



## The effects of dietary cholecalciferol and $1\alpha$ -hydroxycholecalciferol levels in a calcium- and phosphorus-deficient diet on growth performance and tibia quality of growing broilers

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**KEY WORDS:**  $1\alpha$ -hydroxycholecalciferol, cholecalciferol, growth, tibia, potency, broiler

**ABSTRACT.** This study aims to evaluate the effects of cholecalciferol (vitamin  $D_3$ , 0, 5, 10, 25, 50, 100, 250, 500, and 1000  $\mu\text{g} \cdot \text{kg}^{-1}$ ) and  $1\alpha$ -hydroxycholecalciferol ( $1\alpha\text{-OH } D_3$ , 0, 2.5, and 5  $\mu\text{g} \cdot \text{kg}^{-1}$ ) on growth performance and tibia quality and to compare the relative potency of  $1\alpha\text{-OH } D_3$  to vitamin  $D_3$  in 1- to 21-day-old female broilers fed a calcium (Ca)- and phosphorus (P)-deficient diet. The basal diet contained 0.50% Ca, 0.25% non-phytate phosphorus (NPP) and was not supplemented with vitamin  $D_3$ , whereas the control diet contained 1.00% Ca, 0.45% NPP, and 25  $\mu\text{g} \cdot \text{kg}^{-1}$  vitamin  $D_3$ . Dietary vitamin  $D_3$  levels significantly affected body weight gain (BWG), feed intake (FI), serum Ca and tibia parameters. The addition of 5  $\mu\text{g} \cdot \text{kg}^{-1}$   $1\alpha\text{-OH } D_3$  resulted in greater BWG, FI, and serum Ca as well as tibia ash weight and content, breaking strength, and Ca and P contents, compared with the birds fed the basal diet. Using BWG, serum Ca, tibia ash weight and content, and tibia Ca and P content as the criteria, the vitamin  $D_3$  requirements of 1- to 21-day-old broilers fed Ca- and P-deficient diets were 64.0, 16.2, 173.0, 65.1, 33.1 and 30.0  $\mu\text{g} \cdot \text{kg}^{-1}$ , and the relative potencies of  $1\alpha\text{-OH } D_3$  to vitamin  $D_3$  were 5.03, 2.19, 18.00, 5.14, 4.09 and 3.33, respectively. These data indicate that high levels of vitamin  $D_3$  can spare the use of P in broiler diets and that the potency of  $1\alpha\text{-OH } D_3$  is higher than that of vitamin  $D_3$ .

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### Introduction

In a basal diet with low levels of calcium (Ca, 0.64%) and total phosphorus (tP, 0.42%), 8- to 20-day-old broilers that were fed 1250  $\mu\text{g} \cdot \text{kg}^{-1}$  of cholecalciferol (vitamin  $D_3$ ) achieved greater body weight gain (BWG) and tibia ash content than birds

fed 37.5  $\mu\text{g} \cdot \text{kg}^{-1}$  of vitamin  $D_3$  (Baker et al., 1998). On the other hand, chickens fed Ca- and P-adequate diets (1.00% Ca, 0.77% tP) with 5  $\mu\text{g} \cdot \text{kg}^{-1}$  of vitamin  $D_3$  exhibited BWG and bone ash values as high as those of chickens that received 30 and 1250  $\mu\text{g} \cdot \text{kg}^{-1}$  vitamin  $D_3$  (Baker et al., 1998). These results indicate that broilers require less vitamin  $D_3$

when their diet contains adequate Ca and P. When the broilers were fed sufficient Ca and P, their body weight and tibia breaking strength were maximized on day 14 with  $250 \mu\text{g} \cdot \text{kg}^{-1}$  of vitamin  $\text{D}_3$ , tibia ash was maximized with  $125 \mu\text{g} \cdot \text{kg}^{-1}$  vitamin  $\text{D}_3$  (Whitehead et al., 2004), and bone area, thickness, and mineral content increased with  $250 \mu\text{g} \cdot \text{kg}^{-1}$  vitamin  $\text{D}_3$  (Kim et al., 2011). With growth improvement, broilers need more vitamin D for bone development and strength. In addition, the environmental pollution caused by P in poultry production requires the increasing attention of animal nutritionists. Qian et al. (1997) found that dietary Ca to tP ratios ranging from 1.1 to 1.4 are critical for the efficient use of phytase and vitamin  $\text{D}_3$  in improving the utilization of phytate P. Rao et al. (2006) reported that in a basal diet with 0.50% Ca and 0.25% of non-phytate phosphorus (NPP), the addition of  $90 \mu\text{g} \cdot \text{kg}^{-1}$  vitamin  $\text{D}_3$  maintains growth performance and bone mineralization in broilers. These data suggest that the addition of high levels of vitamin  $\text{D}_3$  can spare the use of P in broiler diets with an appropriate Ca to P ratio. The vitamin  $\text{D}_3$  requirement of broilers for growth and bone quality in Ca- and P-sub-adequate diets is unclear, however.

The compounds  $1\alpha$ -hydroxycholecalciferol ( $1\alpha$ -OH  $\text{D}_3$ ) and 25-hydroxycholecalciferol (25-OH  $\text{D}_3$ ) are vitamin D metabolites. Recently, 25-OH  $\text{D}_3$  has been approved for use in feed for fattening chickens, laying hens and turkeys in Europe. It is possible that  $1\alpha$ -OH  $\text{D}_3$  will become a feed additive in the future. Kasim and Edwards (2000) evaluated the effects of different vitamin  $\text{D}_3$  sources on growth and tibia ash using broiler chickens fed Ca- and P-adequate diets (1.0% Ca, 0.7% tP). In other studies, similar basal diets with sufficient Ca and P were used to compare the efficacy of vitamin  $\text{D}_3$  with that of  $1\alpha$ -OH  $\text{D}_3$  (Edwards et al., 2002) and that of vitamin  $\text{D}_3$  with that of 25-OH  $\text{D}_3$  (Yarger et al., 1995; Fritts and Waldroup, 2003). Vitamin  $\text{D}_3$  (Qian et al., 1997) and  $1\alpha$ -OH  $\text{D}_3$  (Han et al., 2012) have high activity at low levels of dietary Ca and P. Thus, comparing the efficacy of vitamin D metabolites should be conducted under Ca- and P-deficient diets. In the present study, the effect of vitamin  $\text{D}_3$  and  $1\alpha$ -OH  $\text{D}_3$  on growth performance and tibia quality was reevaluated on the basis of diets with 0.50% Ca and 0.25% NPP.

The objective of the presented study is to determine the vitamin  $\text{D}_3$  requirement of 1- to 21-day-old broilers fed Ca- and P-deficient diets and to reevaluate the relative potency of  $1\alpha$ -OH  $\text{D}_3$  compared with that of vitamin  $\text{D}_3$ .

## Material and methods

### Birds, diets, management

All procedures used in the present experiment were approved by the Animal Care Committee of Shangqiu Normal University. On the day of hatching, 480 female Ross broilers were individually weighed and randomly assigned to 12 treatments, four cages ( $70 \text{ cm} \times 70 \text{ cm} \times 30 \text{ cm}$ ) with 10 birds per treatment. The lighting system consisted of 23 h of light from days 0 to 21. The room temperature was maintained at  $33^\circ\text{C}$  from days 0 to 3 and then gradually reduced by  $3^\circ\text{C}$  per week to a final temperature of  $24^\circ\text{C}$ .

Twelve experimental diets were prepared. Vitamin  $\text{D}_3$  and  $1\alpha$ -OH  $\text{D}_3$  were supplied by Tongxiang Tianhecheng Food Technology Co., Ltd. (Jiaxing, China) and Taizhou Healtech Chemical Co., Ltd. (Taizhou, China), respectively. The crystalline vitamin  $\text{D}_3$  and  $1\alpha$ -OH  $\text{D}_3$  were dissolved in ethanol and then brought to final concentrations of  $200 \text{ mg} \cdot \text{l}^{-1}$  of vitamin  $\text{D}_3$  and  $10 \text{ mg} \cdot \text{l}^{-1}$  of  $1\alpha$ -OH  $\text{D}_3$  in a solution of 5% ethanol and 95% propylene glycol (Han et al., 2009).

The basal diet contained 0.50% Ca, 0.25% NPP and was without added vitamin  $\text{D}_3$ . It was supplemented with 5, 10, 25, 50, 100, 250, 500 and  $1000 \mu\text{g} \cdot \text{kg}^{-1}$  of vitamin  $\text{D}_3$  or 2.5 and  $5 \mu\text{g} \cdot \text{kg}^{-1}$  of  $1\alpha$ -OHD $_3$  (Table 1). A diet with adequate

**Table 1.** Ingredients and nutrient composition of the experimental diets

Component	Diet <sup>3</sup>	
	basal	control
Ingredient, g · kg <sup>-1</sup>		
maize	638.7	600.9
soya bean meal	290.0	290.0
soya bean oil	10.0	10.0
lard	7.2	20.5
soya bean protein isolate	31.3	36.5
limestone	6.9	13.9
dicalcium phosphate	7.2	19.5
L-lysine·HCl	1.8	1.8
DL-methionine	0.6	0.6
trace mineral premix <sup>1</sup>	1.0	1.0
vitamin premix <sup>2</sup>	0.3	0.3
choline chloride	2.0	2.0
NaCl	3.0	3.0
Nutrient composition		
metabolizable energy, MJ · kg <sup>-1</sup>	12.55	12.55
analysed crude protein, %	20.5	20.4
analysed calcium, %	0.55	1.08
analysed total phosphorus, %	0.50	0.68
calculated non-phytate phosphorus, %	0.25	0.45

<sup>1</sup> the trace-mineral premix provided the following, per kg of diet: mg: iron 100, zinc 100, copper 8, manganese 120, iodine 0.7, selenium 0.3; <sup>2</sup> the vitamin premix provided the following, per kg of diet: IU: vit. A 8000, vit. E 20; mg: menadione 0.5, thiamine 2.0, riboflavin 8.0, niacin 3.5, pyridoxine 0.01; vit. B<sub>12</sub> 0.01, pantothenic acid 10.0, folic acid 0.55, biotin 0.18; <sup>3</sup> to the control diet  $25 \mu\text{g} \cdot \text{kg}^{-1}$  vit.  $\text{D}_3$  was added

levels of Ca (1.00%), NPP (0.45%) and vitamin D<sub>3</sub> (25 µg · kg<sup>-1</sup>) was used as the control. The birds were given access to mash diets and water *ad libitum* during the 21-day experiment.

### Sample collection

On day 21, the broilers were individually weighed. Two chickens were randomly chosen from each replicate for blood and tibia collection. Serum samples (5 ml) were collected using 5 ml anticoagulated syringes (Shanghai K&G International Co. Ltd., Shanghai, China) by cardiac puncture and then centrifuged for 10 min at 3000 g at 20°C.

The birds were sacrificed by exsanguination, plucked, eviscerated, abdominal fat was removed, and the carcasses were weighed. The left and right tibias were excised and frozen at -20°C for further analysis (breaking strength, ash weight, and content of ash, Ca, and P).

### Sample analysis

The Ca and inorganic phosphate (Pi) in blood serum were immediately determined using a Shimadzu CL-8000 Analyzer (Shimadzu Corp., Kyoto, Japan) following the manufacturer's instructions. According to the method by Hall et al. (2003), the left tibia was boiled for 5 min to loosen the muscle tissue. The meat, connective tissue, and fibula bone were removed from the tibia. The tibia was placed in a container with ethanol for 48 h to remove water and polar lipids. The bones were then further extracted in anhydrous ether for 48 h to remove non-polar lipids. The tibias were dried at 105°C for 24 h, weighed, and their weight recorded. Tibia ash content was determined by ashing the bone in a muffle furnace for 18 h at 600°C.

The right tibia was used to analyse the breaking strength. The tibia breaking strength was determined using an all-digital electronic universal testing machine (Shenzhen Hengen Instrument Co. Ltd., Shenzhen, China). The tibias were cradled on two support points 4 cm apart. Using a 50 kg load cell and a crosshead speed of 10 mm · min<sup>-1</sup>, the force was applied to the midpoint of the same facet of each tibia (Jendral et al., 2008).

Ca in diets was determined according to the EDTA titration method. Total P in diets was determined by photometric methods after reaction with ammonium molybdate and ammonium metavanadate. The crude protein content in diets was determined according to the Kjeldhal method on a PN-1430 apparatus (Barcelona, Spain).

### Statistical analyses

Replicate means are the experimental units in the statistical analysis. All data were analysed with an ANOVA procedure of SAS software (SAS Institute, 2001). The means were compared with Tukey's test at  $p < 0.05$ . Nonlinear regression analysis was conducted using the PROC NLIN procedure of SAS to estimate the vitamin D<sub>3</sub> requirement based on the selected variables, including BWG, serum Ca, tibia ash weight and content, and tibia Ca and P contents. The model was:  $y = ax^2 + bx + c$ , where  $y$  is the response and  $x$  is the variable. The relative potencies of 1α-OH D<sub>3</sub> to vitamin D<sub>3</sub> were determined by the slope ratio method (Littell et al., 1997). The model is:  $y = a + b_1x_1 + b_2x_2$ , where  $y$  is the response,  $x_1$  is vitamin D<sub>3</sub>, and  $x_2$  is 1α-OH D<sub>3</sub>.

### Results

The levels of dietary vitamin D<sub>3</sub> and 1α-OH D<sub>3</sub> significantly affected BWG, feed intake (FI), and serum Ca but did not influence the feed conversion ratio (FCR), slaughter yield (SY), and serum Pi in 1- to 21-day-old broilers (Table 2). BWG and FI responded linearly to vitamin D<sub>3</sub> ranging from 0 to 50 µg · kg<sup>-1</sup> and to 1α-OH D<sub>3</sub> from 0 to 5 µg · kg<sup>-1</sup>. Serum Ca also increased linearly to vitamin D<sub>3</sub> ranging from 0 to 10 µg · kg<sup>-1</sup> and to 1α-OH D<sub>3</sub> from 0 to 5 µg · kg<sup>-1</sup>. Serum Pi in broiler chickens fed 25 µg · kg<sup>-1</sup> of vitamin D<sub>3</sub> was higher than that of birds fed the basal diet ( $p < 0.05$ ). No significant differences were observed in BWG, FI, or serum Ca among the broilers fed 10 to 1000 µg · kg<sup>-1</sup> vitamin D<sub>3</sub> or 5 µg · kg<sup>-1</sup> 1α-OH D<sub>3</sub>, and those fed the control diets.

The tibia ash weight of the broilers increased linearly from 0.42 to 0.71 g/tibia when the dietary vitamin D<sub>3</sub> increased from 0 to 100 µg · kg<sup>-1</sup> (Table 3). Tibia ash content (g · 100 g<sup>-1</sup>) also enhanced linearly to vitamin D<sub>3</sub> ranging from 0 to 25 µg · kg<sup>-1</sup>. Broilers fed 25 to 1000 µg · kg<sup>-1</sup> vitamin D<sub>3</sub> achieved a significantly greater tibia breaking strength than the birds fed the basal diet. The content of tibia Ca and P in broilers fed 25 µg · kg<sup>-1</sup> vitamin D<sub>3</sub> was significantly higher than that of birds fed the basal diet ( $p < 0.05$ ). The addition of 5 µg · kg<sup>-1</sup> 1α-OH D<sub>3</sub> resulted in greater tibia ash weight and content, breaking strength, and Ca and P content, compared with the birds fed the basal diet. No significant differences in tibia ash weight, breaking strength, or Ca and P contents were observed between the broilers fed with 5 µg · kg<sup>-1</sup> 1α-OH D<sub>3</sub> or 25 to 1000 µg · kg<sup>-1</sup> vitamin D<sub>3</sub> and the control diets.

Using BWG, serum Ca, tibia ash weight and content, and tibia Ca and P contents as the criteria,

**Table 2.** Effects of vitamin D<sub>3</sub> and 1 $\alpha$ -OH D<sub>3</sub> on growth performance of 1- to 21-day-old broilers

Group	D <sub>3</sub> $\mu\text{g} \cdot \text{kg}^{-1}$	1 $\alpha$ -OH D <sub>3</sub> $\mu\text{g} \cdot \text{kg}^{-1}$	Growth <sup>2</sup>				Serum <sup>3</sup>		
			BWG $\text{g} \cdot \text{bird}^{-1}$	FI $\text{g} \cdot \text{bird}^{-1}$	FCR (FI/BWG)	SY %	Ca $\text{mg} \cdot 100 \text{ ml}^{-1}$	Pi $\text{mg} \cdot 100 \text{ ml}^{-1}$	
1	Basal diet		563 <sup>d</sup>	910 <sup>c</sup>	1.62	68.89	6.50 <sup>c</sup>	5.47 <sup>b</sup>	
2	5		581 <sup>cd</sup>	938 <sup>bc</sup>	1.62	68.05	7.55 <sup>b</sup>	5.83 <sup>ab</sup>	
3	10		617 <sup>abc</sup>	1041 <sup>ab</sup>	1.69	70.05	8.64 <sup>a</sup>	6.11 <sup>ab</sup>	
4	25		638 <sup>ab</sup>	1070 <sup>a</sup>	1.68	69.79	8.14 <sup>ab</sup>	6.75 <sup>a</sup>	
5	50		641 <sup>ab</sup>	1082 <sup>a</sup>	1.69	69.10	8.20 <sup>ab</sup>	6.25 <sup>ab</sup>	
6	100		634 <sup>ab</sup>	1052 <sup>a</sup>	1.66	69.07	8.54 <sup>ab</sup>	6.41 <sup>ab</sup>	
7	250		635 <sup>ab</sup>	1099 <sup>a</sup>	1.73	68.07	8.27 <sup>ab</sup>	6.21 <sup>ab</sup>	
8	500		638 <sup>ab</sup>	1073 <sup>a</sup>	1.68	68.95	8.53 <sup>ab</sup>	6.45 <sup>ab</sup>	
9	1000		647 <sup>ab</sup>	1085 <sup>a</sup>	1.68	68.67	8.41 <sup>ab</sup>	6.38 <sup>ab</sup>	
10		2.5	602 <sup>bcd</sup>	1023 <sup>abc</sup>	1.70	68.86	8.36 <sup>ab</sup>	5.90 <sup>ab</sup>	
11		5.0	618 <sup>abc</sup>	1041 <sup>ab</sup>	1.69	70.40	8.45 <sup>ab</sup>	5.82 <sup>ab</sup>	
12	Control diet		648 <sup>a</sup>	1067 <sup>a</sup>	1.65	71.83	8.69 <sup>a</sup>	6.72 <sup>a</sup>	
SEM			4	10	0.01	0.25	0.10	0.08	
P value			<0.001	<0.001	0.435	0.092	<0.001	0.012	
Source of variation			Probabilities						
Vitamin D <sub>3</sub> level			<0.001	<0.001	0.392	0.669	<0.001	0.057	
1 $\alpha$ -OH D <sub>3</sub> level			0.021	0.006	0.313	0.368	<0.001	0.400	

<sup>a-d</sup> means in the same column without a common superscript differ significantly ( $p < 0.05$ ); <sup>1</sup> the basal diet contained 0.50% Ca and 0.25% NPP, no added vitamin D<sub>3</sub>. The control diet contained 1.00% Ca, 0.45% NPP, and 25  $\mu\text{g} \cdot \text{kg}^{-1}$  vitamin D<sub>3</sub>; BWG – body weight gain, FI – feed intake, FCR – feed conversion ratio, SY – slaughter yield (carcass weight as a percentage of live body weight), Ca – calcium, Pi – inorganic phosphate; <sup>2</sup> data are means of 4 replicate cages consisting of 10 birds per replicate cage; <sup>3</sup> data are means of 4 replicates consisting of 2 birds per replicate

**Table 3.** Effects of vitamin D<sub>3</sub> and 1 $\alpha$ -OH D<sub>3</sub> on tibia quality of 1- to 21-day-old broilers

Group	D <sub>3</sub> $\mu\text{g} \cdot \text{kg}^{-1}$	1 $\alpha$ -OH D <sub>3</sub> $\mu\text{g} \cdot \text{kg}^{-1}$	Ash		Breaking-strength, N	Ca $\text{g} \cdot 100 \text{ g}^{-1}$	P $\text{g} \cdot 100 \text{ g}^{-1}$		
			$\text{g}/\text{tibia}$	$\text{g} \cdot 100 \text{ g}^{-1}$					
1	Basal diet		0.42 <sup>c</sup>	40.96 <sup>d</sup>	43.67 <sup>d</sup>	14.12 <sup>b</sup>	6.73 <sup>b</sup>		
2	5		0.55 <sup>bc</sup>	41.34 <sup>cd</sup>	63.48 <sup>bcd</sup>	15.13 <sup>ab</sup>	7.39 <sup>ab</sup>		
3	10		0.58 <sup>bc</sup>	42.48 <sup>bcd</sup>	61.13 <sup>cd</sup>	15.44 <sup>ab</sup>	7.70 <sup>a</sup>		
4	25		0.61 <sup>ab</sup>	44.05 <sup>ab</sup>	77.20 <sup>abc</sup>	16.19 <sup>a</sup>	8.06 <sup>a</sup>		
5	50		0.65 <sup>ab</sup>	42.65 <sup>bcd</sup>	69.45 <sup>abc</sup>	15.89 <sup>ab</sup>	7.62 <sup>ab</sup>		
6	100		0.71 <sup>ab</sup>	43.24 <sup>bc</sup>	78.30 <sup>abc</sup>	16.15 <sup>a</sup>	7.65 <sup>ab</sup>		
7	250		0.66 <sup>ab</sup>	43.61 <sup>ab</sup>	73.67 <sup>abc</sup>	15.16 <sup>ab</sup>	7.83 <sup>a</sup>		
8	500		0.62 <sup>ab</sup>	42.16 <sup>bcd</sup>	67.59 <sup>abc</sup>	15.21 <sup>ab</sup>	7.70 <sup>a</sup>		
9	1000		0.67 <sup>ab</sup>	43.61 <sup>ab</sup>	80.54 <sup>abc</sup>	15.14 <sup>ab</sup>	7.79 <sup>a</sup>		
10		2.5	0.67 <sup>ab</sup>	43.33 <sup>bc</sup>	69.55 <sup>abc</sup>	15.27 <sup>ab</sup>	7.54 <sup>ab</sup>		
11		5.0	0.69 <sup>ab</sup>	43.78 <sup>ab</sup>	86.84 <sup>a</sup>	15.98 <sup>a</sup>	7.72 <sup>a</sup>		
12	Control diet		0.77 <sup>a</sup>	45.57 <sup>a</sup>	82.51 <sup>ab</sup>	16.49 <sup>a</sup>	8.18 <sup>a</sup>		
SEM			0.02	0.21	1.95	0.13	0.07		
P value			<0.001	<0.001	<0.001	0.003	0.002		
Source of variation			Probabilities						
Vitamin D <sub>3</sub> level			<0.001	<0.001	<0.001	0.003	0.008		
1 $\alpha$ -OH D <sub>3</sub> level			<0.001	0.002	<0.001	0.087	0.057		

<sup>a-d</sup> means in the same column without a common superscript differ significantly ( $p < 0.05$ ); <sup>1</sup> the basal diet contained 0.50% Ca and 0.25% NPP, no added vitamin D<sub>3</sub>. The control diet contained 1.00% Ca, 0.45% NPP, and 25  $\mu\text{g} \cdot \text{kg}^{-1}$  vitamin D<sub>3</sub>; data are means of 4 replicates consisting of 2 birds per replicate; Ca – calcium, P – phosphorus

**Table 4.** Vitamin D<sub>3</sub> requirements of 1- to 21-day-old broilers fed Ca- and P-deficient diets

Indices	Formula	P value	X, D <sub>3</sub> , $\mu\text{g} \cdot \text{kg}^{-1}$	D <sub>3</sub> requirement, $\mu\text{g} \cdot \text{kg}^{-1}$
Body weight gain	$y = -0.02050x^2 + 2.6250x + 574.5$	<0.001	0 to 100	64.0
Serum calcium	$y = -0.00939x^2 + 0.3035x + 6.4335$	<0.001	0 to 25	16.2
Tibia ash weight	$y = -0.00001x^2 + 0.00346x + 0.5044$	<0.001	0 to 250	173.0
Tibia ash content	$y = -0.00056x^2 + 0.0729x + 41.3806$	0.002	0 to 100	65.1
Tibia calcium	$y = -0.00194x^2 + 0.1283x + 14.3039$	0.002	0 to 50	33.1
Tibia phosphorus	$y = -0.00149x^2 + 0.0893x + 6.8624$	0.003	0 to 50	30.0

**Table 5.** Relative potencies of 1 $\alpha$ -OH D<sub>3</sub> to vitamin D<sub>3</sub> in 1- to 21-day-old broilers fed Ca- and P-deficient diets<sup>1</sup>

Indices	Formula	P value	x <sub>1</sub> $\mu\text{g} \cdot \text{kg}^{-1}$	x <sub>2</sub> $\mu\text{g} \cdot \text{kg}^{-1}$	Relative potency
Body weight gain	$y = 582.1757 + 1.4412x_1 + 7.2444x_2$	< 0.001	0 to 50	0 to 5	5.03
Serum calcium	$y = 6.7471 + 0.1835x_1 + 0.4015x_2$	< 0.001	0 to 10	0 to 5	2.19
Tibia ash weight	$y = 0.5247 + 0.0021x_1 + 0.0378x_2$	< 0.001	0 to 100	0 to 5	18.00
Tibia ash content	$y = 41.1253 + 0.1170x_1 + 0.6018x_2$	< 0.001	0 to 25	0 to 5	5.14
Tibia calcium	$y = 14.4813 + 0.0740x_1 + 0.3025x_2$	0.006	0 to 25	0 to 5	4.09
Tibia phosphorus	$y = 7.0305 + 0.0456x_1 + 0.1518x_2$	0.003	0 to 25	0 to 5	3.33

<sup>1</sup>x<sub>1</sub> = D<sub>3</sub>, x<sub>2</sub> = 1 $\alpha$ -OH D<sub>3</sub>

the vitamin D<sub>3</sub> requirements of 1- to 21-day-old broilers fed Ca- and P-deficient diets were 64.0, 16.2, 173.0, 65.1, 33.1 and 30.0  $\mu\text{g} \cdot \text{kg}^{-1}$  (Table 4), and the relative potencies of 1 $\alpha$ -OH D<sub>3</sub> to vitamin D<sub>3</sub> were 5.03, 2.19, 18.00, 5.14, 4.09 and 3.33, respectively (Table 5).

## Discussion

The addition of 60  $\mu\text{g} \cdot \text{kg}^{-1}$  of vitamin D<sub>3</sub> increased the BWG of broilers fed with Ca- and P-deficient diets, reaching levels similar to those of birds fed control diets (Rao et al., 2006). Driver et al. (2005) also found that Ca- and P-deficient broilers fed 1 $\alpha$ -OH D<sub>3</sub> and phytase had equal growth performance and tibia ash weight compared with birds fed diets with adequate Ca and P. In the present study, no significant difference was observed in the growth rate as well as ash weight, breaking strength, and Ca and P contents of the tibia among the broilers fed 25 to 1000  $\mu\text{g} \cdot \text{kg}^{-1}$  vitamin D<sub>3</sub> and those fed the control diets.

Phosphorous deficiency reduces carcass weight (Angel et al., 2006) and yield in broilers (Chen and Moran, 1995). When phytase was added, the carcass yield increased (Pillai et al., 2006). By contrast, the slaughter yield of the broilers was not significantly affected by the supplementation of vitamin D<sub>3</sub> or 1 $\alpha$ -OH D<sub>3</sub> in the present experiment. Scheideler and Ferket (2000) also found that the dietary P levels (NPP 0.30 vs. 0.42%) did not influence the weight of the carcass, thigh and breast meat of the broilers.

Dietary vitamin D<sub>3</sub> (Rao et al., 2006), 1,25-(OH)<sub>2</sub> D<sub>3</sub> (Edwards, 2002), and 1 $\alpha$ -OH D<sub>3</sub> (Edwards et al., 2002; Han et al., 2009) increased the serum Ca and Pi concentrations in broilers. In contrast, Driver et al. (2008) reported that 1 $\alpha$ -OH D<sub>3</sub> did not affect the serum Pi content in non-obese diabetic mice. In the present study, the increase of dietary vitamin D<sub>3</sub> enhanced serum Ca and Pi contents, whereas serum P did not depend on the 1 $\alpha$ -OH D<sub>3</sub> level.

Studies have shown that vitamin D<sub>3</sub> (Rao et al., 2009), 1 $\alpha$ -OH D<sub>3</sub> (Snow et al., 2004),

25-OH D<sub>3</sub> (Aburto et al., 1998) and 1,25-(OH)<sub>2</sub> D<sub>3</sub> (Edwards, 2002) increase bone weight, length, and ash in broilers. These data indicate that vitamin D metabolites increase bone growth and mineral deposition in poultry. Fritts and Waldroup (2003) reported no significant differences in tibia ash weight among the broilers fed 25, 50 and 100  $\mu\text{g} \cdot \text{kg}^{-1}$  vitamin D<sub>3</sub>. Similar results were found in the present experiment. When the vitamin D<sub>3</sub> level ranged from 25 to 1000  $\mu\text{g} \cdot \text{kg}^{-1}$ , no significant differences were observed in tibia ash weight, breaking strength, and Ca and P contents of the tibia of the broilers.

The NRC (1994) recommendation on vitamin D<sub>3</sub> for broilers is 5  $\mu\text{g} \cdot \text{kg}^{-1}$  in Ca- and P-adequate diets, which may be lower than the practical requirement. Research has shown that about 25 and 50  $\mu\text{g} \cdot \text{kg}^{-1}$  of vitamin D<sub>3</sub> were needed to maximize the body weight and bone ash of 42-day-old broilers, respectively (Fritts and Waldroup, 2003). When broilers were fed with Ca- and P-deficient diets, the addition of 1250  $\mu\text{g} \cdot \text{kg}^{-1}$  vitamin D<sub>3</sub> resulted in greater tibia ash than the addition of 0 and 37.5  $\mu\text{g} \cdot \text{kg}^{-1}$  of vitamin D<sub>3</sub> (Baker et al., 1998). The addition of 6660  $\mu\text{g} \cdot \text{kg}^{-1}$  vitamin D<sub>3</sub> yielded higher toe ash contents and Ca and P retention than the addition of 66 and 660  $\mu\text{g} \cdot \text{kg}^{-1}$  of vitamin D<sub>3</sub> (Qian et al., 1997). At 42 d, the body weight of broilers fed 100  $\mu\text{g} \cdot \text{kg}^{-1}$  of vitamin D<sub>3</sub> was significantly greater than of birds fed 25 or 50  $\mu\text{g} \cdot \text{kg}^{-1}$  (Fritts and Waldroup, 2005). These data suggest that the vitamin D<sub>3</sub> requirements of broilers in the Ca- and P-deficient diets were more than those of the birds fed Ca- and P-adequate diets. Rao et al. (2009) reported, however, that the vitamin D<sub>3</sub> requirements of broilers fed Ca- and P-deficient diets ranged from 16 to 25  $\mu\text{g} \cdot \text{kg}^{-1}$ . This value may be lower than the actual requirement. Using weight gain and tibia ash weight and content as the criteria in the present experiment, we found that the vitamin D<sub>3</sub> requirements were 64.0, 173.0 and 65.1  $\mu\text{g} \cdot \text{kg}^{-1}$  in broilers fed Ca- and P-deficient diets, respectively.

Previous research has shown that 1 $\alpha$ -OH D<sub>3</sub> is 4.5 times more active than vitamin D<sub>3</sub> in respect to the BWG of broiler chickens (Soares et al., 1978),

8.6 times in respect to tibia ash increase (Boris et al., 1977), and at least 10 times in terms of mobilizing bone calcium and raising plasma calcium concentrations in Leghorn cockerels (Haussler et al., 1973). Using body weight, plasma Ca, tibia ash weight and percentage, and incidence of rickets as criteria, Edwards et al. (2002) reported that the relative biological values of  $1\alpha$ -OH  $D_3$  to vitamin  $D_3$  were 10.08, 9.50, 11.26, 4.48, and 4.50, respectively. The relative potency of  $1\alpha$ -OH  $D_3$  to vitamin  $D_3$  on body weight in our study was similar to the results of Soares et al. (1978) and lower in BWG and plasma Ca, whereas higher in tibia ash weight and content, than those reported by Edwards et al. (2002). The differences may have been caused by the age and sex of the birds and the dietary Ca and P contents. Edwards et al. (2002) used 1- to 16-day-old mixed-sex broilers fed 1.0% Ca and 0.7% tP, whereas the present experiment used 1- to 21-day-old female birds fed 0.50% Ca and 0.48% tP. Our results are in accordance with other studies where the relative efficacy of  $1\alpha$ -OH  $D_3$  to vitamin  $D_3$  in terms of tibia ash weight was higher than for body weight.

## Conclusions

Dietary vitamin  $D_3$  and  $1\alpha$ -OH  $D_3$  improved growth performance and serum Ca as well as the tibia quality of broiler chickens. Using BWG, serum Ca, tibia ash weight and content, and tibia Ca and P contents as the criteria, the vitamin  $D_3$  requirements of 1- to 21-day-old broilers fed Ca- and P-deficient diets were 64.0, 16.2, 173.0, 65.1, 33.1 and 30.0  $\mu\text{g} \cdot \text{kg}^{-1}$ , and the relative potencies of  $1\alpha$ -OH  $D_3$  to vitamin  $D_3$  were 5.03, 2.19, 18.00, 5.14, 4.09 and 3.33, respectively. These data indicate that high levels of vitamin  $D_3$  can spare the use of P in broiler diets and that the potency of  $1\alpha$ -OH  $D_3$  is higher than that of vitamin  $D_3$ .

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