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25-hydroxycholecalciferol affects growth performance, bone calcium content and intestinal calcium transporter gene expression in broiler chickens

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ABSTRACT. This study evaluated the effect of 25-hydroxycholecalciferol (25-hydroxyvitamin D_3, 25-OH-D_3) on growth rate, femur calcium (Ca) content, and mRNA abundance of intestinal Ca transporters in broiler chickens aged 1–21 days. A total of 420 one-day-old female Arbor Acres broilers were assigned to six treatments. Diet 1 did not contain 25-OH-D_3 (0 μg/kg 25-OH-D_3); diets 2–6 were based on diet 1, but additionally supplemented with 3.125, 6.25, 12.5, 25, and 50 μg/kg 25-OH-D_3. The results showed that the addition of 3.125–50 μg/kg 25-OH-D_3 increased body weight gain, feed intake, bone mass, and ash, Ca, and phosphorus content in the femur of 1–21-day-old broilers compared to diet 1 (0 μg/kg 25-OH-D_3). Supplementing 6.25–50 μg/kg 25-OH-D_3 increased transcript levels of Ca-binding protein D28k (*CaBP-D28k*) in the duodenum, jejunum, and ileum compared to diet 1. The level of *CaBP-D28k* mRNA expression in the three intestinal segments increased by 1171-, 2091-, and 46-fold, respectively, after the addition of 50 μg/kg 25-OH-D_3. The levels of plasma membrane Ca ATPase 1b (*PMCA1b*) mRNA in the jejunum and ileum increased after supplementing 12.5–50 μg/kg 25-OH-D_3. The abundance of sodium-Ca exchanger 1 (*NCX1*) mRNA increased in the duodenum after the addition of 12.5 and 50 μg/kg 25-OH-D_3. No differences were observed in mRNA levels of *CaBP-D28k*, *PMCA1b*, and *NCX1* in the jejunum and ileum in broilers fed 12.5–50 μg/kg 25-OH-D_3. These data demonstrated that 25-OH-D_3 improved the growth rate and promoted transcription of the intestinal Ca transporter gene in broiler chickens aged 1–21 days. The requirement of broilers for 25-OH-D_3 was at least 12.5 μg/kg based on gene expression levels of intestinal Ca transporters genes.

Introduction

25-hydroxycholecalciferol (25-hydroxyvitamin D_3, 25-OH-D_3) is a derivative of cholecalciferol (vitamin D_3). The bioactivity of 25-OH-D_3 in broiler diets is two times higher compared to vitamin D_3 (Soares et al., 1995; Han et al., 2016). In recent years, the feed additive 25-OH-D_3 has been produced and authorized for use in poultry in China.

Maternal supplementation of 25-OH-D_3 during lactation improves growth rate, leg bone quality, and bone calcium (Ca) content in suckling piglets, which correlates with Ca absorption in the piglet intestine (Zhang et al., 2019). In poultry, Ca is the most commonly added mineral element in feed. The effect of 25-OH-D_3 on intestinal Ca absorption needs to be clarified. There is a number of active Ca transporters in the poultry intestine, including transient receptor potential channel (TRPV6), Ca-binding protein D28k (CaBP-D28k), plasma membrane Ca ATPase 1b (PMCA1b), and sodium-Ca exchanger 1 (NCX1) (Proszkowiec-Weglarz and Angel, 2013). These transporters are involved in Ca absorption in intestinal epithelial cells. TRPV6 is present in the brush border membranes of the intestinal cells in laying hens (Yang et al., 2011). *TRPV6* has not been cloned in the broiler intestine (Rousseau et al., 2016). CaBP-D28k is located in the cytoplasm of poultry intestinal cells and is responsible for transepithelial Ca transport. NCX1 and PMCA1b have been detected in the basolateral membrane, and are involved in Ca transport from intestinal cells to the blood. Gene expression levels of *CaBP-D28k*, *NCX1*, and *PMCA1b* in the broiler gut have been previously analysed and reported (Centeno et al., 2004; Rousseau et al., 2016; Han et al., 2022). However, the effect of 25-OH-D_3 on intestinal Ca transporter gene expression has not been studied. Therefore, the present study investigated the effect of 25-OH-D_3 dose on the growth rate and gene expression levels of intestinal Ca transporters in broilers.

Material and methods

Animals and diets

The animal experiment was conducted in accordance with the guidelines of the Animal Ethics Committee of Henan Agricultural University and Shangqiu Normal University (2020-0915).

A total of 420 one-day-old female Arbor Acres broilers were assigned to six groups, with five replicates. Each replicate contained 14 broilers. The experiment lasted from day 1 to day 21 of the chickens' life. All chickens were weighed on days 1 and 21. The difference in body weight (BW) on days 21 and 1 was defined as body weight gain (BWG). Feed intake (FI) was determined daily. The FI to BWG ratio in broilers aged 1–21 days was defined as the feed conversion ratio (FCR). Dead broilers were weighed to correct FI and FCR.

Table 1. Ingredient and nutrient composition of diet 1 (as-fed basis)

Item	Amount
Ingredient, g/kg	
maize	574.0
soybean meal (43% CP)	320.0
soybean oil	24.7
soybean protein powder (63% CP)	41.2
limestone	12.2
dicalcium phosphate	19.4
L-lysine·HCl (98%)	1.8
DL-methionine (99%)	1.4
mineral additive[1]	0.1
vitamin additive[2]	0.2
choline chloride (50%)	2.0
salt	3.0
Nutrient composition, g/kg	
metabolizable energy, kcal/kg	2973.0
crude protein	212.3
analysed calcium	9.8
analysed total phosphorus	7.0
non-phytate phosphorus	4.5
lysine	11.0
methionine	5.1

[1] provided per kg of diet: mg: iron 80, manganese 60, zinc 40, copper 8, iodine 0.35, selenium 0.15; [2] provided per kg of diet: mg: niacin 35, DL-α-tocopheryl acetate 20, pantothenic acid 10, riboflavin 8, retinyl palmitate 4.4, pyridoxine 3.5, thiamine 2, folic acid 0.55, menadione 0.5, biotin 0.18, cobalamin 0.01

Diet 1 did not contain 25-OH-D_3 (0 μg/kg 25-OH-D_3) (Table 1), and diets 2–6 were based on diet 1 and additionally supplemented with 3.125, 6.25, 12.5, 25, and 50 μg/kg 25-OH-D_3. The powder feed additive 25-OH-D_3 was supplied by Shandong Haineng Bioengineering Co., Ltd (Rizhao, SD, China). The content of 25-OH-D_3 in the feed additive was 12.5 mg/g, as determined by high-performance liquid chromatography.

Broilers were raised in pens (140 cm × 70 cm × 35 cm) and supplied with mash feed. The photoperiod consisted of 23 h of light from 1 to 3 days and 18 h of light from 4 to 21 days. Room temperature was maintained at 32 °C during days 1–3, 30 °C during days 4–7, and 28 °C during days 8–21.

Bone sample collection and analysis

On day 21, 10 broilers were randomly selected from five replicates of each treatment. The broilers were euthanized by cervical dislocation. Femurs were collected and stored at −20 °C until analysis. Bone mass, and ash, Ca, and phosphorus (P) contents in the femur were analysed. Bone mass was determined after drying the femurs at 105 °C

for 36 h. Bone ash was weighed after burning the dried femurs in a muffle furnace at 600 °C for 48 h. The ash to bone weight ratio indicated the percentage of ash in the bone. Ca content in the bone and feed was measured according to the method of AOAC (2005). Phosphorus content was analysed using the method of Rutherfurd et al. (2004). Ca and P percentages referred to the ratios of Ca and P weight to bone mass.

Collection of intestinal mucosa samples

The small intestine was separated and divided into three segments (duodenum, jejunum, and ileum) on day 21 after the broilers were euthanized. The segment from the pylorus to the distal pancreas loop was considered the duodenum. The segment from the distal duodenum to Meckel's diverticulum was considered the jejunum. The portion from the distal jejunum to the ileocecal junction was considered the ileum. The intestinal segments were cut longitudinally with scissors and washed with ice-cold saline. Intestinal mucosa was scraped with a slide. The mucosa sample was collected into a 1.5 ml centrifuge tube, frozen in liquid nitrogen, and stored in a freezer at −80 °C.

Real-time polymerase chain reaction (PCR)

Total RNA was extracted from the intestinal mucosa of broilers using TRIzol reagent (Takara, Dalian, LN, China). Total RNA concentration was analysed by spectrophotometry. The purity of total RNA was evaluated by OD260/280 measurements and values in the range of 1.8–2.0 indicated pure RNA.

Reverse transcription was conducted using the reverse transcription reagent kit (Takara, Dalian, LN, China) and primers for glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), *CaBP-D28k*, *NCX1*, and *PMCA1b* were synthesized by Sangon Biotech Co., Ltd (Shanghai, China; Table 2).

Real-time PCR amplifications of *CaBP-D28k*, *NCX1*, and *PMCA1b* genes were conducted using the Roche Lightcycler 480 Real-time PCR system (Roche Ltd., Basel, Switzerland) and the SYBR premix PCR kit (Takara, Dalian, LN, China). PCR products for *GAPDH* were used as an internal standard. For PCR, a 10 µl reaction mixture containing cDNA (0.5 µl), forward primer (0.5 µl), reverse primer (0.5 µl), TB Green (5 µl) and RNase-free water (3.5 µl) was used. The PCR programme was as follows: denaturation at 95 °C for 5 min, followed by 35 cycles (95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s), and final extension at 72 °C for 10 min. Each reaction was repeated three times. Gene expression levels were determined using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

Statistical analysis

Analysis of variance (ANOVA) and polynomial comparison implemented in SAS 9.0 software (SAS Institute, 2002) were used to assess the effect of 25-OH-D_3 dose on growth rate, bone development, and gene expression levels of intestinal Ca transporters. Tukey's test was used to analyse the differences between the six groups ($P < 0.05$).

Results

Growth performance

The initial BW of each broiler was 41.8–42.6 g. The addition of 3.125–50 µg/kg 25-OH-D_3 increased FI and BWG and reduced FCR in broilers aged 1–21 days compared to diet 1 (0 µg/kg 25-OH-D_3) ($P < 0.05$; Table 3). Increasing the 25-OH-D_3 dose from 3.125 to 50 µg/kg did not improve FI, BWG or FCR in broilers ($P > 0.05$). The addition of 25-OH-D_3 did not affect chicken mortality ($P > 0.05$).

Bone mineralization

The addition of 3.125–50 µg/kg 25-OH-D_3 increased bone mass, ash, and mineral (Ca and P) weight, as well as the percentage of minerals in

Table 2. Primer sequences used for real-time PCR

Gene	Accession no.	(Forward/Reverse) Sequence 5'→3'	Size, bp
Calcium-binding protein D28k (*CaBP-D28k*)	NM_205513.1	F: AGATCTGGCACCACTACGAC R: TGAGCAAGCTCAACGATTCCT	187
Plasma membrane calcium ATPase 1b (*PMCA1b*)	NM_001168002.3	F: AGCTCAAGATGGTGCAGCTA R: AACAAACCTGCTTTGCCAATCT	165
Sodium-calcium exchanger 1 (*NCX1*)	NM_001079473.1	F: TCACCTTCTTCTTCTTCCCAATCT R: GCAACCTTTCCGTCCATCTC	158
Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*)	NM_204305.1	F: GAACATCATCCCAGCGTCCA R: ACGGCAGGTCAGGTCAACAA	133

Table 3. Effect of dietary 25-hydroxycholecalciferol (25-OH-D_3) levels on growth performance of broiler chickens at 1–21 days of age[1]

25-OH-D_3, µg/kg	BWG, g	FI, g	FCR, g/g	Mortality, %
0	391[b]	645[b]	1.65[a]	1.43
3.125	701[a]	950[a]	1.36[b]	1.43
6.25	747[a]	991[a]	1.33[b]	0
12.5	748[a]	968[a]	1.29[b]	0
25	751[a]	1000[a]	1.33[b]	2.86
50	741[a]	989[a]	1.33[b]	2.86
SEM	24.7	25.2	0.024	0.631
P-value				
linear	<0.05	<0.05	<0.05	0.40
quadratic	<0.05	<0.05	<0.05	0.25

BWG – body weight gain, FI – feed intake, FCR – feed conversion ratio, SEM – standard error of the mean; [1] values are means of five replicates (14 chickens per replicate, n = 5); [ab] – means within a column with different superscripts are significantly different at $P < 0.05$

the femur compared to diet 1 (0 µg/kg 25-OH-D_3) ($P < 0.05$; Table 4). Increasing 25-OH-D_3 doses from 3.125 to 6.25 µg/kg increased the weight of bone and minerals ($P < 0.05$), but they did not affect the percentage of minerals ($P > 0.05$). No differences in femur mineralization (except for ash weight) were observed in broilers supplied with 6.25–50 µg/kg 25-OH-D_3 ($P > 0.05$).

(0 µg/kg 25-OH-D_3) ($P < 0.05$). Notably, the abundance of *CaBP-D28k* mRNA in the duodenum, jejunum, and ileum increased 1171-, 2091-, and 46-fold, respectively, after the addition of 50 µg/kg 25-OH-D_3. The level of *CaBP-D28k* mRNA in the duodenum was elevated when 25-OH-D_3 doses were increased from 6.25 to 50 µg/kg ($P < 0.05$). In contrast, increasing the dose of 25-OH-D_3 from 12.5 to 50 µg/kg had no effect on the abundance of *CaBP-D28k* mRNA in the jejunum and ileum ($P > 0.05$).

The dose of 25-OH-D_3 affected mRNA levels of intestinal *PMCA1b* (Figures 1B, 2B, and 3B). Supplementing 50 µg/kg 25-OH-D_3 enhanced the expression of *PMCA1b* mRNA in the duodenum by 36% compared to diet 1 (0 µg/kg 25-OH-D_3) ($P < 0.05$). The addition of 12.5–50 µg/kg 25-OH-D_3 increased the abundance of *PMCA1b* mRNA in the jejunum by 146–180%, and in the ileum by 34–40% ($P < 0.05$). No differences in *PMCA1b* mRNA levels in the jejunum and ileum were observed in broilers fed 12.5–50 µg/kg 25-OH-D_3.

Dietary 25-OH-D_3 also affected mRNA levels of the intestinal *NCX1* gene (Figures 1C, 2C, and 3C). Supplementation with 12.5 and 50 µg/kg 25-OH-D_3 increased mRNA levels of *NCX1* in the duodenum by 290% and 160%, respectively,

Table 4. Effect of dietary 25-hydroxycholecalciferol (25-OH-D_3) levels on femur development in broiler chickens at 1–21 days of age[1]

25-OH-D_3, µg/kg	Bone weight, g	Mineral weight, g			Mineral percentage, %		
		Ash	Ca	P	Ash	Ca	P
0	0.72[c]	0.254[d]	0.093[c]	0.049[c]	35.7[b]	13.1[b]	6.91[c]
3.125	1.07[b]	0.467[c]	0.167[b]	0.088[b]	43.7[a]	15.6[a]	8.16[b]
6.25	1.29[a]	0.576[ab]	0.213[a]	0.112[a]	44.7[a]	16.5[a]	8.64[ab]
12.5	1.34[a]	0.596[a]	0.226[a]	0.119[a]	44.7[a]	16.9[a]	8.91[a]
25	1.26[a]	0.552[ab]	0.209[a]	0.112[a]	43.9[a]	16.5[a]	8.88[a]
50	1.25[a]	0.525[bc]	0.213[a]	0.114[a]	42.2[a]	17.0[a]	9.12[a]
SEM	0.041	0.0221	0.0089	0.0047	0.65	0.29	0.148
P-value							
linear	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
quadratic	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05

SEM – standard error of the mean; [1] values are means of five replicates (two chickens per replicate, n = 5); [a–d] – means within a column with different superscripts are significantly different at $P < 0.05$

Expression of calcium transporter genes

Dietary 25-OH-D_3 upregulated mRNA levels of the *CaBP-D28k* gene in the duodenum, jejunum, and ileum of broilers (Figures 1A, 2A, and 3A). The addition of 6.25–50 µg/kg 25-OH-D_3 enhanced mRNA expression of the three intestinal *CaBP-D28k* genes compared to diet 1 compared to diet 1 (0 µg/kg 25-OH-D_3) ($P < 0.05$). The addition of 25 µg/kg 25-OH-D_3 enhanced *NCX1* mRNA expression in the jejunum by 92% ($P < 0.05$). Broilers provided with 50 µg/kg 25-OH-D_3 had higher mRNA levels of the *NCX1* gene in the ileum than those supplied with 3.125 µg/kg 25-OH-D_3 ($P < 0.05$).

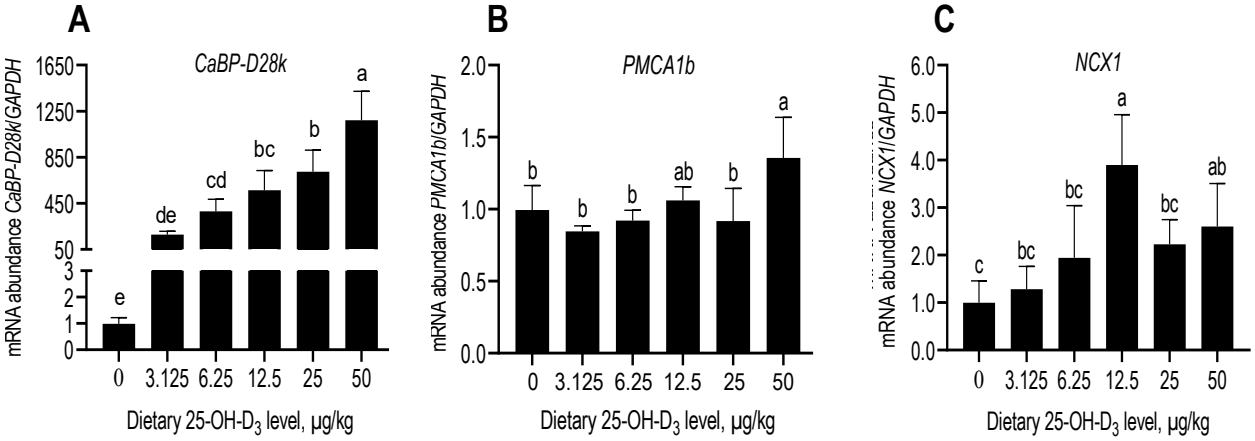

Figure 1. Effect of dietary 25-hydroxycholecalciferol (25-OH-D_3) levels on mRNA abundance of duodenal calcium transporters in broiler chickens at 1–21 days of age. (A) *CaBP-D28k* – calcium-binding protein D28k (linear $P < 0.05$, quadratic $P = 0.07$); (B) *PMCA1b* – plasma membrane calcium ATPase 1b (linear $P < 0.05$, quadratic $P < 0.05$); (C) *NCX1* – sodium-calcium exchanger 1 (linear $P < 0.05$, quadratic $P < 0.05$); *GAPDH* – glyceraldehyde-3-phosphate dehydrogenase. Values are means of five replicates (two chickens per replicate, n = 5) ± SD (standard deviation). $^{a–e}$ – data with different superscripts indicate significant differences ($P < 0.05$)

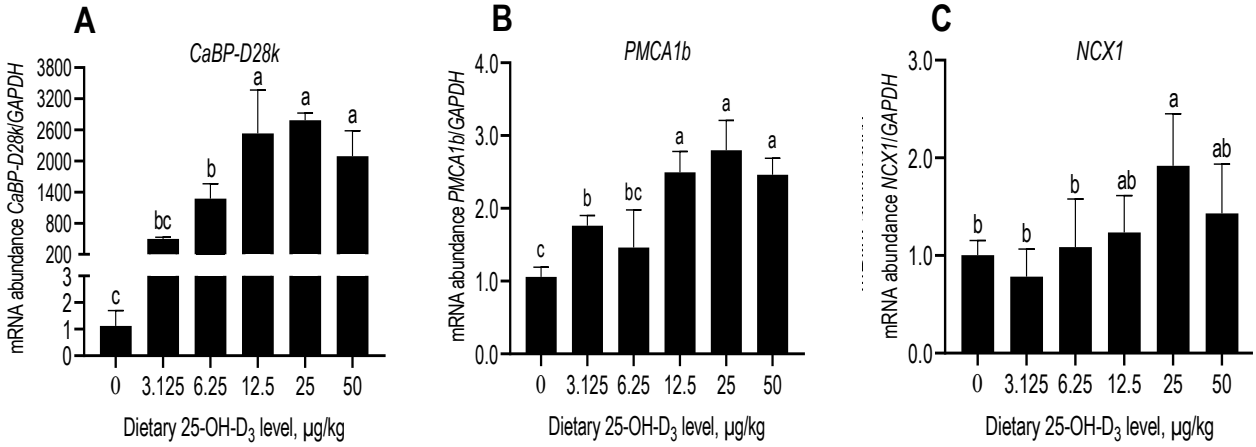

Figure 2. Effect of dietary 25-hydroxycholecalciferol (25-OH-D_3) levels on mRNA abundance of jejunal calcium transporters in broiler chickens at 1–21 days of age. (A) *CaBP-D28k* – calcium-binding protein D28k (linear $P < 0.05$, quadratic $P < 0.05$); (B) *PMCA1b* – plasma membrane calcium ATPase 1b (linear $P < 0.05$, quadratic $P < 0.05$); (C) *NCX1* – sodium-calcium exchanger 1 (linear $P < 0.05$, quadratic $P = 0.92$); *GAPDH* – glyceraldehyde-3-phosphate dehydrogenase. Values are means of five replicates (two chickens per replicate, n = 5) ± SD (standard deviation). $^{a–c}$ – data with different superscripts indicate significant differences ($P < 0.05$)

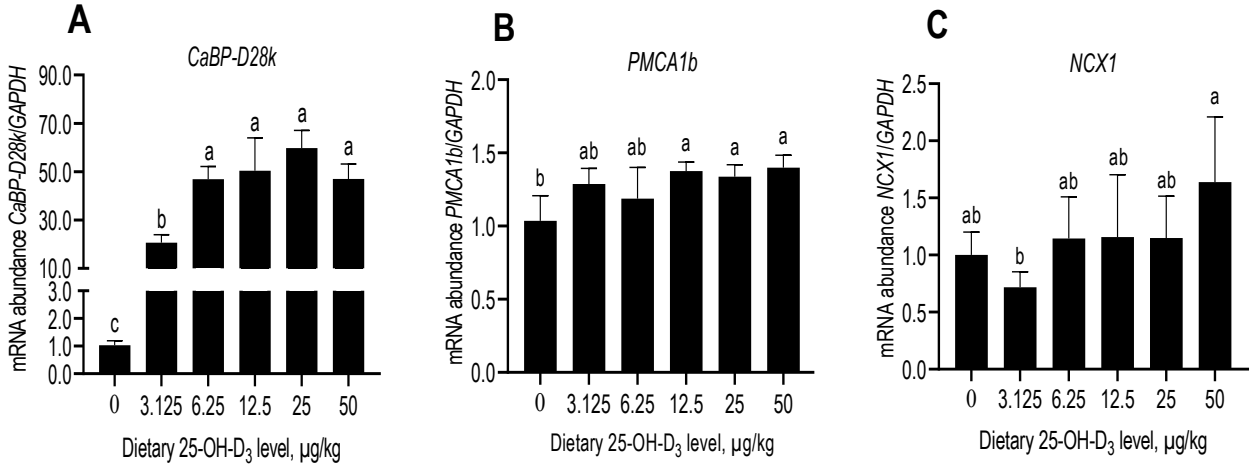

Figure 3. Effect of dietary 25-hydroxycholecalciferol (25-OH-D_3) levels on mRNA abundance of ileal calcium transporters in broiler chickens at 1–21 days of age. (A) *CaBP-D28k* – calcium-binding protein D28k (linear $P < 0.05$, quadratic $P < 0.05$); (B) *PMCA1b* – plasma membrane calcium ATPase 1b (linear $P < 0.05$, quadratic $P = 0.20$); (C) *NCX1* – sodium-calcium exchanger 1 (linear $P < 0.05$, quadratic $P = 0.18$); *GAPDH* – glyceraldehyde-3-phosphate dehydrogenase. Values are means of five replicates (two chickens per replicate, n = 5) ± SD (standard deviation). $^{a–c}$ – data with different superscripts indicate significant differences ($P < 0.05$)

Discussion

Broiler FI in this study was higher in the group supplemented with 3.125–50 μg/kg 25-OH-D_3 compared to the control group without this supplement. With increasing FI, more nutrients were digested, absorbed and deposited in the broilers' bodies, thereby increasing BWG in the 25-OH-D_3 group. These results were consistent with the studies of Bar et al. (2003) and Chen et al. (2017), who reported BWG and FI improvements after increaing 25-OH-D_3 doses from 1.25 to 5 μg/kg. Thus, dietary 25-OH-D_3 increased the growth rate of broiler chickens. In contrast, higher 25-OH-D_3 doses (from 3.125 to 50 μg/kg) did not further improve the growth rate of broilers in the present study. A similar result was reported in a study on vitamin D_3, where increasing dietary vitamin D_3 levels (from 2 to 195 μg/kg) did not affect the growth rate of broilers (Cho et al., 2020). BWG, FI, and FCR of 10–21-day-old broilers were not affected by the 2.5–80-μg/kg dose of vitamin D_3 (Leyva-Jimenez et al., 2019). The recommended vitamin D_3 supplementation for 1–21-day-old broilers are 5 (NRC, 1994) and 25 μg/kg (Feeding Standard of Chicken in China, 2004). The bioactivity of 25-OH-D_3 is two times higher than vitamin D_3 in broiler diets (Han et al., 2016). Thus, broilers need less 25-OH-D_3 than vitamin D_3 for growth. The appropriate amounts of 25-OH-D_3 were in the range of 12.5–25 μg/kg in 1–21-day-old broiler diets (Fritts and Waldroup, 2003). When 25-OH-D_3 supplementation meets broiler requirements, an additional dose of 25-OH-D_3 had no positive effect on growth performance.

The addition of 3.125–50 μg/kg 25-OH-D_3 in the present study increased FI of broilers compared to the diet without 25-OH-D_3 supplementation. Ingested amounts of Ca, P, and other minerals were elevated after 25-OH-D_3 addition, and thus, bone mass and ash content also increased. Similar results were obtained by Chen et al. (2017) and Zhang et al. (2020), who reported that bone weight and ash content in the tibia increased after the addition of 3.125–15 μg/kg 25-OH-D_3. Thus, supplementing 25-OH-D_3 improved leg bone quality of broiler chickens compared to animals fed 25-OH-D_3-deficient diets. Notably, an increased supplementation of 25-OH-D_3 (12.5–50 μg/kg) did not affect femur development in the present research. Similarly, Fritts and Waldroup (2003) have reported that increasing 25-OH-D_3 concentration from 12.5 to 100 μg/kg did not increase the percentage of ash in the tibia of broilers. The minimum concentration of 25-OH-D_3 to properly support skeletal mineralization of broilers was approximately 12.5 μg/kg (Chen et al., 2017). Increased 25-OH-D_3 dosing over the required level did not improve bone development.

Maternal 25-OH-D_3 was shown to increase leg bone Ca content by improving intestinal Ca absorption in suckling piglets (Zhang et al., 2019). A positive response to 25-OH-D_3 in the form of Ca absorption in the isolated duodenal loops of chickens was observed by Phadnis and Nemere (2003). In contrast, the relationship between the expression of Ca transporter genes and dietary 25-OH-D_3 dosage in broilers has not been investigated. Thus, the present study was conducted to characterize these processes.

CaBP-D9k is expressed in the cytoplasm of mammalian intestinal cells, while CaBP-D28k is present in poultry intestinal epithelial cells. Maternal supplementation of 25-OH-D_3 during lactation increased the abundance of intestinal *CaBP-D9k* mRNA in suckling piglets (Zhang et al., 2019). Similarly, the addition of 12.5–50 μg/kg 25-OH-D_3 in the present study enhanced the expression of *CaBP-D28k* mRNA in the duodenum, jejunum, and ileum 562–1171-, 2091–2787-, and 46–59-fold, respectively, compared to diet 1 (0 μg/kg 25-OH-D_3). 25-OH-D_3 promoted *CaBP-D28k* gene transcription, especially in the duodenum and jejunum, and stimulated Ca transport. The Ca content in the femur increased with the improvement of intestinal Ca absorption due to 25-OH-D_3 supplementation. There were no differences in *CaBP-D28k* mRNA abundance in the jejunum and ileum in broilers fed 12.5–50 μg/kg 25-OH-D_3. Thus, the optimal dose of 25-OH-D_3 for broilers aged 1–21 days would be at least 12.5 μg/kg based on intestinal *CaBP-D28k* gene expression.

PMCA1b is the main transporter for Ca extrusion in the basolateral membrane of enterocytes (Wongdee and Charoenphandhu, 2015). The magnitude of Ca transport mediated by PMCA1b is approximately five times higher than that mediated by NCX1 in the rat intestine (Ghijsen et al., 1983). The addition of 50 μg/kg 25-OH-D_3 in the present research increased *PMCA1b* mRNA abundance in the duodenum, jejunum, and ileum by 36%, 146%, and 40% compared to diet 1 (0 μg/kg 25-OH-D_3), respectively. Ca transport from intestinal cells to the blood was positively correlated with the expression levels of the intestinal *PMCA1b* gene in rats (Armbrecht et al., 1999). Hence, 25-OH-D_3 promoted intestinal *PMCA1b* gene expression, stimulated

Ca absorption, and contributed to Ca retention in the femurs of broilers.

NCX1 is involved in Ca extrusion from intestinal cells into the blood. The level of duodenal *NCX1* mRNA increased by 290% and 160% in the current study after adding 12.5 and 50 μg/kg 25-OH-D_3 to diet 1 (0 μg/kg 25-OH-D_3), respec-tively. The level of jejunal *NCX1* mRNA increased by 92% after supplementing 25 μg/kg 25-OH-D_3. The final product of 25-OH-D_3 in chickens is 1,25-dihydroxycholecalciferol (1,25-$(OH)_2$-D_3) (Rasmussen et al., 1972), and both compounds have similar biological function. Studies in rodents demonstrated that 1,25-$(OH)_2$-D_3 increased Ca transport mediated by NCX1 by stimulating transcription of the *NCX1* gene in the duodenum of mice (Khuituan et al., 2012; 2013; Wongdee and Charoenphandhu, 2015). Thus, 25-OH-D_3 promoted the transcription of the intestinal *NCX1* gene, and thus Ca absorption, and increased femur Ca content after conversion to 1,25-$(OH)_2$-D_3 in the kidneys of broiler chickens.

Conclusions

Supplementation of 25-OH-D_3 improved growth performance and promoted transcription of the intestinal Ca transporter gene in broiler chickens fed a 25-OH-D_3-deficient diet. The 25-OH-D_3 requirement of broilers aged 1–21 days was at least 12.5 μg/kg based on the expression of the intestinal Ca transporter gene.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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