^{bc}	4.24^{bc}	0.70^{b}	0.32^{cd}	36.30^{a}	12.71^{a}	6.42^{ab}
	2.5	0.93^{ab}	4.46^{ab}	0.73^{ab}	0.36^{abc}	38.79^{a}	14.14^{a}	7.14^{a}
	5	1.09^{a}	4.70^{a}	0.79^{a}	0.43^{a}	39.39^{a}	14.20^{a}	7.37^{a}
SEM[3]		0.03	0.08	0.02	0.02	0.97	0.44	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
	Quadratic	0.316	0.146	0.043	0.846	0.014	0.022	0.016
1α-OH-D_3	Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.001	<0.001	0.002	<0.001	<0.001	<0.001	0.002

[a–e] Means in the same column without a common superscript significantly differ ($P<0.05$).
[1] Data are the means of 5 replicate cages consisting of 1 chick per replicate cage.
[2] D_3=cholecalciferol, 1α-OH-D_3=1α-hydroxycholecalciferol, P=phosphorus.
[3] SEM=pooled standard error of the mean.

Table 7. **Relative bioavailability (RBV) of 1α-OH-D_3 to D_3 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	R^2	RBV
Growth	Body weight gain	$y=404.0918+16.5774x_1+79.2993x_2$	<0.001	0.71	4.78
	Feed intake	$y=712.1154+19.0561x_1+90.4751x_2$	<0.001	0.72	4.75
	Feed efficiency	$y=0.5590+0.0064x_1+0.0288x_2$	<0.001	0.39	4.50
	Plasma Ca	$y=7.1910+0.1109x_1+0.4672x_2$	<0.001	0.51	4.21
Tibia	Breaking-strength	$y=34.1606+2.6993x_1+15.0712x_2$	<0.001	0.77	5.58
	Weight	$y=1.0024+0.0305x_1+0.1574x_2$	<0.001	0.74	5.16
	Length	$y=5.0449+0.0728x_1+0.3216x_2$	<0.001	0.73	4.42
	Width	$y=0.5118+0.0053x_1+0.0249x_2$	<0.001	0.41	4.70
	Ash weight	$y=0.3188+0.0197x_1+0.0990x_2$	<0.001	0.78	5.03
	Ash content	$y=30.9996+0.6894x_1+3.0759x_2$	<0.001	0.64	4.46
	Ca content	$y=10.4936+0.2609x_1+1.2272x_2$	<0.001	0.63	4.70
	P content	$y=5.9826+0.1210x_1+0.5800x_2$	<0.001	0.65	4.79
Femur	Weight	$y=0.7842+0.0236x_1+0.1201x_2$	<0.001	0.76	5.09
	Length	$y=3.6572+0.0643x_1+0.2848x_2$	<0.001	0.69	4.43
	Width	$y=0.5925+0.0052x_1+0.0166x_2$	0.007	0.24	3.19
	Ash weight	$y=0.2470+0.0141x_1+0.0759x_2$	<0.001	0.78	5.38
	Ash content	$y=31.0489+0.6078x_1+3.1665x_2$	<0.001	0.61	5.21
	Ca content	$y=10.3304+0.2345x_1+1.2355x_2$	<0.001	0.62	5.27
	P content	$y=5.7303+0.1075x_1+0.5713x_2$	<0.001	0.58	5.31
Metatarsus	Weight	$y=0.6035+0.0219x_1+0.1094x_2$	<0.001	0.68	5.00
	Length	$y=3.7008+0.0568x_1+0.2303x_2$	<0.001	0.70	4.05
	Width	$y=0.5796+0.0081x_1+0.0481x_2$	<0.001	0.62	5.94
	Ash weight	$y=0.1752+0.0123x_1+0.0582x_2$	<0.001	0.74	4.73
	Ash content	$y=28.8116+0.4988x_1+2.6572x_2$	<0.001	0.49	5.33
	Ca content	$y=9.4990+0.2115x_1+1.1921x_2$	<0.001	0.49	5.64
	P content	$y=5.4692+0.1058x_1+0.4533x_2$	<0.001	0.42	4.28

[1] $x_1=D_3$, x_2=1α-OH-D_3, P=phosphorus.

as criteria, the RBV of 1α-OH-D_3 to D_3 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-D_3 to D_3 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 D_3 (Qian *et al*., 1997) or 1α-OH-D_3 (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 D_3 or 1α-OH-D_3 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 D_3 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 D_3 or 1α-OH-D_3.

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 D_3 or 1α-OH-D_3 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 D_3 or 1α-OH-D_3 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 D_3 or 1α-OH-D_3.

Bone Mineralization

Bone ash weight and content increased with the increase in D_3 (Fritts and Waldroup, 2003; Rao *et al*., 2007) or 1α-OH-D_3 (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 D_3 or 1α-OH-D_3. Tibia breaking-strength was also enhanced by D_3 or 1α-OH-D_3 in this study, which was consistent with our previous results (Han *et al*., 2009a, b, 2012b). These results suggest that D_3 and 1α-OH-D_3 promote bone growth and mineralization by increasing the absorption and retention of Ca and P.

The RBV Value

The biological activity of 1α-OH-D_3 was higher than that of D_3 in this study. This result agrees with the findings of Edwards *et al*. (2002a), who found that 1α-OH-D_3 was approximately 8 times as active as D_3 in increasing growth. However, the RBV of 1α-OH-D_3 to D_3 in the present study were lower than that reported by Edwards *et al*. (2002a).

The RBV values of 1α-OH-D_3 to D_3 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-D_3 to D_3 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-D_3 to D_3 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 D_3 and 1α-OH-D_3, 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-D_3 to D_3 in previous research.

In conclusion, the increasing levels of D_3 or 1α-OH-D_3 improved growth performance, plasma Ca and Pi, and bone quality of the broilers. These data indicate that the RBV of 1α-OH-D_3 to D_3 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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