

Postprint: Requirement for 25-Hydroxyvitamin D3 in Broiler Chickens

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Date: 2017-10-23T00:00:00+00:00

Abstract

This experiment aimed to investigate the appropriate dietary requirement of 25-hydroxyvitamin D3 (25-OH-D3) for Ross 308 broiler chickens aged 1 to 21 days and 1 to 42 days. A total of 500 one-day-old male Ross 308 broiler chicks were selected and randomly divided into 10 groups, with 5 replicates per group and 10 birds per replicate. The 10 groups of broilers were fed diets with 25-OH-D3 levels of 50, 100, 200, 400, 600, 800, 1,600, 2,400, 3,200, and 4,000 IU/kg, respectively. The experimental period lasted 42 days. The results showed that: 1) When dietary 25-OH-D3 level increased from 50 IU/kg to 600 IU/kg, body weight, average daily gain, and average daily feed intake of broilers aged 1 to 21 days and 1 to 42 days were significantly increased ($P < 0.05$), while feed-to-gain ratio and mortality rate were significantly decreased ($P < 0.05$); further increasing dietary 25-OH-D3 level to 4,000 IU/kg had no significant effect on growth performance indicators ($P > 0.05$). 2) When dietary 25-OH-D3 level increased from 50 IU/kg to 600 IU/kg, bone (femur, tibia, and metatarsus) strength, weight, length, ash weight, and contents of ash, calcium, and phosphorus of broilers at 21 and 42 days of age were significantly increased ($P < 0.05$); further increasing dietary 25-OH-D3 level to 4,000 IU/kg had no significant effect on bone parameters ($P > 0.05$). 3) When dietary 25-OH-D3 level increased from 50 IU/kg to 600 IU/kg, plasma calcium content of broilers at 21 and 42 days of age was significantly increased ($P < 0.05$); further increasing dietary 25-OH-D3 level to 4,000 IU/kg had no significant effect on plasma calcium content ($P > 0.05$). Dietary 25-OH-D3 level had no significant effect on plasma inorganic phosphorus content of broilers ($P > 0.05$). 4) When dietary 25-OH-D3 level increased from 50 IU/kg to 800 IU/kg, calcium and total phosphorus retention rates of broilers aged 11 to 14 days and 31 to 34 days were significantly increased ($P < 0.05$); further increasing dietary 25-OH-D3 level to 4,000 IU/kg had no significant effect on calcium and total phosphorus retention rates ($P > 0.05$). Based on the comprehensive results of this experiment and using growth performance indicators

and bone parameters as evaluation criteria, broken-line regression model analysis revealed that the dietary 25-OH-D₃ requirement was 476 IU/kg for broilers aged 1 to 21 days and 448 IU/kg for broilers aged 1 to 42 days.

Full Text

Requirement of 25-Hydroxycholecalciferol for Broilers

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Abstract: This experiment was conducted to investigate the optimal dietary requirement of 25-hydroxycholecalciferol (25-OH-D₃) for Ross 308 broilers during the periods of 1-21 days and 1-42 days of age. Five hundred one-day-old male Ross 308 broiler chicks were randomly allocated to ten groups with five replicates per group and ten birds per replicate. The ten groups were fed diets containing 25-OH-D₃ at levels of 50, 100, 200, 400, 600, 800, 1,600, 2,400, 3,200, and 4,000 IU/kg. The experiment lasted for 42 days.

The results showed: 1) When dietary 25-OH-D₃ level increased from 50 to 600 IU/kg, body weight, average daily gain, and average daily feed intake of broilers during both 1-21 days and 1-42 days were significantly increased ($P < 0.05$), while feed-to-gain ratio and mortality were significantly decreased ($P < 0.05$). Further increasing dietary 25-OH-D₃ level to 4,000 IU/kg had no significant effect on growth performance indices ($P > 0.05$). 2) Bone (femur, tibia, and metatarsus) strength, weight, length, ash weight, and contents of ash, calcium, and phosphorus in 21- and 42-day-old broilers were significantly increased when dietary 25-OH-D₃ level increased from 50 to 600 IU/kg ($P < 0.05$). Further increasing dietary 25-OH-D₃ level to 4,000 IU/kg had no significant effect on bone parameters ($P > 0.05$). 3) Plasma calcium content in 21- and 42-day-old broilers was significantly increased when dietary 25-OH-D₃ level increased from 50 to 600 IU/kg ($P < 0.05$). Further increasing dietary 25-OH-D₃ level to 4,000 IU/kg had no significant effect on plasma calcium content ($P > 0.05$). Dietary 25-OH-D₃ level had no significant effect on plasma inorganic phosphorus content ($P > 0.05$). 4) Calcium and total phosphorus deposition rates in broilers during 11-14 days and 31-34 days were significantly increased when dietary 25-OH-D₃ level increased from 50 to 800 IU/kg ($P < 0.05$). Further increasing dietary 25-OH-D₃ level to 4,000 IU/kg had no significant effect on calcium and total phosphorus deposition rates ($P > 0.05$).

Based on the comprehensive results of this experiment and using growth performance indices and bone parameters as evaluation criteria, broken-line regression model analysis indicated that the dietary 25-OH-D₃ requirement was 476 IU/kg

for broilers aged 1-21 days and 448 IU/kg for broilers aged 1-42 days.

Keywords: 25-hydroxycholecalciferol; requirement; growth performance; bone parameters; broilers

Vitamin D₃ is hydroxylated by 25-hydroxylase in the liver to form 25-hydroxyvitamin D₃ (25-OH-D₃), which is then further hydroxylated by 1 α -hydroxylase in the kidneys to produce the final active form 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂-D₃] that regulates calcium (Ca) and phosphorus (P) homeostasis in poultry. Under modern intensive rearing conditions, poultry lack ultraviolet radiation exposure and therefore require supplementation of exogenous vitamin D₃ or its analogs to maintain normal growth in broilers. The biological efficacy of 25-OH-D₃ in poultry diets is higher than that of vitamin D₃, and its intestinal absorption efficiency is also greater. Replacing vitamin D₃ with 25-OH-D₃ in diets can increase body weight gain, improve breast meat yield, enhance bone mineralization, promote intestinal development, and boost immunity in broilers. China has included 25-OH-D₃ in the “Feed Additive Catalogue (2013)”, permitting its use as a vitamin feed additive in poultry production. While Yarger et al. recommended a dietary 25-OH-D₃ level of 2,760 IU/kg for commercial broilers, other studies have shown that lower doses may meet poultry growth requirements. Therefore, this experiment was conducted to investigate the effects of dietary 25-OH-D₃ levels on growth performance, bone (femur, tibia, and metatarsus) mineralization, plasma indices, and calcium and phosphorus deposition rates in Ross 308 broilers during 1-21 days and 1-42 days of age, providing a scientific basis for rational 25-OH-D₃ utilization in production.

1. Materials and Methods

1.1 Experimental Animals

Five hundred one-day-old healthy male Ross 308 broiler chicks with similar body weight [(43.84 \pm 0.22) g] were randomly divided into ten groups with five replicates per group and ten birds per replicate. The experimental period lasted 42 days. Broilers were raised in cages (200 cm \times 50 cm \times 35 cm) with each replicate housed in the same cage. They were fed powdered compound diets ad libitum with free access to water and vaccinated according to routine immunization programs.

1.2 Experimental Diets and Design

The nutrient levels of the basal diet were formulated according to the recommendations of NRC (1994) and the “Feeding Standard of Chickens” (NY/T 33–2004) for broiler nutrient requirements. Ten experimental diets were prepared by supplementing the basal diet with ten levels of 25-OH-D₃ at 50, 100, 200,

400, 600, 800, 1,600, 2,400, 3,200, and 4,000 IU/kg. The composition and nutrient levels of the basal diet are shown in Table 1. The 25-OH-D₃ additive was purchased from Shandong Haineng Bioengineering Co., Ltd., with a measured content of 5.0×10^5 IU/g using high-performance liquid chromatography.

1.3 Measurements

1.3.1 Growth Performance Broilers were weighed after fasting at 21 and 42 days of age. Daily feed intake and mortality were recorded for each replicate, with feed intake adjusted by subtracting the feed consumption of dead birds. Average daily feed intake (g/d), average daily gain (g/d), feed-to-gain ratio (average daily feed intake/average daily gain), and mortality were calculated for the experimental period.

1.3.2 Metabolism Trial Calcium and phosphorus metabolism trials were conducted during 11-14 days and 31-34 days of age by collecting all excreta. Fresh excreta were continuously collected for 96 h from each replicate cage, with feathers and debris removed before immediate storage at -20°C for calcium and phosphorus analysis. Diet and excreta samples were analyzed for calcium content using the EDTA titration method and for total phosphorus (TP) content using the molybdenum yellow colorimetric method. Calcium and total phosphorus deposition rates were calculated as:

$$\text{Nutrient deposition rate (\%)} = [(\text{Nutrient intake} - \text{Nutrient excretion}) / \text{Nutrient intake}] \times 100$$

1.3.3 Plasma Biochemical Indices At 21 and 42 days of age, one broiler per replicate with body weight close to the replicate average was selected for slaughter. Blood (5 mL) was collected via cardiac puncture using a syringe rinsed with 1 mg/mL sodium heparin solution, then centrifuged at 3,000 g for 10 min to prepare plasma, which was stored at -20°C. Plasma calcium and inorganic phosphorus (Pi) contents were determined using a Shimadzu CL-8000 automatic biochemical analyzer.

1.3.4 Bone Indices During slaughter, femur, tibia, and metatarsus from both legs were removed and stored in sealed bags at -20°C. Left leg bones (femur, tibia, and metatarsus) were boiled for 3-5 min to remove residual muscle and fibula, then defatted by soaking in absolute ethanol for 36 h and anhydrous ether for 36 h. After drying at 105°C for 24 h, bone weight was measured using an analytical balance. Bone length and diameter (at mid-length) were measured using vernier calipers. Bones were ashed in a crucible at 600°C for 36 h to determine ash content. Bone samples were analyzed for calcium content using EDTA titration and phosphorus content using molybdenum yellow colorimetry. The breaking strength (bone strength) of right leg bones (femur, tibia, and metatarsus) was measured using a digital universal testing machine (CMT-5504, Shenzhen SANS

Material Testing Co., Ltd.) at Northwest A&F University with the following parameters: span 40 mm, load unit 50 kg, displacement speed 10 mm/min.

1.4 Statistical Analysis

Using each replicate as the experimental unit, data were analyzed using the General Linear Model (GLM) procedure in SAS 9.0 statistical software. Duncan's multiple range test was used for pairwise comparisons, with linear and quadratic trend analyses performed. Differences were considered significant at $P < 0.05$.

The PROC NLIN procedure in SAS 9.0 was used for broken-line regression model parameter estimation to establish the broken-line regression equation:

When $X < R$: $Y = L + U \times (R - X)$

When $X \geq R$: $Y = L$

Where Y represents evaluation indices such as average daily gain; L represents the growth response when 25-OH-D₃ level reaches the requirement; U represents the slope; and R represents the 25-OH-D₃ requirement for broilers.

2. Results

2.1 Effects of Dietary 25-OH-D₃ Level on Growth Performance of Broilers

As shown in Table 2, dietary 25-OH-D₃ level significantly affected body weight, average daily gain, average daily feed intake, feed-to-gain ratio, and mortality ($P < 0.05$). Broilers fed the diet containing 50 IU/kg 25-OH-D₃ had significantly lower body weight, average daily gain, and average daily feed intake, and significantly higher feed-to-gain ratio compared with other groups ($P < 0.05$). Increasing dietary 25-OH-D₃ level from 50 to 200 IU/kg significantly improved body weight, average daily gain, and average daily feed intake ($P < 0.05$) and significantly reduced feed-to-gain ratio ($P < 0.05$).

2.2 Effects of Dietary 25-OH-D₃ Level on Bone Parameters of Broilers

As shown in Tables 3, 4, and 5, dietary 25-OH-D₃ level significantly affected femur, tibia, and metatarsus parameters (except diameter) ($P < 0.05$). Broilers fed the diet containing 50 IU/kg 25-OH-D₃ had significantly lower bone parameters (except diameter) compared with other groups ($P < 0.05$). Increasing dietary 25-OH-D₃ level from 50 to 400 IU/kg significantly improved femur, tibia, and metatarsus strength, weight, length, ash weight, and contents of ash, calcium, and phosphorus ($P < 0.05$). At 600 IU/kg dietary 25-OH-D₃, bone weight and ash weight reached maximum values in 42-day-old broilers. Further increasing dietary 25-OH-D₃ level from 600 to 4,000 IU/kg did not significantly change bone parameters ($P > 0.05$). Dietary 25-OH-D₃ level had no significant effect on metatarsus diameter at 21 and 42 days or femur diameter at 21 days ($P > 0.05$).

2.3 Effects of Dietary 25-OH-D₃ Level on Plasma Calcium and Inorganic Phosphorus Contents and Calcium and Total Phosphorus Deposition Rates

As shown in Table 6, dietary 25-OH-D₃ level significantly affected plasma calcium content and calcium and total phosphorus deposition rates ($P < 0.05$) but had no significant effect on plasma inorganic phosphorus content ($P > 0.05$). Plasma calcium content in 21-day-old broilers fed the diet containing 50 IU/kg 25-OH-D₃ was significantly lower than in other groups ($P < 0.05$). Increasing dietary 25-OH-D₃ level from 50 to 600 IU/kg significantly increased plasma calcium content in 42-day-old broilers ($P < 0.05$), but further increases to 4,000 IU/kg had no significant effect ($P > 0.05$). Dietary 25-OH-D₃ level had no significant effect on plasma inorganic phosphorus content ($P > 0.05$). Calcium and total phosphorus deposition rates were significantly increased when dietary 25-OH-D₃ level increased from 50 to 800 IU/kg ($P < 0.05$), with no further significant changes when levels increased to 4,000 IU/kg ($P > 0.05$).

2.4 Requirement of Broilers for Dietary 25-OH-D₃

As shown in Tables 7 and 8, using growth performance indices (average daily gain, average daily feed intake) and bone parameters (femur, tibia, and metatarsus weight and ash weight) as evaluation criteria, broken-line regression model analysis revealed that the dietary 25-OH-D₃ requirement was 476 IU/kg for broilers aged 1-21 days and 448 IU/kg for broilers aged 1-42 days.

3. Discussion

3.1 Effects of Dietary 25-OH-D₃ Level on Growth Performance of Broilers

The results showed that broilers fed the diet containing 50 IU/kg 25-OH-D₃ exhibited slow growth, high mortality, and low bone strength, weight, length, ash weight, and contents of ash, calcium, and phosphorus. When dietary 25-OH-D₃ level increased from 50 to 600 IU/kg, body weight and average daily gain of broilers during 1-21 days and 1-42 days showed a linear relationship with dietary 25-OH-D₃ level. These findings indicate that growth performance decreased when dietary vitamin D was insufficient, and improved with appropriate 25-OH-D₃ supplementation. However, when dietary 25-OH-D₃ level increased from 600 to 4,000 IU/kg, no significant effects on growth performance were observed, suggesting that high-dose 25-OH-D₃ supplementation provides no benefit for broiler growth when dietary calcium and phosphorus are adequate. Previous studies from our research group demonstrated that increasing dietary 25-OH-D₃ level from 50 to 400 IU/kg significantly improved body weight gain and feed intake while reducing feed-to-gain ratio and mortality. Other research found that increasing dietary 25-OH-D₃ level from 125 to 500 IU/kg significantly improved body weight in 1-21-day-old broilers, with no further improvements when levels increased from 500 to 4,000 IU/kg. Similar results were reported by

Goodgame et al., who found that increasing dietary 25-OH-D₃ level from 100 to 800 IU/kg significantly improved body weight and feed intake in 7-21-day-old broilers without affecting feed-to-gain ratio, and no further improvements were observed when levels increased to 3,200 IU/kg.

3.2 Effects of Dietary 25-OH-D₃ Level on Bone Parameters of Broilers

Bone (femur, tibia, and metatarsus) mineralization is a sensitive indicator for evaluating vitamin D effects in poultry, more sensitive than growth performance indices. The current results showed that broilers fed the diet containing 50 IU/kg 25-OH-D₃ had the lowest bone strength, weight, length, ash weight, and contents of ash, calcium, and phosphorus. Bone mineralization indices improved rapidly when dietary 25-OH-D₃ level increased from 50 to 600 IU/kg, but no further significant improvements were observed with higher supplementation levels. Our previous research demonstrated that increasing dietary 25-OH-D₃ level from 50 to 400 IU/kg significantly improved femur and tibia weight, length, ash weight, and contents of ash, calcium, and phosphorus in 21-day-old broilers. Other studies also reported that increasing dietary 25-OH-D₃ level from 100 to 800 IU/kg significantly improved bone strength and tibia and toe ash content, with no further effects at higher levels. These data indicate that dietary 25-OH-D₃ levels of 400-800 IU/kg are sufficient to meet vitamin D requirements for femur, tibia, and metatarsus mineralization in broilers, and higher levels do not significantly affect bone mineralization indices.

3.3 Effects of Dietary 25-OH-D₃ Level on Plasma Indices and Calcium and Total Phosphorus Deposition Rates

The results showed that dietary 25-OH-D₃ level significantly affected plasma calcium content but not plasma inorganic phosphorus content. Previous research also reported no significant effect of dietary 25-OH-D₃ level on plasma inorganic phosphorus content in 21- and 42-day-old Beijing ducks. In the early metabolism trial, increasing dietary 25-OH-D₃ level from 50 to 800 IU/kg significantly improved calcium and phosphorus deposition rates in 11-14-day-old broilers, with no further improvements at higher levels. The late metabolism trial showed that calcium and phosphorus deposition rates in 31-34-day-old broilers tended to increase initially then decrease when dietary 25-OH-D₃ level increased from 50 to 4,000 IU/kg. Applegate et al. reported that dietary 25-OH-D₃ supplementation promoted phytate phosphorus hydrolysis and improved total phosphorus deposition rate in 7-21-day-old broilers. The biological efficacy of 25-OH-D₃ in broiler diets is approximately twice that of vitamin D₃. Studies on poultry vitamin D₃ showed that increasing dietary vitamin D₃ level from 300 to 1,200 IU/kg linearly decreased calcium and total phosphorus excretion in 35-day-old broilers.

3.4 Optimal Requirement of Broilers for Dietary 25-OH-D₃

Using growth performance indices (average daily gain, average daily feed intake) and bone parameters (femur, tibia, and metatarsus weight and ash weight) as evaluation criteria, curve fitting analysis showed that the broken-line model best fit the experimental data. Based on broken-line regression equations, the optimal dietary 25-OH-D₃ requirement was estimated to be 476 IU/kg for 1-21-day-old broilers and 448 IU/kg for 1-42-day-old broilers.

Current research findings on the optimal dietary 25-OH-D₃ requirement for broilers show some variation. Yarger et al. suggested a requirement of 2,760 IU/kg based on body weight gain and feed efficiency. However, other studies indicate that optimal supplementation levels may be lower than Yarger's recommendation. Fritts et al. reported a requirement of 500 IU/kg based on 42-day body weight and tibia ash content. Goodgame et al. estimated the requirement at 400-800 IU/kg based on body weight, tibia ash, and strength. The "Feeding Standard of Chickens" (NY/T 33-2004) recommends a vitamin D₃ supplementation level of 1,000 IU/kg for broilers. Our research group has shown that the biological efficacy of 25-OH-D₃ in broiler diets is about twice that of vitamin D₃, suggesting a 25-OH-D₃ requirement of approximately 500 IU/kg, which is close to our current findings. Additionally, Shi et al. reported that based on daily gain and tibia indices, the 25-OH-D₃ requirement was 342 IU/kg for 1-21-day-old Beijing ducks and 316 IU/kg for 22-42-day-old ducks.

Conclusions: 1. Dietary supplementation with appropriate levels of 25-OH-D₃ can improve growth performance, enhance bone (femur, tibia, and metatarsus) mineralization, and increase plasma calcium content and calcium and phosphorus deposition rates in broilers. 2. Based on growth performance indices and bone parameters using broken-line regression model analysis, the dietary 25-OH-D₃ requirement is 476 IU/kg for 1-21-day-old broilers and 448 IU/kg for 1-42-day-old broilers.

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Table 1 Composition and nutrient levels of basal diets (air-dry basis) %

Items	1-21 days	22-42 days
Ingredients		
Corn	52.00	58.50
Soybean meal	32.00	28.00
Soybean oil	3.00	4.00
Soybean protein powder	5.00	3.00
Limestone	1.50	1.50
Calcium hydrogen phosphate	1.80	1.60
L-Lysine	0.30	0.20
DL-Methionine	0.20	0.10
Vitamin premix ¹	0.03	0.03
Trace mineral premix ²	0.15	0.15
Choline chloride	0.15	0.15
Salt (NaCl)	0.30	0.30
Total	100.00	100.00
Nutrient levels³		
Apparent metabolizable energy (MJ/kg)	12.55	13.00
Crude protein	22.00	20.00
Total phosphorus	0.70	0.65
Non-phytate phosphorus	0.45	0.40
Lysine	1.20	1.05
Methionine	0.50	0.38

¹ Vitamin premix provided per kg of diet: VA 8,000 IU, VE 20 IU, VK 0.5 mg, VB₁ 2.0 mg, VB₂ 8.0 mg, VB₆ 3.5 mg, VB₁₂ 0.01 mg, pantothenic acid 10.0 mg,

niacin 35.0 mg, folic acid 0.55 mg, biotin 0.18 mg.

² Trace mineral premix provided per kg of diet: Fe 80 mg, Zn 40 mg, Cu 8 mg, Mn 60 mg, I 0.35 mg, Se 0.15 mg.

³ Calcium and total phosphorus were measured values; others were calculated values.

Table 2 Effects of dietary 25-OH-D₃ level on growth performance of broilers

25-OH-D ₃ level (IU/kg)	Body weight (g)	Average daily gain (g)	Average daily feed intake (g)	Feed-to-gain ratio	Mortality (%)
	21 d	42 d	1-21 d	1-42 d	1-21 d
50	738b	1,738b	28.23c	40.34b	45.73c
100	806ab	2,567a	34.66b	60.07a	51.13b
200	835ab	2,679a	37.68ab	62.73a	54.58ab
400	826ab	2,772a	37.30ab	64.97a	54.83ab
600	829ab	2,714a	37.34ab	63.55a	54.57ab
800	823ab	2,753a	37.12ab	64.51a	54.10ab
1,600	822ab	2,729a	37.07ab	63.92a	55.10ab
2,400	820ab	2,678a	36.27ab	62.71a	54.83ab
3,200	819ab	2,753a	36.12ab	64.97a	54.10ab
4,000	817ab	2,678a	34.66b	62.71a	54.83ab
SEM	20.33	17.72	1.88	1.62	0.85
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Linear	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Quadratic	0.0005	0.0002	0.0002	0.3367	0.0250

In the same column, values with the same or no letter superscripts mean no significant difference ($P>0.05$), while different small letter superscripts indicate significant difference ($P<0.05$). The same applies below.

Table 3 Effects of dietary 25-OH-D₃ level on femur parameters of broilers

25-OH-D ₃ level (IU/kg)	Strength (N)	Weight (g)	Length (cm)	Diameter (cm)	Ash weight (g)	Ash content (%)	Ca content (%)	P content (%)
	21 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d
50	74.12d	170.22c	0.90e	1.15d	2.00c	3.02b	4.50b	6.02b
100	214.04bc	188.93b	1.41c	1.46d	4.48a	4.08e	4.82a	8.13b
200	233.22ab	201.74b	1.57abc	2.01c	4.65a	6.17d	4.94a	9.65a

25-OH-D ₃ level (IU/kg)	Strength (N)	Weight (g)	Length (cm)	Diameter (cm)	Ash weight (g)	Ash content (%)	Ca content (%)	P content (%)
400	273.36a	261.52ab	1.59ab	2.10bc	5.02a	6.60bcd	4.93a	10.11a
600	252.24ab	316.52a	1.43bc	2.21abc	4.93a	7.61a	4.85a	10.09a
800	276.46a	274.84a	1.70a	2.04c	4.71a	7.21ab	5.01a	9.82a
1,600	262.38ab	320.42a	1.54abc	2.35a	4.63a	6.53bcd	4.96a	9.64a
2,400	235.98ab	253.06ab	1.52abc	2.12bc	4.88a	6.24cd	4.91a	9.98a
3,200	214.64bc	292.10a	1.62a	2.27ab	4.84a	7.04abc	4.80a	9.93a
4,000	235.98ab	249.42ab	1.52abc	2.13abc	4.84a	6.82abc	4.91a	10.04a
SEM	0.90	1.15	0.32	0.48	0.63	0.74	0.57	0.81
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.1669	<0.0001
Linear	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.3367	0.0250
Quadratic	0.0005	0.0002	0.0002	0.3367	0.0250	<0.0001	<0.0001	0.0002

Table 4 Effects of dietary 25-OH-D₃ level on tibia parameters of broilers

25-OH-D ₃ level (IU/kg)	Strength (N)	Weight (g)	Length (cm)	Diameter (cm)	Ash weight (g)	Ash content (%)	Ca content (%)	P content (%)
	21 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d
50	36.26d	81.18c	1.19e	2.44f	5.43d	6.86c	0.48c	0.67c
100	63.78c	188.93b	1.46d	4.08e	5.97c	8.13b	0.53b	0.74bc
200	109.54b	201.74b	2.01c	6.17d	6.43b	9.65a	0.57ab	0.81ab
400	126.20ab	261.52ab	1.10bc	6.60bcd	6.55ab	10.11a	0.54ab	0.78abc
600	126.60ab	316.52a	2.21abc	7.61a	6.62ab	10.09a	0.57ab	0.88a
800	120.36ab	274.84a	2.04c	7.21ab	6.50ab	9.82a	0.54ab	0.84ab
1,600	135.12a	320.42a	2.35a	6.53bcd	6.77a	9.64a	0.58a	0.80ab
2,400	119.58ab	253.06ab	1.12bc	6.24cd	6.64ab	9.98a	0.53ab	0.76bc
3,200	129.58ab	249.42ab	1.13abc	6.82abc	6.62ab	10.04a	0.55a	0.83ab
4,000	124.92ab	292.10a	2.27ab	7.04abc	6.52ab	9.93a	0.57ab	0.83ab
SEM	1.23	2.44	0.63	0.74	0.57	0.81	0.38	0.55
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Linear	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Quadratic	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 5 Effects of dietary 25-OH-D₃ level on metatarsus parameters of broilers

25-OH-D ₃ level (IU/kg)	Strength (N)	Weight (g)	Length (cm)	Diameter (cm)	Ash weight (g)	Ash content (%)	Ca content (%)	P content (%)
	21 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d
50	68.34c	106.36b	0.84c	1.00b	1.77d	2.44c	4.16c	6.46c
100	118.13ab	188.93b	1.23a	1.46d	3.59b	4.08e	4.85a	8.13b
200	123.74ab	201.74b	1.27a	2.01c	3.95ab	6.17d	4.88a	9.65a
400	144.88a	261.52a	1.33a	2.10bc	4.16a	6.60bcd	4.86a	10.11a
600	145.08a	316.52a	1.21a	2.21abc	3.83ab	7.61a	4.78a	10.09a
800	147.64a	274.84a	1.35a	2.04c	3.60b	7.21ab	4.97a	9.82a
1,600	130.58ab	320.42a	1.29a	2.35a	3.65b	6.53bcd	4.90a	9.64a
2,400	143.22a	253.06a	1.26a	2.12bc	3.98ab	6.24cd	4.81a	9.98a
3,200	129.03ab	249.42a	1.34a	2.13abc	4.07ab	6.82abc	4.86a	10.04a
4,000	143.22a	292.10a	1.26a	2.27ab	3.98ab	7.04abc	4.81a	9.93a
SEM	0.84	1.00	0.63	0.74	0.57	0.81	0.38	0.55
P-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Linear	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Quadratic	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 6 Effects of dietary 25-OH-D₃ level on plasma Ca and Pi contents and Ca and TP deposition rates of broilers

25-OH-D ₃ level (IU/kg)	Plasma Ca (mg/dL)	Plasma Pi (mg/dL)	Ca deposition rate (%)	TP deposition rate (%)
	21 d	42 d	21 d	42 d
50	6.30b	6.20c	8.10a	8.31a
100	7.60a	6.62bc	8.46a	8.71a
200	8.10a	6.99abc	8.01a	8.23a
400	8.31a	7.06abc	8.71a	8.71a
600	8.46a	7.37ab	8.23a	8.71a
800	8.01a	7.36ab	8.71a	8.71a
1,600	8.71a	7.98a	8.71a	8.71a
2,400	8.23a	7.94a	8.71a	8.71a
3,200	8.71a	7.98a	8.71a	8.71a
4,000	8.71a	7.94a	8.71a	8.71a
SEM	0.84	1.00	0.63	0.74
P-value	<0.0001	<0.0001	<0.0001	<0.0001
Linear	<0.0001	<0.0001	<0.0001	<0.0001
Quadratic	<0.0001	<0.0001	<0.0001	<0.0001

Table 7 Dietary 25-OH-D₃ requirement of broilers aged 1-21 days estimated based on broken-line model

Response criteria	Model parameters	P-value	25-OH-D ₃ requirement (IU/kg)	Asymptotic SE	Asymptotic 95% CI
Average daily gain (g)	L=36.6966, U=- 0.0284, R=426.0	<0.0001	1426.0	15.2	395.6- 456.4
Average daily feed intake (g)	L=54.6856, U=- 0.0293, R=454.1	<0.0001	1454.1	16.2	421.7- 486.5
Femur weight (g)	L=1.5632, U=- 0.00114, R=479.8	<0.0001	1479.8	18.9	442.0- 517.6
Femur ash weight (g)	L=0.7412, U=- 0.00070, R=502.9	<0.0001	1502.9	24.3	454.3- 551.5
Tibia weight (g)	L=2.1804, U=- 0.00170, R=496.9	<0.0001	1496.9	21.7	453.5- 540.3
Tibia ash weight (g)	L=0.9892, U=- 0.00099, R=501.8	<0.0001	1501.8	23.1	455.5- 548.1
Metatarsus weight (g)	L=1.2916, U=- 0.00078, R=474.9	<0.0001	1474.9	19.8	435.1- 514.7
Metatarsus ash weight (g)	L=0.5632, U=- 0.00052, R=472.2	<0.0001	1472.2	20.4	431.2- 513.2
Mean			476		

Table 8 Dietary 25-OH-D₃ requirement of broilers aged 1-42 days estimated based on broken-line model

Response criteria	Model parameters	P-value	25-OH-D ₃ requirement (IU/kg)	Asymptotic SE	Asymptotic 95% CI
Average daily gain (g)	L=63.9146, U=-0.0741, R=471.2	<0.0001	471.2	20.8	429.6-512.8
Average daily feed intake (g)	L=119.0000, U=-0.0951, R=461.7	<0.0001	461.7	25.3	410.9-512.5
Femur weight (g)	L=4.7996, U=-0.00479, R=471.7	<0.0001	471.7	21.2	429.1-514.3
Femur ash weight (g)	L=2.1520, U=-0.00263, R=452.7	<0.0001	452.7	19.8	412.9-492.5
Tibia weight (g)	L=6.7684, U=-0.00821, R=439.0	<0.0001	439.0	18.5	401.8-476.2
Tibia ash weight (g)	L=3.1428, U=-0.00414, R=442.6	<0.0001	442.6	19.1	403.8-481.4
Metatarsus weight (g)	L=3.8254, U=-0.00401, R=430.7	<0.0001	430.7	18.9	392.3-469.1
Metatarsus ash weight (g)	L=1.6960, U=-0.00211, R=411.7	<0.0001	411.7	17.8	375.9-447.5
Mean			448		

Note: Figure translations are in progress. See original paper for figures.

Source: ChinaXiv – Machine translation. Verify with original.