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Original Article

The Effect of Alpha-Hydroxycholecalciferol and Phytase on the Performance, Carcass Characteristics, Blood Factors, and Expression of Vdr and Cabp-D28k Genes in Broiler Chickens

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■Keywords

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ABSTRACT

We evaluated the effect of alpha-hydroxycholecalciferol (1α-OH-D3) and phytase supplementation on the performance, carcass characteristics, blood factors, and the expression of vitamin D receptors (VDR) and Calbindin 28k (CaBP-D28k) genes in broiler chickens. In the current experiment, 400 one-day-old male chicks of the Cobb 500 strain were allocated into a completely randomized design with 4 treatments and 5 replicates (20 chicks per replicate). Experimental treatments were: 1- Control (no additional supplements), 2- Addition of 14 mg/kg DM 1α-OH-D3 to the diet, 3- Addition of 5 mg/kg phytase, and 4- Addition of 14 mg/kg 1α- OH-D3 + 5 mg/k phytase. Compared to the control diet, adding 1α-OH-D3 and phytase could significantly improve the feed conversion ratio and weight gain ($p \leq 0.05$). The combination also decreased alkaline phosphatase (ALP) activity relative to the control ($p \leq 0.05$) The relative expression of VDR and CaBP-D28k genes in the treatments that used 1α-OH-D3 and 1α-OH-D3 along with the phytase enzyme had a significant elevation compared to the control group ($p<0.05$). Overall, using 1α-OH-D3 along with the phytase enzyme can lead to a higher growth performance than using each one of them separately. The use of 1α-OH-D3 together with phytase led to a greater improvement of the growth performance and expression of the VDR and CaBP-D28k genes when compared to their separate use in broiler chickens' diets.

INTRODUCTION

Genetic improvement in broiler chickens' potential growth rate has significantly increased the importance of nutrient supply for such animals (Poorghasemi *et al*., 2017). This is particularly true for vitamins and minerals that are essential for most of the metabolic reactions that are crucial for driving growth. One key area where the undersupply of nutrients is important in broilers is the development of skeletal abnormalities. Between 20 and 30 percent of broiler flocks in the world suffer from these skeletal abnormalities, which have significant welfare and productivity consequences (Xue *et al*., 2016; Ahmadi *et al*., 2018; Meena *et al*., 2022). A key reason for the high incidence of skeletal abnormalities in the first weeks after birth is thought to be a lack of pancreas development, which leads to limited secretion of lipase in the first few days after hatching, and therefore reduced absorption of vitamin D3 during that period (Whitehead *et al*., 2004). Another major issue driving the high incidence of skeletal abnormalities is the high intake of phytates, the main form of phosphorus storage in grains. Poultry lacks the necessary microflora to hydrolyze phytate phosphorus, and so only utilize phytate phosphorus to a limited extent. Moreover, phytate also limits the availability of other key nutrients for

bone development, particularly calcium, zinc, copper, and essential amino acids (Rousseau *et al*., 2016).

Both of these issues can be addressed by supplementing diets. For vitamin D, simply increasing the supply in the diet can increase uptake. However, as broiler growth rates have increased, it has become increasingly clear that simply supplementing vitamin D is no longer sufficient to prevent vitamin D deficiency. Conversely, adding activated metabolites (i.e. hydroxylated) of vitamin D3 to broiler chickens' diets can partially compensate the lack of this vitamin (Shojadoost *et al*., 2021), with research showing that 1α-OHD3 is more effective than other sources of vitamin D at increasing calcium absorption and maintaining bone health. Furthermore, 1α-OH-D3 can be effective at improving the absorption of phytate phosphorus. This effect can be further improved by the addition of phytase to the diet: since it hydrolyzes phytate, phytase increases the availability of dietary phosphorus and reduces the impact of phytate on the absorption of other nutrients. However, the use of phytase is not a panacea, as a significant proportion of phytate will not be hydrolyzed, with reports suggesting that supplementation with phytase results, on average, in about 29% of the phytate being hydrolyzed (Ghaly *et al*., 2017; Manopriya *et al*., 2022).

The biological effects of vitamin D3 can be mediated by the binding of active vitamin D to vitamin D receptors (VDR) (El-Khasemi and Faye, 2019). In chickens, vitamin D regulates Calbindin 28k (CaBP-D28k), an intracellular Ca^{2+}-binding protein that transports Ca from the apical toward the basolateral membrane (Wu *et al*., 2021). The addition of vitamin D3 also increases CaBP-D28k mRNA expression in the small intestine (Wang *et al*., 2022). CaBP-D28k protein plays a role in Ca^{2+} transport in the kidneys, eggshell gland and intestine in birds, which can be effective in phosphorus absorption.

One of the characteristics of 1α-OH-D3 is that it acts as a substitute for the phytase enzyme of foreign origin, which can be effective on the absorption of minerals and bird growth. Since past research mostly focused on the separate application of 1α-OH-D3 metabolite and phytase, and considering there are few results on the use of their mixture with broiler chickens, the present research can be important from an innovation standpoint. Therefore, the present experiment was conducted to evaluate the effect of 1α-OH-D3 and phytase separately and together on the performance, carcass characteristics, blood factors and the expression of *VDR* and *CaBP-D28k* genes in broiler chickens.

MATERIALS AND METHODS

This experiment was conducted with 400 one-day-old male chickens (Cobb 500 strain), using a completely randomized design with four treatments and five replicates (20 chickens for each replicate) for 42 days. The experimental treatments were: 1- Control (with no 1α-OH-D3 and phytase), 2- Control + 14 mg/kg 1α-OH-D3 in the diet, 3- Control + 5 mg/kg phytase and 4- Control + 14 mg/kg 1α- OH-D3 + 5 mg/k phytase. The two supplements used were crystalline 1α- OH-D3 (Vitamin Derivatives, Georgia USA) and phytase (Bonfezyme, Bioluence, Tehran, Iran). Chickens were initially weighed and the ones with similar average weights were distributed in each replicate. Water and feed were provided freely during the rearing period. Three control diets were used: starter (1-10 days old), grower (11-25 days old), and finisher (26-42 days old), with compositions based on the recommendations of the Cobb 500 Breeding Guide (Table 1).

The chickens were weighed at the end of the rearing period (42 days old), and weight gain, feed conversion ratio, and feed intake were measured for the 42 day period, corrected for losses. After the rearing period, three chickens close to the average weight for each replicate were selected, weighed, and slaughtered. After feather removal and emptying of the digestive system, the weight was measured for the thighs, chest, wings, and neck of the carcass, as well as for the liver, heart, and abdominal fat, using a digital scale (0.1 ± 0.0 g). The percentage of each of the measured parts was calculated relative to the live weight. At 21 and 42 days of age, 3 chickens were randomly chosen from each of the replicates, and blood sample collection was done from the wing vein into blood tubes without anticoagulant. These were immediately transported to the laboratory, and the samples underwent centrifugation at 3000 rpm for 15 minutes to separate the serum. Finally, serum calcium, phosphorus, and alkaline phosphatase levels were evaluated using an RA-XT Auto-Analyzer (Technicon, city, USA) and a Pars Azmon commercial kit (Zahedi *et al.,* 2023).

Three to 5 g of duodenum (for extraction of VDR mRNA) and 3 to 5 g of liver (for extraction of CaBP-D28k mRNA) genes were subsequently collected, each sample was placed in individual RNA-free sterile microtubes, and then transferred to the laboratory at 4˚C, and frozen at -80 °C until RNA extraction. The RNA extraction was undertaken according to the manufacturing company's guidelines, using a Favorgen (PingPung, Taiwan) kit

Table 1 – Dietary composition for different rearing periods.

Ingredients (%)	Starter (1-10 days old)	Grower (11-24 days old)	Finisher (25-42 days old)
Corn	49.11	53.51	58.11
Soybean meal	38.70	35.00	27.00
Wheat	5.00	5.52	9.05
Soybean oil	1.70	1.70	2.00
Corn gluten	1	0	0
Sodium bicarbonate	0.03	0.1	0.15
$CaCO_3$	1.80	1.73	1.60
Mono calcium phosphate	1.20	1.07	0.84
NaCl	0.33	0.27	0.25
Mineral premix[1]	0.25	0.25	0.25
Vitamin premix[2]	0.25	0.25	0.25
L-Lysine-HCl	0.23	0.23	0.19
DL-Methionine	0.3	0.28	0.26
L-Threonine	0.1	0.09	0.05
Total	100	100	100
Chemical analyses			
Metabolizable energy (kcal/kg)	2950	3000	3080
Crude protein (%)	22	20	17.5
Lysine (%)	1.24	1.15	0.95
Methionine (%)	0.6	0.55	0.51
Methionine + Cysteine (%)	0.87	0.8	0.73
Threonine (%)	0.8	0.74	0.6
Arginine (%)	1.34	1.3	1.05
Isoleucine (%)	0.82	0.75	0.68
Valine (%)	1	0.88	0.71
Ca (%)	0.96	0.9	0.8
Available Phosphorus (%)	0.36	0.33	0.28
Na (%)	0.16	0.16	0.16

[1]Supplied the following per kilogram of feed: Mn (as Manganese oxide): 39.68 g; Fe: (as ferrous sulfate): 20 g; Cu (as Copper sulfate): 4 g; Zn (as Zinc oxide): 33.88 g; Se (as sodium selenite): 0.08 g, I (calcium iodate): 0.396 g and Betain: 200 g.

[2]Supplied the following per kilogram of feed (without vitamin D3): vitamin A: 3600000 IU; Niacin: 11.88 g; vitamin E: 7200 IU; vitamin K3: 0.8 g; Thiamin: 0.7 g; vitamin H2 (Biotin): 0.04 g; Riboflavin: 2.64 g; Pantothenic acid: 3.92 g; Pyridoxine: 1.176 g; Folic acid: 0.4 g; vitamin B12: 0.006 g; Antioxidant: 400 mg.

with specifications "Cat. No: FABRK001" and "Lot No: BIB05118C10". The extracted RNA concentration was determined by the spectrophotometric method, and an OD260/280 ratio between 1.8 and 2 was used to evaluate the purity of the extracted RNA. All samples were then kept at -80 °C. The stages of the gene expression study included cDNA generation and assessment of gene expression using qualitative real-time polymerase chain reaction (PCR).

A reverse transcription reaction was done with 1 gμ RNA and a primescript reverse transcription reagent kit (QIAGEN, 205311) following the manufacturer's instructions. Qualitative real-time PCR reaction (qPCR) was done with a QIAGEN SYBR Premix PCR Kit, with the help of specific primers designed for *VDR* and *CaBP-D28k* genes at a reaction volume of 10 μL, including 5 Lμ of SYBR Green Premix I PCR mix (QIAGEN, 204052) (2X), 0.4 μL from the reverse and forward primers (10 μL), 2.3 μL of RNA free deionized water, and 1 μL of cDNA,. The reaction temperature program comprised 95 °C for 60 seconds, 40 cycles with 95 °C for 10 seconds, primer binding temperatures of 60 and 64 °C for VDR and CaBP-D28k sites, respectively, and 72 °C for 30 seconds, with the ultimate extension temperature of 72°C for 5 minutes. The relative expression level of the gene was checked based on the resulting cycle threshold (Ct) curves and by comparison with the beta-actin gene as House Keeping through the $2^{-\Delta\Delta CT}$ template.

The sequence of primers used in the research for *VDR* genes was based on Hsiang Hsiao *et al*. (2018), while that of CaBP-D28k was based on Han *et al*. (2021), as detailed in Table 2.

Table 2 – List of primers used.

Gene	Primer Sequence (5′ to 3′)	Product size
VDR	Forward: CGTGAGAAGCAAATTCAGCA Reverse: GAGGTCCAGGTTGGAAAACA	157 bp
CaBP-D28k	Forward: AGATCTGGCACCACTACGAC Reverse:TGAGCAAGCTCAACGATTCCT	187 bp
β-actin	Forward: CCACCGCAAATGCTTCTAAAC Reverse: GAAGGTGTCAGCAGTCTT	175 bp

STATISTICAL ANALYSIS

Data were analyzed by the statistical model $Y_{ij} = \mu + T_i + \varepsilon_{ij}$ and the general linear models (GLM) procedure of SAS.9.4 software (SAS, 2004). Y_{ij} is the numerical value of each observation, μ is the average of the population, T_i indicates the effect of experimental groups, and ε_{ij} indicates the effect of the experimental error. Duncan's test was used at the 0.05 level to compare the averages.

RESULTS

Table 3 presents the impact of treatments on broiler chickens' performances. The weight gain of chickens that received 1α-OH-D3 in isolation and with phytase indicated a significant increase in relation to the control (p<0.05). The conversion ratio was significantly improved in relation to the controls only in chickens that received 1α-OH-D3 along with the phytase enzyme (p<0.05).

Table 3 – The effect of treatments on broiler chickens' performances at 42 days.

Treatments	Weight gain (g)	Feed consumption (g)	Conversion ratio
A	2600.1±204.96^{b}	4717.1±1.48	1.82±0.07^{a}
B	2816.5±130.81^{a}	4924.4±7.13	1.74±0.09^{ab}
C	2673.6±94.41^{b}	4851.6±4.46	1.81±0.08^{a}
D	2955.1±55.23^{a}	4958.7±2.91	1.67±0.02^{b}
SEM	30.64	37.54	0.025
p-value	0.0469	0.1849	0.0313

A: Control (no additional supplements), B: Addition of 14 mg/kg DM 1α-OH-D3 in diet, C: Addition of 5 mg/kg phytase, and D: Addition of 14 mg/kg 1α- OH-D3 + 5 mg/k phytase.

Means in the same column with at least one common letter have no significant difference (p<0.05)

SEM: standard error of the mean.

Table 4 presents the results of treatments on the percentage of carcass parts and internal organs. The percentage of thighs in the treatments containing the mixture of 1α-OH-D3 and phytase showed a significant increase in comparison with the controls (p<0.05). There was a significant increase in breast percentages in all treatments in comparison to the controls (p<0.05). Furthermore, the abdominal fat percentage in the treatment containing the mixture of 1α-OH-D3 and phytase was significantly reduced in relation to the controls (p<0.05).

Table 4 – The effect of experimental treatments on the carcass characteristics of broiler chickens at 42 days (%).

Treatments	Thigh (%)	Breast (%)	Wing (%)	Neck (%)	Liver (%)	Heart (%)	Abdominal fat (%)
A	19.78±0.71^{b}	22.15±2.31^{b}	5.67±0.59	4.29±0.08	1.64±0.14	0.53±0.04	1.42±0.34^{a}
B	20.31±1.2^{b}	29.30±1.78^{a}	5.84±0.59	5.01±0.09	1.51±0.19	0.43±0.03	1.19±0.43^{ab}
C	20.04±1.95^{b}	27.34±1.6^{a}	5.74±0.25	4.74±0.02	1.53±0.13	0.52±0.1	1.31±0.21^{ab}
D	28.52±0.34^{a}	29.66±2.03^{a}	6.02±0.63	5.39±0.07	1.41±0.15	0.55±0.11	0.80±0.31^{b}
SEM	0.25	0.47	0.11	0.18	0.04	0.02	0.09
p-value	0.048	0.034	0.7633	0.1819	0.2322	0.1632	0.0409

A: Control (no additional supplements), B: Addition of 14 mg/kg DM 1α-OH-D3 in diet, C: Addition of 5 mg/kg phytase, and D: Addition of 14 mg/kg 1α- OH-D3 + 5 mg/k phytase.

Mean values in the same column with at least one common letter are not significantly different (p<0.05)

SEM: standard error of the mean.

Table 5 presents the results of treatments on the blood biochemical factors of broilers on the 21st and 42nd days of rearing. On the 21st day of age, a significant increase in relation to the control was observed in the concentration of blood phosphorus of chickens that received phytase (p<0.05). On the 42nd day of age, the amount of Ca and P in the blood serum of the chickens that received 1α-OH-D3 along with phytase showed a significant increase in comparison with the controls (p<0.05). Moreover, the amount of ALP at the end of

Table 5 – The effect of the experimental treatments on the biochemical parameters of the blood of broiler chickens.

Treatments	21 days of age			42 days of age		
	Ca (mg/dl)	P (mg/dl)	ALP (U/L)	Ca (mg/dl)	P (mg/dl)	ALP (U/L)
A	9.84±1.28	5.46±1.06^{b}	11009.00±10362.79	8.43±1.28^{b}	5.52±1.35^{b}	3337.60±2889.85^{a}
B	10.65±1.10	5.75±1.28^{ab}	6495.00±5277.00	8.96±1.1^{ab}	5.58±1.05^{b}	2137.70±932.08^{ab}
C	11.04±1.41	6.78±1.16^{a}	68861.00±8528.07	8.81±2.01^{ab}	5.90±0.83^{ab}	2144.20±984.73^{ab}
D	11.17±2.01	6.18±1.47^{ab}	5203.00±4854.55	9.46±1.41^{a}	6.58±0.54^{a}	1558.50±465.82^{b}
SEM	0.24	0.21	1227.59	0.17	0.17	266.06
p-value	0.2061	0.0399	0.2213	0.0326	0.0246	0.0111

A: Control (no additional supplements), B: Addition of 14 mg/kg DM 1α-OH-D3 in diet, C: Addition of 5 mg/kg phytase, and D: Addition of 14 mg/kg 1α- OH-D3 + 5 mg/k phytase.

Means in the same column with at least one common letter are not significantly different (p<0.05)

SEM: standard error of the mean.

the period in the chickens that had received 1α-OH-D3 along with phytase had a significant decrease in relation to the controls (p<0.05).

The impact of treatments on *VDR* and *CaBP-D28k* gene expression is respectively presented in Figures 1 and 2. The difference in expression of *VDR* and *CaBP-D28k* genes in the treatments that used 1α-OH-D3 and 1α-OH-D3 along with phytase enzyme indicated a significant increase in comparison with the controls (p<0.05).

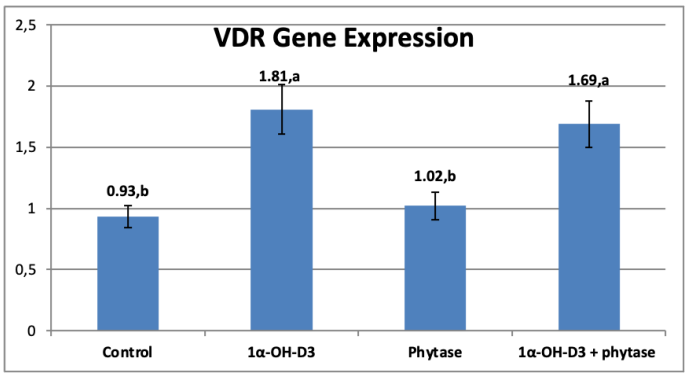

Figure 1 – Effect of the experimental diets on *VDR* gene expression in broiler chickens

Means in the same column with at least one common letter are not significantly different (p < 0.05).

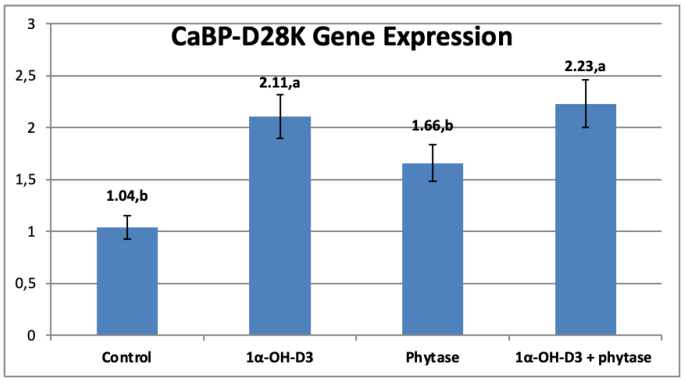

Figure 2 – Effect of the experimental diets on *VDR* gene expression in broiler chickens

Means in the same column with at least one common letter are not significantly different (p<0.05).

DISCUSSION

Chickens had a higher weight gain when 1α-OH-D3 was used in isolation, as well as with phosphatase enzyme in the treatments. Consistent with our results, Edwards (2002) observed that the weight gain of broiler chickens increased with 1α-OH-D3 consumption, and this increase was similar to a group of chickens that received 1,25-(OH)2-D3. Han *et al*. (2009) and Liem *et al*. (2009) reported that the addition of a 1α-OH-D3 metabolite to the feed of broilers improved their performance.

Previous researchers have reported the 1α-OH-D3 bioavailability to be about 8-10 times that of cholecalciferol (Haussler *et al*., 1973; Edwards *et al*., 2002). Also, Edwards (2002) stated in his report that 1α-OH-D3 bioactivity in broilers is comparable to that of 1,25-(OH)2-D3.

1α-OH-D3 is hydroxylated to 1,25-(OH)2-D3 by 25-hydroxylase enzyme in the liver of broilers. The final active form of 1α-OH-D3 is 1,25-(OH)2-D3 (Hu *et al.*, 2020).

The absorption efficiency of 1α-OH-D3 is higher than vitamin cholecalciferol, and it is converted to 1,25-(OH)2-D3 more quickly in the liver. Since the 1-hydroxylated metabolite of vitamin D has finished one step of hydroxylation, it improves the absorption of calcium and phosphorus through a faster absorption, which can be expected to improve growth performance (Christakos *et al*., 2016).

Han *et al*. (2009) reported an interaction effect of 1α-OH-D3 and phytase enzyme improving the feed conversion ratio of broiler chickens. Various studies have shown that calcium in the intestine reduces the ability to dissolve phytate (Tamim *et al*., 2004). Metabolites of 1α-OH-D3 increase calcium absorption and the ability to dissolve phytates, thus increasing intestinal phytase activity. This can be confirmed by the significant reduction of the conversion ratio in the present experiment.

As seen in the results, breast percentage increased when vitamin D metabolite and phytase were used alone or in combination in the diet. Also, the mixture of 1α-OH-D3 and phytase could increase the percentage of thigh in the broilers. For optimal growth, broilers continuously need the active metabolite of vitamin D3 to absorb calcium and phosphorus. With an increase in the age of the bird, vitamin D metabolism is disturbed due to the decrease in the capacity of liver and kidney hydroxylase enzymes to activate cholecalciferol, or the internal synthesis of active metabolites of vitamin D3 (Ghasemi *et al*., 2019). Since 1α-OH-D3 is hydroxylated more efficiently and quickly, together with phytase it can increase calcium and phosphorus absorption. As a result, the amount of saponified calcium with fatty acids in the digestive tract is reduced, and the absorption of fatty acids is improved, which can help improve the growth of carcass components (Momeneh *et al*., 2018).

Furthermore, the simultaneous consumption of 1α-OH-D3 and phytase has a great effect on the mineralization and strength of the tibia due to the absorption of calcium and phosphorus, and their

reduction may reduce the ash content of the leg. In the present experiment, better calcium and phosphorus absorption may be effective in improving the weight of the thighs, possibly due to an effect on the weight of the femur (Khajali *et al*. 2010).

The use of phytase improves protein digestibility and absorption of essential amino acids, especially lysine, and may increase the production of meat and carcasses in broiler chickens (Marchal *et al*., 2021).

In our experiment, the chickens receiving the mixture of 1α-OH-D3 and phytase showed a lower abdominal fat percentage compared to other chickens. The results of some studies have shown that vitamin D3 can prevent the accumulation of fat in the liver by regulating the circulation of free fatty acids, inhibiting lipogenesis, and strengthening the oxidation of fats (Yin *et al*., 2012; Asadi Kermani *et al.*, 2023). Regarding the molecular mechanism of the effects of vitamin D3, vitamin D3 can increase the expression of ATG16L1, which is part of the ATG5-ATG12 complex and is necessary for the formation of autophagosomes, thereby reducing fat accumulation in the liver and regulating the organ's lipid metabolism (Fujita *et al*., 2008).

The findings of other studies have also confirmed the benefits of phytase enzyme supplementation in reducing abdominal fat. Phosphorus phytate reduces the function of digestive enzymes, including pancreatic lipase function. As a result, adding phytase can lead to optimal lipase activity in the digestive system and to a reduction of abdominal fat (Pieniazek *et al*., 2016).

Adding phytase along with 1α-OH-D3 significantly increased blood calcium and phosphorus compared to the control group.

1,25-(OH)2-D3 as the active form of vitamin D can increase calcium and phosphorus absorption (Hsiang Hsiao *et al*., 2018). It increases the calcium and phosphorus absorption from the lumen into the plasma due to its effect on the small intestine' enterocytes. This vitamin, along with parathyroid hormones, can improve calcium reabsorption from the distal tubules, which will finally lead to an increase in the calcium and phosphorus blood levels (Han *et al*., 2016). Since 1α-OH-D3 is quickly converted into the active form of vitamin D, it can increase the amount of phosphorus and calcium in the blood.

Some researchers have shown that the use of phytase increases blood phosphorus concentration and the possibility of phosphorus storage in bone tissue (Ciurescu *et al*., 2020; Shi *et al*., 2024).

In this study, alkaline phosphatase was affected by the consumption of 1α-OH-D3 along with phytase. Alkaline phosphatase is a zinc-containing metalloenzyme that is considered an indicator of liver damage. Moreover, it is one of the endogenous phosphatases, which, in the small intestine, cause the release of phosphorus from inositol monophosphate, and the production of inositol, which plays a key role in bone mineralization (Zanu *et al*., 2020).

It was reported that increasing phosphorus availability may reduce birds' need to use endogenous phosphatases (Daramola *et al*., 2017). Since the use of 1α-OH-D3 along with phytase has increased the level of available phosphorus in the blood in the course of our experiment, it has probably been able to reduce the amount of alkaline phosphatase.

The level of expression of *VDR* and *CaBP-D28k* genes in the treatments that used the vitamin D metabolite, alone or mixed with phytase enzyme, indicated a significant elevation compared to the control and the treatment that contained phytase alone. Centeno *et al*. (2011) stated that reducing the level of vitamin D in mice caused a decrease in *VDR* gene expression in their experiments.

Yang *et al*. (2020) stated that the optimal levels of 1α-hydroxycholecalciferol (1α-OH-D3) and 1,25-(OH)2-D3 enhanced *VDR* gene expression in the intestine of broiler chickens.

Yang *et al*. (2019) showed in their experiments that vitamin D increased *CaBP-D28k* expression levels in broilers, which is in line with our results. In another study, the expression level of intestinal CaBP-D28k in broiler chickens after receiving 1,25-(OH)2-D3 was 50 and 433.7 times higher than in chickens with vitamin D deficiency (Clemens *et al*., 1988);

Previous studies have shown that vitamin D binds to VDR in the small intestine to regulate phosphorus and calcium absorption. The absorption of phosphorus and calcium in the small intestine is increased by 1α-OH-D3. When 1α-OH-D3 is converted to 1,25-(OH)2-D3, it can bind to VDR (Xu *et al*., 2002; Yang *et al*., 2020).

The vitamin D receptor is found in intestinal epithelial cells (Han *et al*., 2018). In the small intestine of animals, there are transcellular and paracellular channels for calcium absorption. In Ca^{2+} transcellular transport, Ca-binding protein-D28k (CaBP-D28k) plays an effective role (Fleet and Schoch, 2010).

CaBP-D28k transports calcium from the apical membrane to the basolateral membrane, which affects calcium absorption (Okano *et al*., 2004).

As a phosphorus transporter protein, type IIb sodium-phosphate cotransporter (NaPi-IIb) can be found in the epithelial cell apical membranes of the broilers' small intestines (Forster *et al*. 2013). Yang *et al*. (2020) observed that 1,25-(OH)2-D3 can regulate NaPi-IIb mRNA expression levels in rat intestines.

After absorption, 1α-OH-D3 quickly turns into the active form of 1,25-(OH)2-D3, which enhances calcium and phosphorus absorption. One of the important features of 1α-OH-D3 is that it acts independently of the phytase enzyme with foreign origin. Phytase acts in the upper parts of the digestive tract with low pH to help digest phytate into phosphate and inositol, while 1α-OH-D3 acts in the lower part of the digestive tract with high pH, which can help improve absorption of calcium and phosphorus (Proszkowiec-Weglarz *et al*., 2019). Therefore, the significant improvement in the expression of the *VDR* and *CaBP-D28k* genes in the treatments of this experiment containing 1α-OH-D3, in isolation or mixed with phytase, can indicate a higher absorption of calcium and phosphorus.

CONCLUSIONS

The results showed that the use of 1α-OH-D3 mixed with phytase, compared to their separate use in the diet, can cause higher growth performance, an increase in the amount of Ca and P in blood serum, and a greater relative expression of the *VDR* and *CaBP-D28k* genes in broiler chickens.

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AUTHOR CONTRIBUTIONS

AH and NE: methodology and writing – original draft preparation; MP: formal analysis and investigation; AA: writing – review and editing. All authors have read and agreed on the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

Data is available upon request.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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