Relative biological value of 1α-hydroxycholecalciferol to 25-hydroxycholecalciferol in broiler chicken diets

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ABSTRACT This study was conducted to evaluate the relative biological value (RBV) of 1α-hydroxycholecalciferol (1α-OH-D_3) to 25-hydroxycholecalciferol (25-OH-D_3) in one- to 21-day-old broiler chickens fed calcium (Ca)- and phosphorus (P)-deficient diets. On the d of hatch, 450 male Ross 308 broiler chickens were weighed and randomly allotted to 9 treatments with 5 replicates of 10 birds per replicate. The basal diet contained 0.50% Ca and 0.25% non-phytate phosphorus (NPP) but was not supplemented with cholecalciferol (vitamin D_3). The levels of Ca and NPP in basal diets were lower than those recommended by NRC (1994). 25-OH-D_3 was fed at zero, 1.25, 2.5, 5.0, and 10.0 μg/kg, and 1α-OH-D_3 was fed at 0.625, 1.25, 2.5, and 5.0 μg/kg. The RBV of 1α-OH-D_3 to 25-OH-D_3 based on vitamin D intake was determined by the slope ratio method. Results showed that 25-OH-D_3 or 1α-OH-D_3 improved the growth performance and decreased the mortality in one- to 21-day-old broilers. A linear relationship was observed between the level of 25-OH-D_3 or 1α-OH-D_3 and mineralization of the femur, tibia, or metatarsus. The RBV of 1α-OH-D_3 to 25-OH-D_3 were 234, 253, and 202% when the weight, ash weight, and Ca percentage of femur were used as criteria. The corresponding RBV of 1α-OH-D_3 to 25-OH-D_3 were 232 to 263% and 245 to 267%, respectively, when tibia and metatarsus mineralization were used as criteria. These data indicate that when directly feeding a hormonally active form of vitamin D as 1α-OH-D_3 proportionally less is needed than when using the precursor (25-OH-D_3) in diets deficient in Ca and P.

Key words: 1α-hydroxycholecalciferol, 25-hydroxycholecalciferol, relative biological value, broiler chicken

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INTRODUCTION

Cholecalciferol (**vitamin D_3**) is used as a commercial source of vitamin D in poultry feed. It is well known that vitamin D_3 undergoes 25-hydroxylation in the liver to 25-hydroxycholecalciferol (**25-OH-D_3**) followed by 1α-hydroxylation in the kidney to 1,25-dihydroxycholecalciferol [**1,25-$(OH)_2$-D_3**]. The synthetic compound 25-OH-D_3 has been authorized for use as a feed additive for broiler chickens in China. Soares et al. (1995) found that 25-OH-D_3 is nearly twice as active as vitamin D_3 in broiler diets. Atencio et al. (2005) found that the relative biological value (**RBV**) of 25-OH-D_3 to vitamin D_3 is 108 to 400% in broiler breeder hen diets. These data indicate that the RBV of 25-OH-D_3 is higher than that of vitamin D_3 in poultry diets.

1α-Hydroxycholecalciferol (**1α-OH-D_3**) is an active derivative of vitamin D. It is hydroxylated by 25-hydroxylase in the liver and metabolized to 1,25-$(OH)_2$-D_3 in the chick (Edelstein et al., 1978). The addition of 1α-OH-D_3 improved the growth performance and bone mineralization in broilers (Biehl and Baker, 1997b). It can be used to prevent tibial dyschondroplasia of broiler chickens (Pesti and Shivaprasad, 2010). Research has shown that the RBV of 1α-OH-D_3 is higher than that of vitamin D_3 (Edwards et al., 2002; Liem, 2009; Han et al., 2013), and it is as effective as 1,25-$(OH)_2$-D_3 in increasing phytate P utilization in broiler chicken diets (Edwards, 2002). However, the relative bioactivity of 1α-OH-D_3 and 25-OH-D_3 has not been examined.

Therefore, the objective of this study was to evaluate the RBV of 1α-OH-D_3 to 25-OH-D_3 in one- to 21-day-old broiler chicken diets.

MATERIALS AND METHODS

Birds, Diets, and Management

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

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Table 1. Ingredients and nutrient composition in the basal diet.

Item	Amount (%)
Ingredient	
Corn	60.64
Soybean meal (43% CP)	32.00
Soybean oil	1.60
Soybean protein powder (65% CP)	3.47
Limestone	0.67
Dicalcium phosphate	0.71
L-lysine·HCl (98%)	0.14
DL-methionine (98%)	0.14
Trace mineral premix [1]	0.10
Vitamin premix [2]	0.03
Choline chloride (50%)	0.20
Sodium chloride	0.30
Total	100.00
Composition (%)	
AME (kcal/kg)	2,975.20
Analyzed crude protein (CP)	21.71
Analyzed calcium (Ca)	0.51
Analyzed total phosphorus (tP)	0.48
Non-phytate phosphorus (NPP)	0.25

[1] The trace mineral premix provided the following (per kg of diet): 80 mg iron; 40 mg zinc; 8 mg copper; 60 mg manganese; 0.35 mg iodine; 0.15 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 B_{12}; 10.0 mg pantothenic acid; 0.55 mg folic acid; 0.18 mg biotin.

On the d of hatch, 450 male Ross 308 broiler chickens were weighed and randomly allotted to 9 treatments with 5 replicates of 10 birds per replicate. Compound 25-OH-D_3 was fed at zero, 1.25, 2.5, 5.0, and 10.0 μg/kg, and 1α-OH-D_3 was fed at 0.625, 1.25, 2.5, and 5.0 μg/kg. The basal diet contained 0.50% Ca and 0.25% non-phytate phosphorus (**NPP**) but was not supplemented with vitamin D_3. The levels of Ca and NPP in basal diets were lower than those recommended by NRC (1994). Birds from one to 21 d of age were reared in stainless steel cages (190 cm × 50 cm × 35 cm). The birds were provided mash diet (Table 1) and water ad libitum. The lighting system consisted of 24 h of light from incandescent bulbs. Room temperature was controlled at 33°C from zero d to 3 d, 30°C from 4 d to 7 d, 27°C from 8 d to 14 d, and 24°C from 15 d to 21 days.

25-OH-D_3 and 1α-OH-D_3

Crystalline 25-OH-D_3 and 1α-OH-D_3 were supplied by Changzhou Book Chemical Co., Ltd. (Changzhou, China) and Taizhou Healtech Chemical Co., Ltd. (Taizhou, China), respectively. The solution of 25-OH-D_3 or 1α-OH-D_3 was prepared as described by Biehl and Baker (1997b). The crystalline 25-OH-D_3 or 1α-OH-D_3 was weighed, dissolved in ethanol, and then diluted by propylene glycol (5% ethanol:95% propylene glycol). The solution concentration was analyzed with the high performance liquid chromatography (**HPLC**) method by Shanghai Fuxin Analysis Technology Center (Shanghai, China). The determined concentrations of the 25-OH-D_3 and 1α-OH-D_3 solution were 10.47 and 9.15 μg/mL, respectively. Then, the solution of 1α-OH-D_3 or 25-OH-D_3 was added to the broiler diets.

Sample Collection

The broiler chickens were weighed on d 21. Body weight and feed consumption were measured. Average daily feed intake (**ADFI**), average daily gain (**ADG**), and feed conversion ratio (**FCR**) were calculated from one to 21 d of age. All dead birds were weighed and the weight was used to correct the ADFI. Two chickens from each replicate with the body weight close to replicate average were selected for collection of femur, tibia, and metatarsus. The leg bones of individual bird were excised and frozen at −20°C.

Sample Analysis

The femur, tibia, and metatarsus were boiled for 5 min to loosen muscle tissues as described by Hall et al. (2003). The meat, connective tissue, and fibula bone were completely removed using scissors and forceps. The bones were then placed in a container of ethanol for 24 h (removing water and polar lipids) after cleaning. Subsequently, the bones were further extracted in anhydrous ether for 24 h (removing non-polar lipids). Bones were dried at 105°C for 24 h before weighing. Bone diameter was determined at the medial point. Bone ash weight was determined by ashing the bone in a muffle furnace for 48 h at 600°C. Bone ash percentage was expressed relative to dried fat-free bone weight.

The Ca and P contents in diets and bones were determined as described by Han et al. (2013). The crude protein content in diets was determined with the method of AOAC (2000) using a PN-1430 Kjeldahl apparatus (Barcelona, Spain).

Statistical Analysis

Replicate means served as the experimental units for statistical analysis. Data were analyzed by the general linear model (**GLM**) of SAS software (SAS Institute, 2002). The RBV of 1α-OH-D_3 to 25-OH-D_3 was determined based on vitamin D intake 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 25-OH-D_3 intake, x_2 is 1α-OH-D_3 intake, and RBV = b_2/b_1. Standard errors (**SE**) of the slopes and slope ratios were estimated using the method of error propagation as described by Littell et al. (1997). Polynomial comparisons were performed to determine the linear and quadratic effects of 1α-OH-D_3 or 25-OH-D_3 levels on growth performance and bone mineralization.

Table 2. Effects of 1α-OH-D$_3$ and 25-OH-D$_3$ on growth performance of broiler chickens from one to 21 d of age.

25-OH-D$_3$ (μg/kg)	1α-OH-D$_3$ (μg/kg)	Average daily feed intake (g)	Average daily gain (g)	Feed conversion ratio (g/g)	Mortality (%)
0	0	22.7	10.8	2.10	30
1.25	–	27.2	14.6	1.86	14
2.5	–	31.3	17.9	1.75	8
5.0	–	46.2	29.7	1.56	0
10.0	–	48.5	31.4	1.54	0
–	0.625	30.3	16.7	1.81	16
–	1.25	39.5	26.0	1.52	2
–	2.5	46.9	29.9	1.57	0
–	5.0	50.8	31.6	1.61	0
SEM		1.5	1.2	0.03	2
P-value					
25-OH-D$_3$	Linear	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.060	0.053	<0.001	0.10
1α-OH-D$_3$	Linear	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.001	0.001	<0.001	0.034

Table 3. Effects of 1α-OH-D$_3$ and 25-OH-D$_3$ on femur mineralization in broiler chickens at 21 d of age.

25-OH-D$_3$ (μg/kg)	1α-OH-D$_3$ (μg/kg)	Weight (mg/bone)	Length (cm/bone)	Diameter (cm/bone)	Ash (mg/bone)	Ash (%)	Ca (%)	P (%)
0	0	481	3.22	0.49	132	27.75	11.32	5.54
1.25	–	635	3.50	0.52	166	28.10	11.73	5.60
2.5	–	695	3.72	0.54	230	33.79	13.28	6.74
5.0	–	999	4.60	0.59	411	42.31	16.49	7.61
10.0	–	1,072	4.62	0.60	460	43.77	16.52	8.12
–	0.625	644	4.03	0.53	220	35.10	12.38	6.69
–	1.25	872	4.30	0.55	353	41.31	14.77	7.72
–	2.5	974	4.56	0.59	405	42.44	15.35	7.70
–	5.0	1,101	4.77	0.61	497	46.04	16.29	8.56
SEM		34	0.08	0.01	20	1.01	0.34	0.16
P-value								
25-OH-D$_3$	Linear	<0.001	<0.001	0.002	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.62	0.52	0.95	0.054	0.13	0.59	0.32
1α-OH-D$_3$	Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.12	0.002	0.80	0.29	<0.001	0.41	0.007

RESULTS

Growth Performance

The lowest values of ADFI and ADG and the highest mortality were observed in one- to 21-day-old broiler chickens fed the basal diets without vitamin D (Table 2). The addition of 25-OH-D$_3$ or 1α-OH-D$_3$ linearly increased the ADFI and ADG, and decreased the FCR and mortality of broilers ($P < 0.05$).

A linear relationship was observed between the level of 25-OH-D$_3$ or 1α-OH-D$_3$ and growth performance ($P < 0.05$). However, a quadratic relationship also existed between dietary 1α-OH-D$_3$ and the growth of birds ($P < 0.05$). Hence, the ADFI, ADG, or FCR can't be used as criteria to estimate the RBV of 1α-OH-D$_3$ to 25-OH-D$_3$.

Bone Mineralization

Vitamin D deficiency in the basal diets resulted in poor growth and mineralization of the femur, tibia, and metatarsus in broilers at 21 d of age (Tables 3, 4, and 5). The addition of 25-OH-D$_3$ or 1α-OH-D$_3$ linearly improved the leg bone mineralization ($P < 0.05$). The percentage of ash, Ca, and P in bones increased after the addition of 25-OH-D$_3$ or 1α-OH-D$_3$. The weight, ash weight, length, and diameter of bones increased accordingly.

A linear relationship was observed between the level of 25-OH-D$_3$ or 1α-OH-D$_3$ and the mineralization of the 3 leg bones ($P < 0.05$). However, a quadratic relationship also existed between dietary 1α-OH-D$_3$ and the percentage of ash and P in the femur ($P < 0.05$). Therefore, the percentage of ash and P in the femur cannot be used as criteria to evaluate the RBV of 1α-OH-D$_3$ to 25-OH-D$_3$. Similar results were found in the tibia and metatarsus.

RBV of 1α-OH-D$_3$ to 25-OH-D$_3$

The criteria with a linear relationship between the level of vitamin D and bone mineralization were used to evaluate the RBV of 1α-OH-D$_3$ to 25-OH-D$_3$ based on vitamin D intake in broiler diets. The RBV was calculated by the slope ratio method (Littell et al., 1997). When femur weight was used as a criterion, the corresponding slopes of 25-OH-D$_3$ and 1α-OH-D$_3$ were

Table 4. Effects of 1α-OH-D_3 and 25-OH-D_3 on tibia mineralization in broiler chickens at 21 d of age.

25-OH-D_3 (μg/kg)	1α-OH-D_3 (μg/kg)	Weight (mg/bone)	Length (cm/bone)	Diameter (cm/bone)	Ash (mg/bone)	Ash (%)	Ca (%)	P (%)
0	0	587	4.67	0.45	175	29.85	11.04	5.41
1.25	–	827	5.30	0.46	259	31.49	11.48	5.92
2.5	–	929	5.40	0.48	309	33.67	11.83	6.19
5.0	–	1,282	6.21	0.56	546	43.03	15.53	7.65
10.0	–	1,384	6.30	0.56	620	45.31	16.38	8.22
–	0.625	809	5.34	0.46	266	33.25	11.23	6.11
–	1.25	1,122	5.81	0.50	441	39.71	13.96	7.28
–	2.5	1,310	6.12	0.59	553	42.61	15.12	7.78
–	5.0	1,355	6.27	0.56	625	46.90	16.82	8.59
SEM		43	0.09	0.01	26	0.96	0.38	0.18
P value								
25-OH-D_3	Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.89	0.39	0.33	0.12	0.054	0.14	0.35
1α-OH-D_3	Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.003	<0.001	0.64	0.22	0.61	0.31	0.53

Table 5. Effects of 1α-OH-D_3 and 25-OH-D_3 on metatarsus mineralization in broiler chickens at 21 d of age.

25-OH-D_3 (μg/kg)	1α-OH-D_3 (μg/kg)	Weight (mg/bone)	Length (cm/bone)	Diameter (cm/bone)	Ash (mg/bone)	Ash (%)	Ca (%)	P (%)
0	0	356	3.35	0.49	83	23.42	8.35	4.50
1.25	–	566	3.78	0.59	131	24.79	8.49	4.62
2.5	–	646	3.93	0.62	185	29.22	11.78	5.38
5.0	–	932	4.56	0.71	333	36.54	13.35	6.73
10.0	–	1,022	4.64	0.74	384	37.90	13.99	7.10
–	0.625	602	3.89	0.60	151	26.49	9.44	5.23
–	1.25	817	4.35	0.67	279	34.43	12.56	6.25
–	2.5	915	4.53	0.74	338	37.23	14.20	6.66
–	5.0	982	4.72	0.74	389	40.07	14.63	7.23
SEM		34	0.07	0.01	17	0.95	0.38	0.16
P value								
25-OH-D_3	Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.83	0.55	0.33	0.060	0.47	0.53	0.14
1α-OH-D_3	Linear	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Quadratic	0.003	<0.001	0.001	0.036	0.16	0.14	0.28

Table 6. Relative biological value (RBV) of 1α-OH-D_3 to 25-OH-D_3 based on vitamin D intake (μg/bird) in broiler chicken diets with the slope ratio method.

Criteria	Intercept	Slope ± SE		*P*-value	R^2	RBV ± SE
		25-OH-D_3	1α-OH-D_3			
Femur mineralization						
Weight (mg/bone)	633.12	49.04 ± 6.09	114.78 ± 13.39	<0.001	0.71	2.34 ± 0.33
Ash weight (mg/bone)	199.72	28.55 ± 3.29	72.28 ± 7.24	<0.001	0.76	2.53 ± 0.32
Ca percentage (%)	12.47	0.47 ± 0.08	0.95 ± 0.17	<0.001	0.55	2.02 ± 0.42
Tibia mineralization						
Ash weight (mg/bone)	267.47	38.52 ± 4.30	89.51 ± 9.45	<0.001	0.75	2.32 ± 0.29
Ash percentage (%)	32.67	1.38 ± 0.16	3.46 ± 0.35	<0.001	0.75	2.51 ± 0.33
Ca percentage (%)	11.58	0.52 ± 0.07	1.25 ± 0.16	<0.001	0.66	2.40 ± 0.38
P percentage (%)	6.00	0.24 ± 0.04	0.63 ± 0.08	<0.001	0.64	2.63 ± 0.47
Metatarsus mineralization						
Ash percentage (%)	26.90	1.24 ± 0.18	3.31 ± 0.40	<0.001	0.67	2.67 ± 0.45
Ca percentage (%)	9.85	0.47 ± 0.08	1.24 ± 0.17	<0.001	0.61	2.64 ± 0.51
P percentage (%)	5.05	0.22 ± 0.03	0.54 ± 0.07	<0.001	0.66	2.45 ± 0.40

49.04 and 114.78, respectively, which indicate a RBV of 234% (deriving 114.78 by 49.04) for 1α-OH-D_3 using 25-OH-D_3 as a reference (Table 6). The RBV of 1α-OH-D_3 to 25-OH-D_3 were 253 and 202% when the ash weight and Ca percentage of the femur were used as criteria. The corresponding RBV were 232 to 263% and 245 to 267%, respectively, when tibia and metatarsus mineralization were used as criteria. The average RBV of 1α-OH-D_3 to 25-OH-D_3 was about 245% for promoting growth performance and bone mineralization in broiler chickens.

DISCUSSION

The optimal Ca-to-NPP ratio is 2.0 to promote growth performance and bone mineralization in broiler chickens (Bar et al., 2003; Rao et al., 2007). Thus, the Ca-to-NPP ratio was maintained at 2.0 in the present study. Vitamin D_3 levels linearly improved growth and bone ash when broilers were fed Ca- and NPP-deficient diets; by contrast, performance was quadratically affected by vitamin D_3 levels when Ca and NPP contents were sufficient (Baker et al., 1998). Therefore, Ca- and NPP-deficient diets were designed in this study and the levels of Ca and NPP were maintained at 0.50 and 0.25%, respectively.

The appropriate levels of 1α-OH-D_3 and 25-OH-D_3 in broiler chicken diets were 5 μg/kg (Pesti and Shivaprasad, 2010) and 10 to 20 μg/kg (Goodgame et al., 2011), respectively. Thus, the level of 1α-OH-D_3 was maintained at 0.625 to 5.0 μg/kg in this study. The tibia ash percentage of broilers fed 6.25 μg/kg of 25-OH-D_3 was greater than that of birds fed 3.125 μg/kg of 25-OH-D_3; however, no further improvement in BWG and tibia ash was observed when the level of 25-OH-D_3 ranged from 6.25 to 100 μg/kg (Fritts and Waldroup, 2003). Therefore, the level of 25-OH-D_3 was maintained at 1.25 to 10.0 μg/kg in the present study.

The present results revealed a linear relationship between the level of 25-OH-D_3 or 1α-OH-D_3 and growth performance or bone mineralization in broiler chickens. The positive effects of 1α-OH-D_3 (Biehl and Baker, 1997b; Edwards et al., 2002; Snow et al., 2004; Han et al., 2009 and 2013) and 25-OH-D_3 (Yarger et al., 1995; Applegate et al., 2003; Fritts and Waldroup, 2003; Ledwaba and Roberson, 2003; Morris et al., 2014) on growth performance and bone mineralization in broiler chickens have been observed in previous studies. Edwards (2002) reported that the addition of 1α-OH-D_3 increased phytate P retention in broiler diets. By contrast, 25-OH-D_3 gave inconsistent responses to improve phytate P utilization in chicks (Edwards, 2002; Applegate et al., 2003; Ledwaba and Roberson, 2003). These data indicate that the capacity of 1α-OH-D_3 to increase phytate P utilization is greater than that of 25-OH-D_3. Remarkably, neither 1α-OH-D_3 nor 25-OH-D_3 affects intestinal phytase activities of broiler chickens (Biehl and Baker, 1997a; Applegate et al., 2003). By contrast, dietary Ca and P levels decrease intestinal phytase activity and phytate P hydrolysis of birds (Applegate et al., 2003; Onyango et al., 2006; Li et al., 2016). It is well known that 1,25-$(OH)_2$-D_3 increases Ca and P absorption. Therefore, the inhibition of dietary Ca and P on intestinal phytase activity declines and phytate P hydrolysis increases after dietary 1α-OH-D_3 or 25-OH-D_3 converts to 1,25-$(OH)_2$-D_3, which contributes to the improvement of growth performance and bone mineralization in chicks.

Only the tibia was used as a criterion in evaluation of the RBV of vitamin D derivatives in previous research. The differences in the growth and mineralization among the femur, tibia, and metatarsus were observed in broilers (Applegate and Lilburn, 2002; Han et al., 2015) and turkeys (Goetting-Fuchs et al., 2012). Thus, femur and metararsus also were used as criteria in the present study. The average RBV of 1α-OH-D_3 to 25-OH-D_3 were 2.30, 2.47, and 2.59 when the femur, tibia, and metatarsus were used as criteria, respectively. The differences in the RBV among the criteria were found. Metatarsus yielded the greatest value, followed by the tibia. The femur gave the lowest RBV.

Soares et al. (1995) found that 25-OH-D_3 is about 2 times as active as vitamin D_3 in broiler chicken diets. Similar results were observed in our research (Han et al., 2016). Atencio et al. (2005) found that the RBV of 25-OH-D_3 to vitamin D_3 is 108 to 400% in broiler breeder hen diets. These data indicate that the RBV of 25-OH-D_3 is higher than that of vitamin D_3 in poultry. The comparison in RBV of 1α-OH-D_3 and vitamin D_3 also has been conducted. Research has shown that 1α-OH-D_3 is approximately 8 times (Edwards et al., 2002), 7 to 15 times (Liem, 2009), and 5 times (Han et al., 2013) as effective as vitamin D_3 for promoting growth performance and bone mineralization in broiler chicken diets. These data indicate that the bioactivity of 1α-OH-D_3 may be higher than that of 25-OH-D_3. The results of the present study confirm our inferences.

CONCLUSION

The RBV of 1α-OH-D_3 to 25-OH-D_3 ranged from 202 to 267% for promoting growth performance and bone mineralization in broiler chickens from one to 21 d of age. These data indicate that when directly feeding a hormonally active form of vitamin D as 1α-OH-D_3, proportionally less is needed than when using the precursor (25-OH-D_3) in broiler diets deficient in Ca and P.

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