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Enhancing calcium and phosphorus utilization in broiler chickens through cholecalciferol in sequential calcium-phosphorus-deficient diets

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ABSTRACT

We hypothesized that both performance and bone mineralization would show improvements when cholecalciferol supplementation was increased above the recommended NRC (1994) [*Nutrient requirements of poultry*, 9th rev. ed. Washington, DC: National Academy Press] level. In this study, male Ross 308 chickens (n = 540) were supplemented with diets containing either a low level of 200 IU/kg or a higher dose of 5,000 IU/kg of cholecalciferol (VD). The results showed that during the starter phase, weight gain was significantly higher in VD5000 and the control compared to VD0, with significantly lower feed intake in VD0. Consequently, FCR was significantly better in VD200 and VD5000 compared to VD0. In the finisher phase, weight gain, feed intake, and FCR were significantly higher in VD200 and VD5000 compared to VD0. Tibia ash, Ca, and P were significantly higher in VD5000 compared to VD200. Carcass weight was significantly higher in VD200 and VD5000 compared to VD0. Total tract N retention and GE were significantly higher in VD5000 compared to VD0. The study concludes that cholecalciferol supplementation at 5000 IU/kg enhances growth performance, tibia mineralization, and nutrient digestibility in broilers fed with 30% reduced Ca and P in their diets.

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Introduction

Genetic selection in broiler chickens has brought about notable enhancements, including accelerated growth rates and improved feed conversion ratios. This process has also redirected attention from leg muscle yield to an emphasis on breast muscle, thereby altering the bird's center of gravity to a more forward position (Sakkas et al. 2019). In the realm of calcium metabolism in birds, vitamin D plays a pivotal role. For animals raised without access to natural light, vitamin supplementation becomes necessary to meet their vitamin D needs (Khan et al. 2023). The function of vitamin D is well-recognized – it enhances the absorption and utilization of dietary calcium and phosphorus (Tizziani et al. 2019). The current recommended level of vitamin D3 supplementation as per the National Research Council (NRC) guidelines from 1994 is 200 IU/kg. However, it's important to note that this value is primarily intended to prevent vitamin deficiency and is significantly lower than the levels typically employed to enhance production (Vazquez et al. 2018).

Calcium (Ca) stands as a fundamental nutrient for avian organisms, actively engaging in a multitude of biochemical functions and contributing significantly to bone formation. The absence or insufficiency of this mineral can yield detrimental consequences. As a result, it becomes imperative to effectively fulfill the nutritional prerequisites at the various stages of avian development. Furthermore, calcium's metabolic processes are intricately interlinked with those of phosphorus (Mello et al. 2012). This connection underscores the need for cautious consideration when formulating well-balanced diets encompassing these minerals. Such precision is vital for attaining optimal dietary utilization, as imbalances can have adverse repercussions on both performance and bone quality (Rao et al. 2006). Birds exhibit a notable efficiency in utilizing calcium even when subjected to sub-optimal levels (Khan et al. 2023). This underscores the adaptability and effectiveness of avian physiological systems in capitalizing on available nutrients (Li et al. 2012).

Sequential feeding involves short-term providing broiler chickens with different diets containing varying nutrient levels in consecutive cycles (Baradaran et al. 2021). Such feeding modifies the gait score of broiler chickens, with the aim of enhancing their welfare and restoring reduced Ca and P in the diet (Bizeray et al. 2002; Leterrier et al. 2008; Baradaran et al. 2021). These feeding strategies potentially provide a deeper insight into nutritional method to enhance nutrient utilization. Following an extensive review of the literature, it has

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become evident that there is a significant gap in research pertaining to the influence of low- and then adequate-phosphorus diets, on growth performance and nutrient digestibility, utilizing synthetic sources of vitamin D3. Consequently, the primary objective of the present study is to assess growth performance, nutrient digestibility, as well as tibia Ca and P contents in broilers supplemented with vitamin D3 under NRC (1994) recommendation or 25 times (5000 IU/kg) higher dose.

Materials and methods

Diets and experimental treatments

The baseline diet was formulated using corn and soybean meal, and it was designed to fulfill the minimum nutritional requirements for the birds, including sufficient levels of P and Ca. This formulation covered both the starter phase (days 1–21) and the finisher phase (days 22–35) of the birds' growth. In contrast, two negative control diets in terms of Ca and P were prepared, each with incremental reductions of 30%. The study encompassed four experimental diets: Control (basal diet, normal 1.00% Ca, and 0.45% P); Diet-1 (0.70% Ca + 0.30% P); Diet-2 (0.70% Ca + 0.30% P + 200 IU/kg cholecalciferol) and Diet-3 (0.70% Ca + 0.30% P + 5000 IU/kg cholecalciferol). The source of vitamin D3 was incorporated into the diet along with the minerals-vitamins premix. The composition and calculated nutritional analysis of the starter and finisher diets are detailed in Table 1.

Performance traits

Throughout the entire duration of the study, the feed supply and consumption were meticulously quantified. At the end of the trial period, the weight growth of the broilers was noted weekly for each group. The values of feed intake and weight gain were employed to calculate the feed conversion ratio (FCR). At day 35 of the experimental trial, two birds from each replicate were selected, weighed, and then slaughtered. The slaughtering process involved bleeding and subsequent feather plucking. The weight of the dressed carcass was then compared relative to the live body weight of the birds.

Table 1. Basal and Ca: P deficient diets composition.

		Low density (Ca:*P* = 0.70%:0.30%)		
Ingredient	Control	VD0	VD200	VD5000
Corn (Grain)	55.84	58.055	58.055	58.055
Corn GM-30%	3.00	3.500	3.500	3.500
Soybean Meal −44%	16.00	11.000	11.000	11.000
Canola	8.00	8.000	8.000	8.000
Corn gluten-60% (Meal)	3.00	3.744	3.744	3.744
Oil	2.07	2.000	2.000	2.000
Poultry By-Product	3.00	3.000	3.000	3.000
Sunflower	5.00	6.000	6.000	6.000
Bone	1.00	0.126	0.126	0.126
Limestone	1.018	0.879	0.879	0.879
DCP	0.653	0.500	0.500	0.500
Salt	0.418	2.475	2.475	2.475
DL-Methionine	0.137	0.157	0.157	0.157
L-Lysine HCL	0.695	0.372	0.372	0.372
Threonine	0.050	0.073	0.073	0.073
Vit:Min Premix	0.120	0.120	0.120	0.120
Total	100	100	100	100
Calculated composition				
CP %	19.00	19.00	19.00	19.00
ME/ kcal/g	2.95	2.95	2.95	2.95
Calcium %	1.0	0.70	0.70	0.70
Available *P*, %	0.45	0.30	0.30	0.30
Threonine, %	0.82	0.80	0.80	0.80
Lysine, %	1.15	1.15	1.15	1.15
Methionine + Cystine, %	0.52	0.90	0.90	0.90
Methionine, %	0.90	0.54	0.54	0.54

Total tract digestibility assay

On the 35th day of the experiment, total tract nitrogen (N) retention was evaluated using acid insoluble ash as a digestibility marker. On the 33rd day of the experiment (five birds per replicate), using acid insoluble marker was blended into the experimental diets and administered to the birds, which were individually housed in metallic cages until the conclusion of the trial. When the experiment reached its 35th day, fecal samples were cleanly collected twice daily and preserved in plastic bags at a temperature of −20°C. Subsequently, samples were milled on 0.5 mm sieve and were used in the Kjeldahl technique to calculate total tract N retention and then multiplied by a conversion factor of 6.25. Furthermore, bomb calorimeter was used to determine the gross energy (GE) following the method outlined by Sandberg et al. (1993).

Tibia measurements

On day 35 of the experiment, the right tibia of two birds from each pen were euthanized. The tibia were removed from the surrounding tissues and dried at a temperature of 100°C overnight. Following this, fat was removed from the tibia in Soxhlet apparatus using 100% ethyl ether. This process followed modified procedures in accordance with the guidelines provided by Watson et al. (2006). The tibia samples were then transported to a laboratory for the measurement of Ca, P and total ash contents.

Statistical analysis

The collected data underwent one-way analysis of variance (ANOVA) using the general linear model (GLM) procedure within the SAS statistical analysis system (SAS 2002). Tukey's test was utilized to differentiate between the means. Statistical significance was established with a *p*-value below 0.05.

Results

The findings of weight gain, feed intake and FCR at the end of starter phase of broilers fed deficient Ca and P diets are given in Table 2. The results showed that weight gain was significantly ($P < 0.01$) higher in VD5000 and the control compared to VD0, however, feed intake was significantly ($P < 0.01$) lower in VD0. Resultantly, FCR was significantly ($P < 0.01$) better in VD200 and VD5000 compared to VD0.

The effects of different levels of cholecalciferol on growth performance of broilers at the end of finisher phase fed deficient Ca and P diets are given in Table 3. The results

Table 2. Weight gain, Feed intake and FCR of broilers fed on vitamin D3 supplemented diets at day 21.

Diets	Weight Gain	Feed Intake	FCR
	g		
Control	743.38[a]	1115.5[a]	1.50[b]
VD0	575.38[c]	1029.0[c]	1.79[a]
VD200	681.15[b]	1071.4[b]	1.58[b]
VD5000	715.50[a]	1087.9[b]	1.52[b]
SEM	3.45	12.37	0.01
P value	0.0001	0.0001	0.0009

Means in the same column with different superscript differ ($P < 0.05$).
Diets contained different levels of Ca and *P* wherein the Control = Ca 1.00% and *P* 0.45%; VD0 = Ca 0.70% and *P* 0.30% + 0IU/kg vitD3; D200 = Ca 0.70% and *P* 0.30% + 200IU/kg vitD3; VD5000 = Ca 0.70% and -*P* 0.30% + 5000 IU/kg vitD3.

Table 3. Overall Weight gain, Feed intake and FCR of broilers fed on vitamin D3 supplemented diets in broilers at day 35.

[1]Diets	Weight Gain	Feed Intake	FCR
	g		
Control	1740.6[a]	2785.1[a]	1.60[b]
VD0	1370.1[b]	2675.0[b]	1.95[a]
VD200	1736.9[a]	2774.3[a]	1.59[b]
VD5000	1750.7[a]	2768.8[a]	1.57[b]
SEM	12.34	65.78	0.12
P value	<0.01	0.002	<0.01

Means in the same column with different superscript differ ($P < 0.05$).
Diets contained different levels of Ca and *P* wherein the Control = Ca 1.00% and *P* 0.45%; VD0 = Ca 0.70% and *P* 0.30% + 0IU/kg vitD3; D200 = Ca 0.70% and *P* 0.30% + 200IU/kg vitD3; VD5000 = Ca 0.70% and *P* 0.30% + 5000 IU/kg vitD3.

Table 4. Tibia Ash, tibia Ca and tibia *P* contents of broilers fed vitamin D3 supplemented diets in broilers.

Diets	Tibia Ash	Tibia Ca	Tibia *P*
	%		
Control	39.789[ab]	30.263[a]	14.245[a]
VD0	34.704[b]	27.709[b]	12.612[b]
VD200	42.448[ab]	28.793[b]	13.878[b]
VD5000	44.971[a]	30.367[a]	15.414[a]
SEM	1.22	1.43	0.91
P value	0.0121	0.0269	0.0098

Means in the same column with different superscript differ ($P < 0.05$).
Diets contained different levels of Ca and *P* wherein the Control = Ca 1.00% and *P* 0.45%; VD0 = Ca 0.70% and *P* 0.30% + 0IU/kg vitD3; D200 = Ca 0.70% and *P* 0.30% + 200IU/kg vitD3; VD5000 = Ca 0.70% and *P* 0.30% + 5000 IU/kg vitD3.

showed that weight gain, feed intake and FCR were significantly ($P < 0.05$) higher in VD200 and VD5000 compared to VD0.

The effects of different levels of cholecalciferol on tibia ash, tibia Ca and P of broilers fed deficient Ca and P diets are given in Table 4.

Table 5. Hot carcass weight, Illeal CP digestibility and AME contents of broilers fed vitamin D3 supplemented diets in broilers.

[1]Diets	Carcass Weight	Total tract N retention	Gross Energy
	G	%	kcal/kg
Control	1294.2[b]	70.424[b]	2902.5[b]
VD0	1022.1[c]	66.606[c]	2848.8[c]
VD200	1318.1[a]	72.117[a]	2853.1[b]
VD5000	1334.5[a]	71.610[ab]	2989.9[a]
SEM	8.64	2.34	17.86
P-value	<0.01	<0.01	0.0002

Means in the same column with different superscript differ ($P < 0.05$).
Diets contained different levels of Ca and *P* wherein the Control = Ca 1.00% and *P* 0.45%; VD0 = Ca 0.70% and *P* 0.30% + 0IU/kg vitD3; D200 = Ca 0.70% and *P* 0.30% + 200IU/kg vitD3; VD5000 = Ca 0.70% and *P* 0.30% + 5000 IU/kg vitD3.

The results showed that tibia ash, Ca and P were significantly ($P < 0.05$) higher in VD5000 compared to VD200.

The effects of different levels of cholecalciferol on carcass weight, Total tract N retention, and Gross Energy of broilers fed deficient Ca and P diets are given in Table 5. The results showed that carcass weight was significantly ($P < 0.01$) higher in VD200 and VD5000 compared to VD0. On the other hand, total tract N retention and GE were significantly ($P < 0.01$) higher in VD5000 compared to VD0.

Discussion

In the current study, it was evident that supplementation of cholecalciferol at NRC (1994) recommended dose was not very effective, however, when the vitamin D3 dose was increased by 25 times (5000IU/kg), the growth performance, mineralization of tibia and nutrients digestibility were enhanced. These findings align with previous research indicating that administering vitamin D3 at doses higher than the recommended level in the NRC (1994) guidelines can be beneficial in enhancing the growth performance of broilers. The study conducted by Fritts and Waldroup in 2005 discovered that broilers that were fed a diet containing 4,000 IU/kg of cholecalciferol exhibited enhanced body weight gain (BWG) in comparison to those that were fed 1,000 IU/kg. In a 2013 study by Sun et al., increasing dietary cholecalciferol levels to 2,000 or 4,000 IU/kg improved walking ability, bone quality, and reduced footpad/hock dermatitis severity in broilers. Likewise, Gómez-Verduzco et al. found that chickens fed diets with 2,000 IU/kg cholecalciferol showed better growth and immune response than those following NRC (1994) recommendations. Given the continuous genetic improvement of broiler chickens and the ongoing development of more efficient exogenous phosphatases, it is essential to re-evaluate the non-phytate phosphorus (NPP) requirements of broiler chickens (Salisbury et al. 2021). The imbalance of Ca and P in broiler rations had an impact on weight gain and feed efficiency during both the starter and finisher phases. Nevertheless, growth performance was restored when cholecalciferol was supplemented at either of the two doses during the finisher phase. In the starter phase, enhanced weight gain and feed intake were noticed in response to VD5000 and VD200 was not much effective at this stage. Previous research has demonstrated that imbalanced Ca and P ratios had a detrimental effect on the body weight gain and feed intake of broilers (Li et al. 2012; Han et al. 2016). A high dietary Ca to non-phytate phosphorus (NPP) ratio had a negative impact on phosphorus utilization due to the formation of Ca-P complexes in the gastrointestinal tract, which were not accessible for the birds. Based on the results obtained in this study, it is evident that restoring Ca and P levels through dietary vitamin D3 supplementation has a significant impact. The expected effect is due to phosphorus playing a crucial role in protein/amino acid synthesis and energy metabolism (Proszkowiec-Weglarz and Angel 2013)., as well as in adjusting appetite and metabolism (Fallah et al. 2020).

In the current study, tibia ash, Ca an P were significantly higher in birds supplemented with either 5 or 10 µg/kg of diets. The dietary calcium (Ca) to phosphorus (P) ratio plays a

significant role in regulating bone mineralization and turnover in mice. This regulation occurs by influencing the transport of calcium and phosphorus in the intestines, as demonstrated in a study by Masuyama et al. (2003). Bone mineralization is of utmost importance as it directly correlates with bone strength. Maximizing bone mineralization serves to reduce mortality, morbidity, and the occurrence of leg weakness. The supplementation of cholecalciferol or its metabolites has consistently shown improvements in bone ash and the deposition of calcium (Ca) and phosphorus (P) (Sun et al. 2013; Vazquez et al. 2018; Kermani et al. 2023).

No study has been published on nutrients digestibility in broilers fed with deficient Ca and P diets and supplemented with cholecalciferol. The increased nutrients digestibility is directly related to the higher level of Ca (Abdollahi et al. 2016). From the results of the present study, it seems that vitamin D3 helps to absorbs Ca and P. further, the nutrients digestibility is linked with higher level of Ca and P which is evident from their deposition on tibia in the group fed with higher level of vitamin D3. Past research has indicated that the consumption of diets lacking essential nutrients adversely affects the efficient utilization of these nutrients (Dilger et al. 2004). Notably, P deficiency has been recognized for its negative influence on energy utilization and the processing of other nutrients. This is due to the fact that P is involved in numerous biological reactions that underpin energy production and related processes. However, further research is needed to understand the impact of vitamin D3 on the nutrients digestibility in broilers under deficient levels of Ca and P.

Conclusion

From the results of the present findings we concluded that supplementation of cholecalciferol at 5000 IU/kg resulted in enhanced growth performance, mineralization of tibia and nutrients digestibility in broilers fed with 30% reduced Ca and P in their diets.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Ethical statement

The experiments were conducted according to The University of Agriculture, Animal Ethics Committee guidelines (Protocol Number, PS-123 dated, 3/4/2021).

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