《Research Note》

Dietary Calcium Levels Reduce the Efficacy of One Alpha-Hydroxycholecalciferol in Phosphorus-Deficient Diets of Broilers

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One alpha-hydroxycholecalciferol (1 α -OH D₃), an analog of cholecalciferol (vitamin D₃), improves growth performance and phosphorus retention in broilers. In the present study, we investigate the effects of dietary calcium (Ca) levels on the efficacy of 1 α -OH D₃ in the phosphorus (P)-deficient corn-soybean meal diets of 1- to 21-d-old broilers. Four hundred female Ross 308 broilers, 1-d-old, were randomly allotted to 10 treatments with 4 cages of 10 birds each. A 5×2 factorial experiment was designed to test 0.40, 0.60, 0.80, 1.00, and 1.20% Ca combined with 0 and 5 μ g/kg of 1 α -OH D₃ in a basal diet (0.25% non-phytate phosphorus (NPP), 0.48% total phosphorus (tP)) without vitamin D₃. The lowest levels of body weight gain (BWG), feed intake (FI), feed efficiency (FE), tibia quality (except of ash and P), as well as the highest value of mortality, were observed at 0.40% Ca. Additional Ca corrected the Ca deficiency and eventually 1.2% Ca caused a P deficiency. The addition of 1 α -OH D₃ improved BWG, FI, FE, serum inorganic phosphate concentration, tP retention, tibia weight, length, breaking-strength, and content of ash, Ca, and P, and decreased mortality. However, 1 α -OH D₃ efficacy responded negatively to dietary Ca level. These results indicate that 1 α -OH D₃ exerts the highest activities at lower concentration of dietary Ca.

Key words: broiler, calcium, one alpha-hydroxycholecalciferol

J. Poult. Sci., 49: 34–38, 2012

Introduction

Analog of vitamin D include cholecalciferol (D₃), 25-hydroxycholecalciferol (25-OH D₃), 1alpha-hydroxycholecalciferol (1 α -OH D₃), and 1,25-dihydroxycholecalciferol (1,25-(OH)₂ D₃). The activity of the four analogs is 1,25-(OH)₂ D₃ > 1 α -OH D₃ > 25-OH D₃ > D₃ (Soares *et al.*, 1995; Edwards, 2002; Edwards *et al.*, 2002). Vitamin D₃ is usually used in animal feed formulation. Recently, 25-OH D₃ is authorized for use in feed for fattening chickens, laying hens, and turkeys in Europe. It is possible that 1 α -OH D₃ become a feed additive in future.

Previous studies have shown that the addition of 1 α -OH D₃ improves the growth and tibia ash of broilers fed a phosphorus-deficient corn-soybean meal diet (Edwards, 1993; Biehl and Baker, 1997a, 1997b; Snow *et al.*, 2004). However, Shirley (2003) and Han *et al.* (2009a) found that 1 α -OH D₃ decreased body weight gain (BWG) and feed

intake (FI) in starter broilers when 1 α -OH D₃ and phytase were added together. The efficacy of 1 α -OH D₃ may be affected by the levels of dietary calcium (Ca) and phosphorus (P). Research on 25-OH D₃ showed that dietary 25-OH D₃ increased phytate P digestion at a lower concentration of dietary Ca (Ledwaba and Roberson, 2003). Edwards (2002) reported that 1 α -OH D₃ did not improve BWG and feed efficiency (FE) in 1- to 16-d-old broilers when the dietary non-phytate phosphorus (NPP) level reached 0.30%, although it increased tibia ash and P utilization. Further study indicated that addition of 5 μ g/kg of 1 α -OH D₃ and 1,000 U/kg of phytase improves growth and tibia ash in broilers fed with Ca- and P-deficient diets (Driver *et al.*, 2005).

Based on the results of previous research, we conclude that 1 α -OH D₃ exerts the highest activity in broiler diets with low levels of Ca and P. However, no report yet exists on the relationship between dietary Ca and 1 α -OH D₃. Thus, the objective of the present study is to investigate the effect of dietary Ca levels on 1 α -OH D₃ efficacy in broilers.

Received: July 18, 2011, Accepted: November 7, 2011

Released Online Advance Publication: November 25, 2011

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

Item	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Ingredient (%)					
Corn	64.47	63.36	62.23	61.11	59.99
Soybean meal	29.00	29.00	29.00	29.00	29.00
Soybean oil	1.00	1.00	1.00	1.00	1.00
Swine lard	0.50	0.89	1.29	1.68	2.08
Soybean powder	3.04	3.19	3.34	3.50	3.65
Limestone	0.40	0.97	1.55	2.12	2.69
Dicalcium phosphate	0.72	0.72	0.72	0.72	0.72
L-Lysine·HCl	0.18	0.18	0.18	0.18	0.18
DL-Methionine	0.06	0.06	0.06	0.06	0.06
Trace mineral premix ¹	0.10	0.10	0.10	0.10	0.10
Vitamin premix ²	0.03	0.03	0.03	0.03	0.03
Choline chloride	0.20	0.20	0.20	0.20	0.20
Sodium chloride	0.30	0.30	0.30	0.30	0.30
Nutrient composition³					
Metabolizable energy (kcal/kg)	3000	3000	3000	3000	3000
Analyzed crude protein (%)	20.5	20.3	20.4	20.3	20.1
Analyzed calcium (%)	0.48	0.71	0.88	1.03	1.20
Analyzed total phosphorus (%)	0.49	0.48	0.47	0.50	0.48
Non-phytate phosphorus (%)	0.25	0.25	0.25	0.25	0.25

¹ The trace mineral premix provided the following (per kg 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 provided the following (per kg 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.

³ Calculated value.

Materials and Methods

Birds, Diets, Feeding, and Management

All procedures used in the present study were approved by the Animal Care Committee of Shangqiu Normal University.

On the day of hatch, 400 females Ross 308 broilers were individually weighed and randomly and equally assigned to 10 treatments with 4 cages (70×70×30 cm) of 10 birds each. A 5×2 factorial experiment was designed to test 0.40, 0.60, 0.80, 1.00, and 1.20% Ca combined with 0 and 5 µg/kg of 1α-OH D₃ in a basal diet (0.25% NPP, 0.48% total phosphorus (**tP**)) without vitamin D₃ (Table 1). The birds were given access to mash feed and water ad libitum during the experiment. The lighting system consisted of 23 h of light from d 0 to 3 and 20 h of light from d 4 to 21. The room temperature was controlled at 33°C from d 0 to 3 and then gradually reduced by 3°C per week to a final temperature of 24°C.

1α-OH D₃

The compound 1α-OH D₃ was supplied by Taizhou Healtech Chemical Co., Ltd. (Taizhou, China). A 1α-OH D₃ solution was prepared by the method of Biehl and Baker (1997b). Briefly, 1α-OH D₃ was dissolved in ethanol, and then diluted to a final concentration of 10 mg/L of 1α-OH D₃ in a solution of 5% ethanol and 95% propylene glycol.

Collection and Analysis of Samples

Excreta sample was collected from beneath each cage between d 17 and 21, dried at 65°C for 24 h, and ground to pass through a 1-mm mesh screen. On d 21, all broilers were weighed individually. To determine the sample dry matter (**DM**), a 5.0-g sample was placed into a drying oven at 100°C

for 24 h. Ca and tP in the diet and excreta examples were determined by the EDTA titration method and the photometric method after reaction with ammonium molybdate and ammonium metavanadate, respectively. Crude protein was determined by the Kjeldhal method (PN-1430, Barcelona, Spain).

For collection of blood and tibias, two chicks were randomly selected from each replicate and weighed. Serum samples (5 mL) were collected into 5-mL anticoagulated syringes (Shanghai K&G International Co. Ltd., Shanghai, China) by cardiac puncture, and centrifuged for 10 min at 3,000×g at 20°C. Serum Ca and inorganic phosphate (**Pi**) were determined with a Shimadzu CL-8000 Analyzer (Shimadzu Corp., Kyoto, Japan) following the manufacturer's instructions.

Birds were killed by exsanguination. The left and right tibias of the individual birds were excised. According to the method by Hall *et al.* (2003), the left tibias were boiled for 5 min to loosen muscle tissue. Then, the meat, connective tissue, and the fibula bone removed completely using scissors and forceps. After the tibias were cleaned, they were put into a container of ethanol for 48 h (removing water and polar lipids). Bones were then further extracted in anhydrous ether for 24 h (removing non-polar lipids). Tibias were dried at 105°C for 24 h and then weighed. Tibia weight was recorded. Tibia diameter was determined at the medial point. 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 analyze the breaking-strength. Tibia breaking-strength 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. Using a 50 kg load cell and a crosshead speed of 10 mm/min, the force was applied to the midpoint of the same facial of each tibia (Jendral *et al.*, 2008).

Statistical Analysis

All data were analyzed with the one-way and two-way ANOVA procedure of SAS software (SAS Institute, 2001). Means were compared by Tukey's test when probability values were significant ($P < 0.05$).

Results and Discussion

Growth Performance

The lowest levels of BWG, FI, and FE, as well as the highest mortality, were found in the 0.40% Ca group (Table 2). Dietary Ca quadratically increased BWG and FE and decreased mortality. 1α -OH D₃ positively affected growth performance. However, growth improvement by 1α -OH D₃ declined as the dietary Ca concentration was increased. When Ca ranged from 0.40 to 0.60%, 1α -OH D₃ significantly improved BWG and FE. At 1.0 and 1.2% Ca, 1α -OH D₃ exhibited no positive effect. Interaction between dietary Ca and 1α -OH D₃ was observed in BWG, FI, FE, and mortality.

Previous research has shown that increasing dietary Ca from 0.33 to 0.90% improves the growth and FE in 1- to 16-d-old broilers fed with 0.45% NPP diets (Driver, 2004). In the present study, the diet with 0.4% Ca and no added 1α -OH D₃ was severely deficient in Ca, to the point of causing mortality. Additional Ca corrected the Ca deficiency and eventually 1.2% Ca caused a P deficiency. Adding 5 μ g/kg of 1α -OH D₃ corrected the Ca deficiency at 0.4%, however, it made the P deficiency worse at 1.2% Ca. Addition of 1α -OH D₃ improved the BWG and FI of broilers fed with low

levels of Ca; however, it had no positive effect at 1.00 and 1.20% Ca. Previous research has shown that the improvement of vitamin D₃ on the growth is negatively influenced by the increase in dietary Ca to tP ratio (Qian *et al.*, 1997). In addition, 1α -OH D₃ reduces the BWG and FI of broilers fed diets with high levels of phytase (Shirley, 2003).

Serum Minerals concentration and Ca and P Retention

The Ca and tP retention declined with the increase of dietary Ca, whereas the serum Ca was not affected (Table 3). Addition of 1α -OH D₃ increased serum Pi concentration and retention of Ca and tP especially at low levels of dietary Ca. No significant interaction between dietary Ca and 1α -OH D₃ was observed in serum minerals concentration and Ca and P retention.

Addition of 1α -OH D₃ increased serum Pi concentration in broilers, which agrees with the findings of Edwards (2002) and Han *et al.* (2009b). Research has shown that 1α -OH D₃ was metabolized rapidly to 1,25-(OH)₂D₃ in chicks (Edelstein *et al.*, 1978). The active 1,25-(OH)₂D₃ increased intestine NaPi-IIb mRNA abundance in suckling rats (Katai *et al.*, 1999) and phosphate transport in chicks (Matsumoto *et al.*, 1980). NaPi-IIb, sodium-dependent phosphate cotransporter, is responsible for the transport of phosphate in intestine. Based on previous research, 1α -OH D₃ maybe facilitate intestine phosphate absorption by stimulating the NaPi-IIb gene expression. The increase of serum Pi by 1α -OH D₃ responded negatively to dietary Ca. In the present study, addition of 1α -OH D₃ increased tP retention, which is agreed with the findings of Biehl and Baker (1997b). However, the improvement of tP retention by 1α -OH D₃ also declined with the dietary Ca. Dietary 1α -OH D₃ improved Ca retention especially at low levels of dietary Ca in our experiment. Edwards (2002) found that 1α -OH D₃ increased Ca retention from 47.2 to 52.5% in 8-day-old broilers.

Table 2. Effect of dietary Ca and 1α -OH D₃ on growth in 1- to 21-d-old broilers¹

Ca ² (%)	1α -OH D ₃ (μ g/kg)	BWG (g/bird)	FI (g/bird)	FE (BWG/FI)	Mortality (%)
0.40	0	316 ^c	829 ^d	0.38 ^d	33 ^a
0.60	0	449 ^{cd}	964 ^{abcd}	0.47 ^{bc}	18 ^{ab}
0.80	0	452 ^{cd}	906 ^{bcd}	0.50 ^{abc}	3 ^b
1.00	0	505 ^{abc}	988 ^{abc}	0.51 ^{ab}	5 ^b
1.20	0	484 ^{bc}	968 ^{abcd}	0.50 ^{abc}	18 ^{ab}
0.40	5	563 ^a	1067 ^a	0.53 ^{ab}	3 ^b
0.60	5	544 ^{ab}	977 ^{abcd}	0.56 ^a	3 ^b
0.80	5	518 ^{abc}	1054 ^{ab}	0.49 ^{abc}	3 ^b
1.00	5	452 ^{cd}	895 ^{cd}	0.51 ^{ab}	8 ^{ab}
1.20	5	399 ^d	916 ^{abcd}	0.44 ^{cd}	13 ^{ab}
SEM ³		12	14	0.01	2
Source of variation		Probabilities			
Ca		0.002	0.644	<0.001	0.061
1α -OH D ₃		<0.001	0.016	0.002	0.010
Ca $\times$ 1α -OH D ₃		<0.001	<0.001	<0.001	0.036

^{a-c} Means in the same column without common superscript differ significantly ($P < 0.05$).

¹ Data are means of 4 replicate cages consisting of 10 birds per replicate cage.

² Ca=calcium, 1α -OH D₃=1alpha-hydroxycholecalciferol, BWG=body weight gain, FI=feed intake, FE=feed efficiency.

³ SEM=pooled standard error of the mean.

Table 3. Effect of dietary Ca and 1 α -OH D₃ on serum Ca and P and their retention in 1- to 21-d-old broilers¹

Ca ² (%)	1 α -OH D ₃ (μ g/kg)	Serum (mg/100 mL)		Retention (%)	
		Ca	Pi	Ca	tP
0.40	0	7.17	4.24	49.12	57.63
0.60	0	7.74	3.73	44.80	46.79
0.80	0	6.23	5.26	33.47	44.62
1.00	0	6.92	3.26	31.05	43.63
1.20	0	6.95	3.10	24.75	44.47
0.40	5	6.14	5.96	52.11	61.15
0.60	5	7.20	5.93	47.48	60.64
0.80	5	6.35	5.70	36.16	59.93
1.00	5	7.11	4.19	34.50	54.95
1.20	5	6.82	3.43	24.03	47.71
SEM ³		0.21	0.23	1.61	1.31
Source of variation		Probabilities			
Ca		0.548	<0.001	<0.001	<0.001
1 α -OH D ₃		0.509	0.001	0.040	<0.001
Ca $\times$ 1 α -OH D ₃		0.906	0.387	0.398	0.052

¹ Data are means of 4 replicates consisting of 2 birds per replicate.² Ca=calcium, 1 α -OH D₃=1alpha-hydroxycholecalciferol, Pi=inorganic phosphorus, tP=total phosphorus.³ SEM=pooled standard error of the mean.**Table 4. Effect of dietary Ca and 1 α -OH D₃ on tibia quality in 1- to 21-d-old broilers¹**

Ca ² (%)	1 α -OH D ₃ (μ g/kg)	Weight (g)	Length (cm)	Diameter (cm)	Breaking- strength (N)	Ash (%)	Ca (%)	P (%)
0.40	0	0.78 ^c	4.59 ^c	0.49	41.34 ^d	39.97	12.87	6.88
0.60	0	1.33 ^{abc}	5.42 ^{abcd}	0.57	62.89 ^{bcd}	39.93	13.95	7.10
0.80	0	1.04 ^{bcde}	4.99 ^{de}	0.52	54.18 ^{bcd}	38.94	14.30	7.01
1.00	0	1.02 ^{cde}	5.14 ^{cde}	0.50	48.89 ^{cd}	38.34	13.66	6.59
1.20	0	1.23 ^{abcd}	5.28 ^{bcde}	0.51	43.73 ^{cd}	37.74	13.19	5.94
0.40	5	1.46 ^a	5.93 ^{ab}	0.50	101.62 ^a	48.64	17.37	8.76
0.60	5	1.43 ^{ab}	6.06 ^a	0.47	91.46 ^{ab}	48.32	18.02	8.62
0.80	5	1.26 ^{abcd}	5.79 ^{abc}	0.46	81.35 ^{abc}	46.29	17.17	8.24
1.00	5	1.13 ^{abcde}	5.66 ^{abcd}	0.44	68.91 ^{abcd}	46.49	17.21	8.20
1.20	5	0.94 ^{de}	5.34 ^{abcd}	0.39	53.78 ^{bcd}	44.65	16.61	8.03
SEM ³		0.04	0.08	0.01	3.85	0.75	0.37	0.17
Source of variation		Probabilities						
Ca		0.004	0.035	0.081	0.012	0.126	0.620	0.089
1 α -OH D ₃		0.004	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Ca $\times$ 1 α -OH D ₃		<0.001	0.005	0.134	0.045	0.956	0.854	0.763

^{a-e} Means in the same column without common superscript differ significantly ($P<0.05$).¹ Data are means of 4 replicates consisting of 2 birds per replicate.² Ca=calcium, 1 α -OH D₃=1alpha-hydroxycholecalciferol, P=phosphorus.³ SEM=pooled standard error of the mean.

Tibia Quality

Dietary Ca affected the tibia parameters, and the lowest values of tibia quality (except of ash and P) were observed at 0.40% Ca, whereas the highest values were found at 0.60% Ca (except of ash and Ca, Table 4). Dietary Ca quadratically increased tibia diameter, breaking-strength, and Ca content. However, 1.2% Ca resulted in the deficiency of tibia P. The addition of 1 α -OH D₃ significantly increased tibia weight at 0.40% Ca, length at 0.40 and 0.80% Ca, and breaking-strength at 0.40% Ca. In contrast, tibia ash, Ca, and P content were improved at all Ca levels. However, 1 α -OH D₃

decreased tibia diameter, causing the tibia to become thinner. The improvement of 1 α -OH D₃ on tibia quality was negatively affected by dietary Ca. The interaction between dietary Ca and 1 α -OH D₃ was observed in tibia weight, length, and breaking-strength.

Qian *et al.* (1997) reported that the toe ash of broilers decreased with the increase of dietary Ca at 0.27% NPP. However, Driver (2004) found that in a basal diet (0.45% NPP), 0.33 to 0.90% Ca increased the tibia ash content. Further research showed that tibia ash content quadratically responds to dietary Ca (Bar *et al.*, 2003). These data suggest that

when the dietary Ca to P ratio is unbalanced, tibia ash increases with the dietary Ca; whereas, when the ratio is balanced, the tibia ash declines with the Ca level. In the present study, the Ca to P ratio was balanced in a low Ca diet; therefore, the tibia ash content decreased with Ca. Addition of 1α -OH D₃ increased tibia ash content. Similar results were found in vitamin D₃ (Qian *et al.*, 1997) and 25-OH D₃ (Ledwaba and Roberson, 2003). The positive effect of 1α -OH D₃ on tibia weight, length, breaking-strength, and ash negatively related to the dietary Ca. In the present study, dietary 0.40% and 1.20% Ca resulted in the low levels of Ca in tibia. Addition of 1α -OH D₃ increased tibia Ca and P content. Tanaka *et al.* (1971, 1972) found the positive effects of 1α -OH D₃ on the absorption and retention of Ca in rats. These findings and our results suggest that the increase of the intestinal absorption of calcium by dietary 1α -OH D₃ improves tibia breaking-strength and calcium content in broiler chickens.

The present experiment showed that by increasing tP retention and improving feed efficiency, 1α -OH D₃ enhances the growth performance and bone quality in 1- to 21-d-old broilers fed P-deficient corn-soybean meal diets. However, the efficacy of 1α -OH D₃ was negatively responded to dietary Ca levels. Under this condition, 1α -OH D₃ exerts the highest activities at the lowest levels of dietary Ca (0.40%).

Acknowledgments

This research was supported by the National Natural Science Foundation of China (No. 31101732), the Henan Province Programs for Science and Technology Development (No. 102102110173), the Natural Science Foundation of Henan Province (No. 2011B230011), and the Youth Foundation of Shangqiu Normal University (No. 2009QN11).

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