1,25-Dihydroxycholecalciferol Improved the Growth Performance and Upregulated the Calcium Transporter Gene Expression Levels in the Small Intestine of Broiler Chickens

Lihua Wu¹, Xiaona Wang¹, Xianliang Lv¹, Lei He²,³, Hongxia Qu², Chuanxin Shi², Liao Zhang², Jinliang Zhang², Zhixiang Wang¹ and Jincheng Han²

¹ College of Animal Science and Technology, Henan Agricultural University, Zhengzhou 450046, Henan, China
² Department of Animal Science, College of Life Science, Shangqiu Normal University, Shangqiu 476000, Henan, China
³ College of Life Sciences, Henan Normal University, Xinxiang 453007, Henan, China

1,25-Dihydroxycholecalciferol (1,25-(OH)₂-D₃) is the final active product of vitamin D. This study aimed to investigate the effects of 1,25-(OH)₂-D₃ on growth performance, bone development, and calcium (Ca) transporter gene expression levels in the small intestine of broiler chickens. On the day of hatching, 140 female Ross 308 broilers were randomly allotted into two treatments with five replicates (14 birds per replicate). Two levels of 1,25-(OH)₂-D₃ (0 and 1.25 µg/kg) were added to the basal diet without vitamin D. Results showed that the addition of 1.25 µg/kg 1,25-(OH)₂-D₃ increased the average daily feed intake and the average daily gain and decreased the feed conversion ratio and mortality in 1- to 19-day-old broiler chickens compared with the basal diet without vitamin D (P<0.05). 1,25-(OH)₂-D₃ also enhanced the length, weight, ash weight, and the percentage contents of ash, Ca, and P in the tibia and femur of broilers (P<0.05). The mRNA expression levels of the plasma membrane Ca ATPase 1b (PMCA1b) in the duodenum and the sodium (Na)/Ca exchanger 1 (NCX1) in the duodenum and the jejunum were not affected by 1,25-(OH)₂-D₃ while the mRNA expression levels of the Ca-binding protein (CaBP-D28k) in the duodenum, jejunum, and ileum of 19-day-old broilers increased to 88.1-, 109.1-, and 2.7-fold, respectively, after adding 1,25-(OH)₂-D₃ (P<0.05). The mRNA expression levels of PMCA1b and NCX1 in the ileum and that of CaBP-D28k in the duodenum, jejunum, and ileum were also enhanced to 1.57-2.86 times with the addition of 1,25-(OH)₂-D₃ (P<0.05). In contrast, the mRNA expression levels of PMCA1b and NCX1 in the ileum and that of vitamin D receptor (VDR) in the small intestine were not affected by 1,25-(OH)₂-D₃ (P>0.05). These data indicate that 1,25-(OH)₂-D₃ upregulated Ca transporter gene transcription and promoted Ca²⁺ absorption in the small intestine, especially in the proximal intestine (duodenum and jejunum), thereby improving growth performance and bone mineralization in broiler chickens.

Key words: broiler chicken, CaBP-D28k, 1,25-dihydroxycholecalciferol, NCX1, PMCA1b, VDR

J. Poult. Sci., 59: 129–136, 2022

Introduction

Vitamin D₃ (VD₃) is used as an essential feed additive to regulate calcium (Ca) absorption in poultry. Vitamin D deficiency inhibits growth and decreases bone mineralization in chickens (Baker et al., 1998; Chen et al., 2017). Supplementation with VD₃ has been shown to improve the average daily feed intake (ADFI) and average daily gain (ADG) in broilers (Baker et al., 1998). The optimal requirement of VD₃ is about 25 µg/kg feed in broilers from 1 to 21 days of age (Chinese Feeding Standard of Chicken, Ministry of Agriculture of China, 2004). VD₃ undergoes 25-hydroxylation in the liver to form 25-hydroxycholecalciferol (25-OH-D₃) and 1α-hydroxylation in the kidney to form the final product 1,25-dihydroxycholecalciferol (1,25-(OH)₂-D₃). The bioactivity of 1,25-(OH)₂-D₃ is higher than that of VD₃ and 25-OH-D₃ (Soares et al., 1995).

1,25-(OH)₂-D₃ binds to the vitamin D receptor (VDR) to regulate Ca absorption in the intestine. Ca absorption in the small intestine of animals includes transcellular and paracellular pathways. The paracellular pathway allows the direct exchange of Ca²⁺ between the intestine and blood via tight 

Copyright © 2022, Japan Poultry Science Association.
junctons (Hoenderop et al., 2005). The Ca\(^{2+}\) transcellular transport consists of three major steps: entry of Ca\(^{2+}\) through the brush border to intestinal cells, movement from the apical membrane to the basal membrane, and extrusion through the basal membrane to the blood (Bar, 2008; Fleet and Schoch, 2010). The first step proceeds down the chemical gradient of Ca\(^{2+}\), which is facilitated by the transient receptor potential cation channels (TRPV5 and TRPV6). The second step is facilitated by the intracellular Ca-binding protein CaBP-D9k (mammals) or CaBP-D28k (poultry). The energy-dependent third step proceeds up the chemical gradient of Ca\(^{2+}\) and is facilitated by the plasma membrane Ca ATPase 1b (PMCA1b) and sodium (Na)/calcium exchanger (NCX1) (Hoenderop et al., 2005; Lytton, 2007). The components of all three steps of Ca\(^{2+}\) transcellular transport are dependent on vitamin D (Bar, 2008).

Studies in rodents have shown that TRPV6, CaBP-D9k, PMCA1b, and NCX1 exist in the small intestine of mammals and that the injection of 1,25-(OH)\(_2\)-D\(_3\) increased the mRNA expression levels of four Ca transporter genes and promoted Ca absorption in the small intestine of mice (Okano et al., 2004; Benn et al., 2008; Khuituan et al., 2012; Chow et al., 2013; Wongdee and Charoenphandhu, 2015).

CaBP-D9k is found in the small intestine of mammals, whereas CaBP-D28k is expressed in the poultry intestine. Four Ca transporter genes (i.e., TRPV6, CaBP-D28k, PMCA1b, and NCX1) are found in the small intestine of laying hens (Sugiyama et al., 2007; Yang et al., 2011; Li et al., 2018). The highest expression levels of TRPV6 mRNA were found in the duodenum of laying hens, followed by the jejunum and ileum (Yang et al., 2011). The protein expression levels of CaBP-D28k and PMCA1b were higher in the duodenum than in the jejunum and ileum (Sugiyama et al., 2007; Li et al., 2018). Vitamin D regulates Ca transporter gene expression levels after binding VDR. The addition or injection of 1,25-(OH)\(_2\)-D\(_3\) increased the mRNA expression levels of CaBP-D28k in the small intestine of white Leghorn cockerels (Clemens et al., 1988; Hall and Norman, 1990) and laying hens (Bar et al., 1990).

However, reports on Ca absorption in broilers are lacking. The relationship between dietary 1,25-(OH)\(_2\)-D\(_3\) and Ca transporter gene expression levels in broiler chickens has not been evaluated. Therefore, this study aimed to investigate the effects of dietary 1,25-(OH)\(_2\)-D\(_3\) on growth performance, leg bone mineralization, and Ca transporter gene expression levels in the small intestine of broiler chickens.

**Materials and Methods**

**Birds, Diets, and Management**

All procedures used in this study were approved by the Animal Welfare and Ethics Committee of Henan Agricultural University and Shangqiu Normal University (Permit Number: 2019-1023).

On the day of hatching, 140 female Ross 308 broilers were randomly allotted to two treatments with five replicates (14 birds per replicate). Two levels of 1,25-(OH)\(_2\)-D\(_3\) (0 and 1.25 µg/kg) were added to the basal diet without vitamin D (Table 1). The basal diet contained 1.00% Ca and 0.45% non-phytate phosphorous (NPP) in accordance with the recommendations of the NRC (1994).

Crystaline 1,25-(OH)\(_2\)-D\(_3\) was supplied by Changzhou Book Chemical Co., Ltd. (Changzhou, China). The 1,25-(OH)\(_2\)-D\(_3\) solution was prepared as described by Han et al. (2012). The crystalline 1,25-(OH)\(_2\)-D\(_3\) was weighed, dissolved in ethanol, and diluted using propylene glycol (5% ethanol; 95% propylene glycol). The solution concentration was analyzed using high-performance liquid chromatography (HPLC) at the Shanghai Fuxin Analysis Technology Center (Shanghai, China). The concentration of 1,25-(OH)\(_2\)-D\(_3\) solution was 10.39 µg/mL.

Broiler chickens aged 1–19 days were reared in stainless-steel cages (140 cm×70 cm×35 cm). Birds were provided ad libitum access to mash feed and water during experiments with 23 h of light and 1 h of darkness from days 1 to 3 and with 20 h of light and 4 h of darkness from days 4 to 19. Room temperature was controlled at 33°C from days 1 to 3, 30°C from days 4 to 7, and 27°C from days 8 to 19.

**Sample Collection and Analysis**

At 19 days of age, all chickens were weighed, and the ADFI, ADG, feed conversion ratio (FCR), and mortality were determined. Two birds per replicate (10 birds per treatment)

---

### Table 1. **Ingredients and nutrient composition of the basal diet (as-fed basis)**

| Ingredient (%) | Basal diet |
|----------------|------------|
| Corn           | 58.10      |
| Soybean meal   | 32.07      |
| Soybean oil    | 2.22       |
| Soybean protein powder (65% CP) | 3.50 |
| Limestone      | 1.36       |
| Dicalcium phosphate | 1.94 |
| L-Lysine HCl (98%) | 0.14 |
| DL-Methionine (98%) | 0.14 |
| Trace mineral premix\(^1\) | 0.01 |
| Vitamin premix\(^2\) | 0.02 |
| Choline chloride (50%) | 0.20 |
| Sodium chloride | 0.30 |
| Metabolizable energy (kcal/kg) | 2951 |
| Crude protein (CP) | 21.07 |
| Calcium (Ca) | 1.00 |
| Analyzed Ca | 1.05 |
| Total phosphorus (P) | 0.69 |
| Analyzed P | 0.69 |
| Non-phytate phosphorus (NPP) | 0.45 |
| Lysine | 1.10 |
| Methionine | 0.50 |

\(^1\) 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, and 0.15 mg selenium.

\(^2\) The vitamin premix (without vitamin D) provided the following (per kg of diet): 8,000 IU vitamin A, 20 IU vitamin E, 0.5 mg mendi-
were randomly selected and euthanized through cervical dislocation to collect the tibia, femur, and mucosal samples from the duodenum, jejunum, and ileum.

Randomly selected chickens were euthanized, and the whole small intestine was isolated immediately from the gastrointestinal tract and cut into three pieces: duodenum (pancreatic loop), jejunum (from the distal duodenal loop to the Meckel’s diverticulum), and ileum (from the Meckel’s diverticulum to the ileocecal junction) (de Verdal et al., 2010). These segments were rinsed with 0.9% ice-cold NaCl solution. The mucosa was scraped off 3 cm at the center of individual segments (i.e., duodenum, jejunum, and ileum) using a glass microscope slide, immediately frozen in liquid nitrogen, and kept at −80°C.

Tibia and femur bones were collected and stored at −20°C. The length and leg bone weight were determined after drying for 24 h at 105°C. The ash weight was determined after ashing for 48 h at 600°C. The percentage contents of ash, Ca, and P were measured as percentages of bone weight. Ca and total P (tP) contents in the diets and bones were determined as described by Han et al. (2018).

**Total RNA Extraction, Reverse Transcription, and Quantitative Real-time Polymerase Chain Reaction (PCR)**

Total RNA was isolated from the duodenal, jejunal, and ileal mucosae of chickens using the Trizol reagent (Takara Biotechnology Co. Ltd., Dalian, China) in accordance with the manufacturer’s instructions. RNA concentration was determined using a spectrophotometer. OD260/280 values ranged from 1.8 to 2.0 to assure the purity of the total RNA. All samples were stored at −80°C. Reverse transcription was performed using 1 µg total RNA with the PrimeScript Reverse Transcription Reagent Kit (Takara Biotechnology Co. Ltd., Dalian, China) in accordance with the manufacturer’s instructions. The primers for CaBP-D28k, PMCA1b, NCX1, VDR, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China, Table 2). Quantitative real-time PCR was performed using the SYBR Premix PCR Kit (Takara Biotechnology Co. Ltd., Dalian, China) in accordance with the manufacturer’s instructions.

**Statistical Analysis**

Replicates served as experimental units in the statistical analysis. All data were analyzed by Student’s t-test using the SAS software (SAS Institute, 2002). Statistical significance was set at P<0.05.

**Results**

**Bone Mineralization**

Dietary vitamin D deficiency resulted in bone deformity and lower length of the tibia and femur in 19-day-old broilers fed the basal diet without vitamin D (Tables 4 and 5). The addition of 1.25 µg/kg 1,25-(OH)2-D3 increased the ADFI and ADG by 16.6% and 60.9%, respectively. 1,25-(OH)2-D3 decreased the FCR and mortality of broilers compared to the basal diet without vitamin D (P<0.05).

**Ca Transporter Gene Expression Levels**

Five Ca absorption-related genes (TRPV6, CaBP-D28k, PMCA1b, NCX1, and VDR) were examined. TRPV6, an apical membrane Ca channel in intestinal cells, was not successfully cloned in this study.
CaBP-D28k is expressed in the cytoplasm of intestinal cells and transports Ca from the apical membrane to the basolateral membrane. The addition of 1,25-(OH)2-D3 increased the mRNA expression levels of CaBP-D28k in the small intestine of broilers at 19 days of age (P<0.05, Tables 6–8). The mRNA expression levels of CaBP-D28k in the duodenum, jejunum, and ileum of birds fed 1.25 µg/kg 1,25-(OH)2-D3 were enhanced to 88.1, 109.1, and 2.7 times, respectively, compared with birds fed the basal diet without vitamin D.

PMCA1b and NCX1 are located in the basolateral membrane of the intestinal cells. Ca2+ extrusion from enterocytes to the blood is performed by PMCA1b and NCX1. The addition of 1,25-(OH)2-D3 increased the mRNA expression levels of PMCA1b in the duodenum of 19-day-old birds by 57% compared with those in birds fed the basal diet (P<0.05) (Tables 6–8). In contrast, 1,25-(OH)2-D3 did not affect the mRNA expression levels of PMCA1b in the jejenum and ileum (P>0.05). The mRNA expression levels of NCX1 in the duodenum and jejunum of broilers fed 1.25 µg/kg 1,25-(OH)2-D3 were higher than those in birds fed the basal diet (P<0.05). The mRNA expression levels of NCX1 in the ileum were not influenced by 1,25-(OH)2-D3 (P>0.05) (Tables 6–8).

### Table 3. Effects of dietary 1,25-(OH)2-D3 levels on the growth performance of broiler chickens from 1 to 19 days of age

| 1,25-(OH)2-D3 (µg/kg) | ADFI (g/chick) | ADG (g/chick) | FCR (g/g) | Mortality (%) |
|-----------------------|----------------|---------------|-----------|---------------|
| 0                     | 34.37a         | 19.10b        | 1.82a     | 10.00a        |
| 1.25                  | 40.08b         | 30.74a        | 1.31b     | 2.86b         |
| SEM                   | 1.39           | 2.15          | 0.10      | 1.67          |
| P value               | 0.029          | <0.001        | 0.001     | 0.020         |

a,b Means in the same column without a common superscript differ (P<0.05). Values are means of five replicates of 14 chickens per replicate (n=5). 1,25-(OH)2-D3, 1,25-dihydroxycholecalciferol; ADFI, average daily feed intake; ADG, average daily gain; FCR, feed conversion ratio; and SEM, standard error of the mean.

### Table 4. Effects of dietary 1,25-(OH)2-D3 levels on the tibia mineralization of broiler chickens at 19 days of age

| 1,25-(OH)2-D3 (µg/kg) | Length (cm) | Weight (g/bone) | Ash (g/bone) | Ash (%) | Ca (g/bone) | P (%) |
|-----------------------|-------------|-----------------|--------------|--------|-------------|-------|
| 0                     | 4.90b       | 0.94b           | 0.33b        | 34.66b | 12.35b      | 5.70b |
| 1.25                  | 5.83a       | 1.47b           | 0.75b        | 50.61a | 18.91a      | 8.82a |
| SEM                   | 0.18        | 0.10            | 0.08         | 2.70   | 1.12        | 0.53  |
| P value               | 0.002       | 0.002           | <0.001       | <0.001 | <0.001      | <0.001|

a,b Means in the same column without a common superscript differ (P<0.05). Values are means of five replicates of two chickens per replicate (n=5). 1,25-(OH)2-D3, 1,25-dihydroxycholecalciferol; Ca, calcium; P, phosphorus; and SEM, standard error of the mean.

### Table 5. Effects of dietary 1,25-(OH)2-D3 levels on the femur mineralization of broiler chickens at 19 days of age

| 1,25-(OH)2-D3 (µg/kg) | Length (cm) | Weight (g/bone) | Ash (g/bone) | Ash (%) | Ca (g/bone) | P (%) |
|-----------------------|-------------|-----------------|--------------|--------|-------------|-------|
| 0                     | 3.72b       | 0.72b           | 0.25b        | 35.60b | 12.90b      | 5.93b |
| 1.25                  | 4.48b       | 1.15b           | 0.58a        | 50.30a | 18.40a      | 8.64a |
| SEM                   | 0.14        | 0.08            | 0.06         | 2.53   | 0.93        | 0.46  |
| P value               | <0.001      | <0.001          | <0.001       | <0.001 | <0.001      | <0.001|

a,b Means in the same column without a common superscript differ (P<0.05). Values are means of five replicates of two chickens per replicate (n=5). 1,25-(OH)2-D3, 1,25-dihydroxycholecalciferol; Ca, calcium; P, phosphorus; and SEM, standard error of the mean.
Growth Performance

Vitamin D is an essential nutrient in poultry diets and its derivatives include VD$_3$, 25-OH-D$_3$, and 1,25-(OH)$_2$-D$_3$. 1,25-(OH)$_2$-D$_3$ is the final product of vitamin D, and its relative bioactivity is higher than that of VD$_3$ and 25-OH-D$_3$ (Soares et al., 1995). Severe vitamin D deficiency results in the slow growth of broilers (Baker et al., 1998; Chen et al., 2017). The growth performance of chicken was improved after adding VD$_3$, 25-OH-D$_3$, or 1,25-(OH)$_2$-D$_3$ to the diets (Aburto et al., 1998; Baker et al., 1998; Fritts and Waldroup, 2003; Chen et al., 2017). Similar results were observed in the present study. Poor performance was observed in birds fed the basal diet without vitamin D. The addition of 1.25 µg/kg 1,25-(OH)$_2$-D$_3$ increased the ADFI and ADG of broilers compared with the basal diet. These data indicate that vitamin D deficiency damaged the growth performance of broilers. Growth of chickens recovered after vitamin D supplementation.

Bone Mineralization

Vitamin D stimulates Ca absorption in the small intestine and promotes Ca retention in the bones of poultry. Vitamin D deficiency causes leg bone deformation and mineralization abnormalities in broilers (Baker et al., 1998; Chen et al., 2017).
PMCA1b and NCX1 are located in the basolateral membrane of the intestinal cells. Ca is extruded from the basolateral membrane into the blood via the action of PMCA1 and NCX1 (Hoenderop et al., 2005). Vitamin D deficiency decreased the mRNA expression levels of PMCA1b and NCX1 in the duodenum of rats (Zhu, 1995). The injection of 1,25-(OH)\(_2\)-D\(_3\) increased the mRNA expression levels of PMCA1b and NCX1 in the duodenum of rats and mice by 69.5\% - 166.0\% (Zhu, 1995; Khuituan et al., 2012; Wongdee and Charoenphandhu, 2015). Similar results have been observed in poultry. Vitamin D deficiency reduced, but 1,25-(OH)\(_2\)-D\(_3\) repletion elevated the mRNA and protein expression levels of NCX1 in Cobb Harding chick duodenum (Centeno et al., 2011). The injection of 1,25-(OH)\(_2\)-D\(_3\) also enhanced the mRNA expression levels of PMCA1b in the duodenum of white leghorn cockerels fed vitamin D-deficient diets (Cai et al., 1993). Similar results were observed in the present study. The addition of 1,25-(OH)\(_2\)-D\(_3\) increased the mRNA expression levels of PMCA1b and NCX1 in the duodenum of broilers. These data indicate that vitamin D stimulates PMCA1b and NCX1 gene expression levels and promotes the extrusion of Ca from the basolateral membrane into the blood. The gene expression levels of PMCA1b and NCX1 were also affected by dietary Ca levels (Centeno et al., 2004; Rousseau et al., 2016), in which low dietary Ca levels increased the mRNA and protein expression levels of PMCA1b and NCX1 in the duodenum of chickens.

The mRNA expression levels of CaBP-D28k in the duodenum and jejunum of broilers increased to 88.1-109.1 times after adding 1,25-(OH)\(_2\)-D\(_3\), whereas the mRNA expression levels of PMCA1b and NCX1 were enhanced to 1.57-2.86 times with the addition of 1,25-(OH)\(_2\)-D\(_3\). Hence, the response of PMCA1b and NCX1 gene expression to vitamin D was not as sensitive as that of CaBP-D28k.

Vitamin D binds to VDR to regulate Ca absorption in the small intestine. VDR is located in the nuclei of intestinal epithelial cells. The mRNA expression levels of VDR are highest in the duodenum of broiler chickens, lower in the jejunum, and lowest in the ileum (Han et al., 2018). Vitamin D deficiency decreased the mRNA and protein expression levels of VDR in the duodenum of rats (Zineb et al., 1998) and chicks (Centeno et al., 2011). The optimal levels of 25-OH-D\(_3\), 1α-hydroxycholecalciferol (1α-OH-D\(_3\)), and 1,25-(OH)\(_2\)-D\(_3\) increased the mRNA expression levels of VDR in the duodenum of rats (Zineb et al., 1998), Cobb Harding chicks (Centeno et al., 2011), and broiler chickens (Han et al., 2018; Yang et al., 2020). In contrast, the addition of 1,25-(OH)\(_2\)-D\(_3\) did not affect the mRNA expression levels of VDR in the small intestine in the present study. The differences between our study and previous research may be due to the dosages of 1,25-(OH)\(_2\)-D\(_3\).

In conclusion, the addition of 1,25-(OH)\(_2\)-D\(_3\) upregulated Ca transporter gene expression levels and stimulated Ca absorption in the small intestine, especially in the proximal intestine (duodenum and jejunum), thereby improving growth performance and bone mineralization in broiler chickens.
Acknowledgments
This work was supported by the National Natural Science Foundation of China (grant numbers 32072753 and U1704107).

Conflict of Interest
The authors declare no conflict of interest.

References
Aburto A, Edwards HM and Britton WM. The influence of vitamin A on the utilization and amelioration of toxicity of cholecalciferol, 25-hydroxycholecalciferol, and 1,25-dihydroxycholecalciferol in young broiler chickens. Poultry Science, 77: 585–593. 1998.

Baker DH, Biehl RR and Emmert JL. Vitamin D₃ requirement of young chicks receiving diets varying in calcium and available phosphorus. British Poultry Science, 39: 413–417. 1998.

Bar A, Striem S, Mayel-Afshar S and Lawson DE. Differential regulation of calbindin-D28K mRNA in the intestine and eggshell gland of the laying hen. Journal of Molecular Endocrinology, 4: 93–99. 1990.

Bar A. Calcium homeostasis and vitamin D metabolism and expression in strongly calcifying laying birds. Comparative Biochemistry and Physiology, 151: 477–490. 2008.

Cai Q, Chandler JS, Wasserman RH, Kumar R and Penniston JT. Vitamin D and adaptation to dietary calcium and phosphate deficiencies increase intestinal plasma membrane calcium pump gene expression. Proceedings of the National Academy of Sciences of the United States of America, 90: 1345–1349. 1993.

Benn BS, Ajabade D, Porta A, Dhawan P, Hediger M, Peng JB, Jiang Y, Oh GT, Jeung EB, Lieben L, Bouillon R, Carmeliet G and Christakos S. Active intestinal calcium transport in the absence of transient receptor potential vanilloid type 6 and calbindin-D9k. Endocrinology, 149: 3196–3205. 2008.

Centeno V, Picotto G, Perez A, Alisio A and Talamoni NTD. Intestinal Na⁺/Ca²⁺ exchanger protein and gene expression are regulated by 1,25(OH)₂D₃ in vitamin D-deficient chicks. Archives of Biochemistry and Biophysics, 509: 191–196. 2011.

Centeno VA, Barboza GEDD, Marchionatti AM, Alisio AE, Dallorso ME, Nasif R and Talmoni NGTD. Dietary calcium deficiency increases Ca²⁺ uptake and Ca²⁺ extrusion mechanisms in chick enterocytes. Comparative Biochemistry and Physiology, 139: 133–141. 2004.

Chen GH, Zhang JL, Wang JG, Zhang N, Qu HX, Wang ZX, Yan YF and Han JC. Requirement of 25-hydroxycholecalciferol for broilers. Chinese Journal of Animal Nutrition, 29: 2335–2347. 2017.

Chow EC, Quach HP, Vieth R and Pang KS. Temporal changes in tissue 1α,25-dihydroxy vitamin D₃, vitamin D receptor target genes, and calcium and PTH levels after 1,25(OH)₂D₃ treatment in mice. American Journal of Physiology-Endocrinology and Metabolism, 304: 977–989. 2013.

Clemens TL, McGlade SA, Garrett KP, Horiiuchi N and Hendy GN. Tissue-specific regulation of avian vitamin D-dependent calcium-binding protein 28-kDa mRNA by 1,25-dihydroxyvitamin D₃. Journal of Biological Chemistry, 263: 13112–13116. 1988.

de Verdal H, Mignon-Grasteau S, Jeulin C, Le Bihan-Duval E, Leconte M, Mallet S, Martin C and Narcy A. Digestive tract measurements and histological adaptation in broiler lines divergently selected for digestive efficiency. Poultry Science, 89: 1955–1961. 2010.

Fleet JC and Schoch RD. Molecular mechanisms for regulation of intestinal calcium absorption by vitamin D and other factors. Critical Reviews in Clinical Laboratory Sciences, 47: 181–195. 2010.

Fritts CA and Waldroup PW. Effect of source and level of vitamin D on live performance and bone development in growing broilers. Journal of Applied Poultry Research, 12: 45–52. 2003.

Hall AK and Norman AW. Regulation of calbindin-D28k gene expression in the chick intestine: effects of serum calcium status and 1,25-dihydroxyvitamin D₃. Journal of Bone and Mineral Research, 5: 331–336. 1990.

Han JC, Zhang JL, Zhang N, Yang X, Qu HX, Guo Y, Shi CX and Yan YF. Age, phosphorus, and 25-hydroxycholecalciferol regulate mRNA expression of vitamin D receptor and sodium-phosphate cotransporter in the small intestine of broiler chicks. Poultry Science, 97: 1199–1208. 2018.

Hoenderop JG, Nilius B and Bindels RJ. Calcium absorption across epithelia. Physiological Reviews, 85: 373–422. 2005.

Khultsan P, Teerapompuntakit J, Wongdee K, Suntornsaratoon P, Konthapakdee N, Sangsaksri J, Sripong C, Krishnamma N and Charoenphandhu N. Fibroblast growth factor-23 abolishes 1,25-dihydroxyvitamin D₃-enhanced intestinal calcium transport in male mice. American Journal of Physiology-Endocrinology and Metabolism, 302: 903–913. 2012.

Li Q, Zhao X, Wang S and Zhou Z. Letrozole induced low estrogen levels affected the expressions of duodenal and renal calcium-processing gene in laying hens. General and Comparative Endocrinology, 255: 49–55. 2018.

Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2–ΔΔCt method. Methods, 25: 402–408. 2001.

Lytton J. Na⁺/Ca²⁺ exchangers: three mammalian gene families control Ca²⁺ transport. Biochemical Journal, 406: 355–382. 2007.

Ministry of Agriculture, China. Feeding Standard of Chicken, 1st ed.; Standards Press of China: Beijing, China, 2004.

NRC (National Research Council). Nutrient Requirements of Poultry. 9th rev. ed. Washington, DC: National Academies Press. 1994.

Okano T, Tsugawa N, Morishita A and Kato S. Regulation of gene expression of epithelial calcium channels in intestine and kidney of mice by 1α,25-dihydroxyvitamin D₃. Journal of Steroid Biochemistry and Molecular Biology, 89–90: 335–338. 2004.

Proszkowiec-Weglarz M, Schreier LL, Miska KB, Angel R, Kahl S and Russell B. Effect of early neonatal development and delayed feeding post-hatch on jejunal and ileal calcium and phosphorus transporter genes expression in broiler chickens. Poultry Science, 98: 1861–1871. 2019.

Rousseau X, Valable AS, Letourneau-Montminy MP, Meme N, Godet E, Magnin M, Nys Y, Duclos MJ and Narcy A. Adaptive response of broilers to dietary phosphorus and calcium restrictions. Poultry Science, 95: 2849–2860. 2016.

SAS Institute. SAS User’s Guide. Version 9 ed. SAS Inst. Inc., Cary, NC, USA. 2002.

Sechman A, Shimada K, Saito N, Ieda T and Ono T. Triiodothyronine (T₃) enhances the stimulatory effect of 1,25-dihydroxyvitamin D₃ on calbindin-D28k mRNA expression in the kidney and intestine but not in cerebellum of the chick. Asian-Australasian Journal of Animal Sciences, 9: 37–44. 1996.

Soares JH, Kerr JM and Gray RW. 25-hydroxycholecalciferol in poultry nutrition. Poultry Science, 74: 1919–1934. 1995.

Sugiya T, Kikuchi H, Hayama S, Nishizawa K and Kusuhara S. Expression and localisation of calbindin D28k in all intestinal segments of the laying hen. British Poultry Science, 48: 233–238. 2007.
Wongdee K and Charoenphandhu N. Vitamin D-enhanced duodenal calcium transport. Vitamins and Hormones, 98: 407-440. 2015.
Yang JH, Hou JF, Farquharson C, Zhou ZL, Deng YF, Wang L and Yu Y. Localisation and expression of TRPV6 in all intestinal segments and kidney of laying hens. British Poultry Science, 52: 507-516. 2011.
Yang X, Wang XN, Zhang N, Qu HX, Shi CX, Zhang JL, Yan YF, Zheng YX, Wang ZX and Han JC. Differentially expressed genes in vitamin D regulating calcium and phosphorus absorption in duodenum of broiler chickens using RNA-sequencing. Chinese Journal of Animal Nutrition, 31: 5202-5213. 2019.
Yang X, Zhang N, Wang XN, Qu HX, Zhang JL, Yan YF, Cheng YH and Han JH. Optimal dietary levels of 1α-hydroxycholecalciferol in broiler chickens from 1 to 42 days of age. Journal of Poultry Science, 57: 124-130. 2020.
Zhu YT. Effects of vitamin D, dietary calcium and vitamin D restriction, pregnancy and lactation on gene expression of calcium transporting factors. PhD Diss. Iowa State University, Ames. 1995.
Zineb R, Zhor B, Odile W and Marthe RR. Distinct, tissue-specific regulation of vitamin D receptor in the intestine, kidney, and skin by dietary calcium and vitamin D. Endocrinology, 139: 1844-1852. 1998.