DOI: 10.5433/1679-0359.2018v39n5p2205

1,25-dihydroxycholecalciferol from *Solanum glaucophyllum* supports normal growth and reduces the negative effects of calcium and phosphorus restriction on broilers' bone tissue

1,25 dihidroxicolecalciferol de origem herbal (*Solanum glaucophyllum*) suporta o desempenho normal e diminui os efeitos negativos da restrição de cálcio e fósforo sobre o desenvolvimento ósseo de frangos de corte

Flávio Medeiros Vieites[1]; Emanuele Brusamarelo[2]; Bruno Serpa Vieira[3*]; Felipe Gomes da Silva[4]; Christiane Silva Souza[5]; Gerusa da Silva Salles Corrêa[4]; João Garcia Caramori Júnior[4]; George Henrique Kling Moraes[5]

Abstract

Feed supplementation with 1,25-dihydroxycholecalciferol (1,25(OH)$_2$D$_3$) has been studied as a means to decrease the incidence of bone abnormalities in broilers and reduce dietary inclusion of calcium and phosphorus. The objective of this research was to determine the optimal level of feed supplementation with 1,25(OH)$_2$D$_3$ from *Solanum glaucophyllum* for growing broilers restricted in calcium and available phosphorus. A total of 648 day-old male chicks were distributed in a completely randomized design with six dietary treatments and six replications of 18 birds. Treatments consisted of one positive control (PC: corn-soybean-meal diet formulated to reach or exceed Rostagno et al. (2011) nutritional recommendations), one negative control (NC: PC diet with 15% reduction of calcium and available phosphorus), and four NC diets supplemented with 0.5, 1.0, 1.5, or 2.0 μg kg^{-1} of 1,25(OH)$_2$D$_3$. Performance and bone development characteristics were evaluated at days 21 and 33. Negative control did not modify broilers' performance in comparison with PC, but birds fed with NC plus 1.0 μg kg^{-1} of 1,25(OH)$_2$D$_3$ showed higher (p=0.01) daily weight gain than PC at day 21. Negative control reduced ($p < 0.01$) tibia weight, ash, breaking strength, and Seedor index at day 21, but supplementation with 1,25(OH)$_2$D$_3$ mitigated all these negative effects. A quadratic effect of 1,25(OH)$_2$D$_3$ level was found for daily weight gain (p=0.03), tibia weight ($p < 0.01$), breaking strength ($p < 0.01$), and Seedor index ($p < 0.01$) at this age. At day 33, NC broilers still had lower tibia weight (p=0.01), ash ($p < 0.01$), and Seedor index ($p < 0.01$) than those fed PC. Only tibia ash did not return to the same value as that observed in PC after NC supplementation with 1,25(OH)$_2$D$_3$. A quadratic effect ($p < 0.01$) of 1,25(OH)$_2$D$_3$ level was found for tibia ash and Seedor index at this age. On average, dietary supplementation of 1.15 μg kg^{-1} of 1,25(OH)$_2$D$_3$ was identified as the optimal level for bone characteristics in this trial. For performance, this level was 0.98 μg kg^{-1}. In conclusion, feed supplementation with 1,25(OH)$_2$D$_3$ from standardized

[1] Prof., Universidade Federal de Juiz de Fora, UFJF, Juiz de Fora, MG, Brasil. E-mail: fmvieites@yahoo.com.br
[2] Discente, Curso de Doutorado em Ciência Animal, Universidade Federal de Mato Grosso, UFMT, Cuiabá, MT, Brasil. E-mail: leli_zootecnia@hotmail.com
[3] Pesquisador Associado, UFMT, Cuiabá, MT, Brasil. E-mail: vieirabs@hotmail.com
[4] Profs., UFMT, Cuiabá, MT, Brasil. E-mail: felipe.melhoramento@gmail.com; gerusacorrea@hotmail.com; caramori.ufmt@gmail.com
[5] Profs., Universidade Federal de Viçosa, UFV, Viçosa, MG, Brasil. E-mail: christiane_s_souza@hotmail.com; ghkmoraes@gmail.com
* Author for correspondence

Received: July 06, 2017 - Approved: May 03, 2018

leaves of *Solanum glaucophyllum* sustains normal growth and reduces the negative effects of calcium and phosphorus restriction on broilers' bone tissue.

Key words: Chickens. Cholecalciferol. Seedor index. Vitamin D_3. Weight gain.

Resumo

A suplementação da dieta com 1,25-dihidroxicolecalciferol (1,25$(OH)_2D_3$) vem sendo estudada como alternativa para redução da incidência de desordens esqueléticas em frangos de corte e diminuição nos níveis dietéticos de cálcio e fósforo. O objetivo deste trabalho foi determinar o melhor nível de inclusão de 1,25$(OH)_2D_3$ de origem herbal (*Solanum glaucophyllum*) para frangos restritos em cálcio e fósforo. Para tanto, 648 pintainhos de corte machos de um dia foram distribuídos em um delineamento inteiramente casualizado com seis tratamentos e seis repetições de 18 aves. Os tratamentos consistiram em um controle positivo (CP: dieta à base de milho e farelo de soja, formulada para atender às recomendações nutricionais de Rostagno et al. (2011)), um controle negativo (CN: dieta CP com 15% de redução nos níveis de cálcio e fósforo disponível), e quatro dietas CN suplementadas com 0,5; 1,0; 1,5 ou 2,0 µg kg^{-1} de 1,25$(OH)_2D_3$. Desempenho produtivo e características ósseas foram avaliados nos dias 21 e 33. O consumo do CN não reduziu o desempenho das aves em comparação ao CP; porém, as aves alimentadas com o CN+1,0 µg kg^{-1} de 1,25$(OH)_2D_3$ apresentaram maior (p=0,01) ganho de peso do que as alimentadas com o CP aos 21 dias. A dieta CN reduziu (p < 0,01) o peso, a matéria mineral, a força de quebra e o índice de Seedor da tíbia aos 21 dias; porém, a suplementação com 1,25$(OH)_2D_3$ reverteu todos esses efeitos adversos. Efeito quadrático dos níveis de 1,25$(OH)_2D_3$ foi observado para ganho de peso (p=0,03), peso da tíbia (p < 0.01), força de quebra (p < 0,01), e índice de Seedor (p < 0,01) nessa idade. Aos 33 dias, frangos alimentados com a dieta CN continuaram apresentando menores peso de tíbia (p=0,01), matéria mineral (p < 0,01) e índice de Seedor (p < 0,01) do que os que receberam a dieta CP. Somente a matéria mineral da tíbia não retornou a valor semelhante ao observado nas aves CP após a suplementação com 1,25$(OH)_2D_3$. Efeito quadrático (p < 0,01) dos níveis de 1,25$(OH)_2D_3$ foi observado para matéria mineral e índice de Seedor da tíbia nessa idade. Em média, 1,15 µg kg^{-1} de 1,25$(OH)_2D_3$ foi determinado como o nível ideal de inclusão para maximizar as características ósseas dos frangos. Para maximizar o desempenho produtivo, o nível ideal foi de 0,98 µg kg^{-1}. Em conclusão, a suplementação da dieta com 1,25$(OH)_2D_3$ de origem herbal (*Solanum glaucophyllum*) suporta o desempenho normal e diminui os efeitos negativos da restrição de cálcio e fósforo sobre o desenvolvimento ósseo de frangos de corte.

Palavras-chave: Colecalciferol. Frango. Ganho de peso. Índice de Seedor. Vitamina D_3.

Introduction

Skeletal disorders have become an important issue in modern poultry production. Locomotive disturbances that lead to lameness or reduced mobility not only cause economical losses for producers and processing plants, but are also considered a "major cause of poor welfare in broilers" (EC, 2000). Many of these problems could be associated with inadequate bone mineralization as a consequence of nutrient restriction or inefficient mineral metabolization (ANGEL, 2007).

Bone-mineral matrix accounts for approximately 65% of total bone weight, and is composed mainly of calcium (Ca) and phosphorus (P) (RATH et al., 2000). Several metabolic pathways are activated in the interval between Ca and P absorption and their deposition into the bones. Most of these pathways are ultimately regulated by 1,25-dihydroxycholecalciferol (1,25$(OH)_2D_3$) and parathormone (LI et al., 2016; VIEITES et al., 2016). Endogenous synthesis of 1,25$(OH)_2D_3$ depends on a two-step hydroxylation of cholecalciferol (vitamin D_3): first in the liver, generating 25(OH)D_3 (PONCHON et al., 1969); second in the kidneys, generating 1,25$(OH)_2D_3$ (FRASER; KODICEK, 1970). Two comprehensive literature reviews (KUMAR, 1986; DELUCA, 2014) have compiled the most important scientific evidence supporting the concept that $1,25(OH)_2D_3$ triggers mechanisms that not only enhance mineral absorption in the intestine, but also decrease mineral excretion in the kidneys.

Given that $1,25(OH)_2D_3$ is the most active form of vitamin D_3 in the organism, Koreleski and Swiatkiewicz (2005) stated that the occurrence of rickets and tibial dyschondroplasia in modern broilers fed adequate levels of vitamin D_3 may indicate that the endogenous conversion of vitamin D_3 into $25(OH)D_3$ and $1,25(OH)_2D_3$ is not sufficient for satisfactory bone mineralization. Based on that, dietary supplementation with $25(OH)D_3$ or $1,25(OH)_2D_3$ has been studied as a means to decrease the incidence of bone abnormalities in commercial broilers (GARCIA et al., 2013; SOUZA; VIEITES, 2014). It can be further hypothesized that the use of these molecules as feed additives might support a reduction in dietary levels of Ca and P, potentially decreasing feed costs and lessening the environmental impact associated with poultry production.

The plant *Solanum glaucophyllum* has long been investigated as a plausible source of $1,25(OH)_2D_3$ (NAPOLI et al., 1977), since its leaves contain a significant amount of this compound (BACHMANN et al., 2013). Previous data from our laboratory (VIEITES et al., 2014) demonstrated that the dietary inclusion of standardized leaves of *Solanum glaucophyllum* (10 µg g^{-1} of $1,25(OH)_2D_3$) up to 2.5 g kg^{-1} is safe for broilers fed normal levels of Ca and P. Additionally, Cheng et al. (2004) demonstrated that supplementation with *Solanum glaucophyllum* up to 5 g kg^{-1} for broilers restricted in Ca and P improves weight gain, plasma P, and tibia ash content. Although other positive results in favor of *Solanum glaucophyllum* have been demonstrated recently (MATHIS et al., 2016), the use of feed additives based on this plant needs further investigation, especially because the literature is still unclear about the most appropriate combination of Ca, P, and $1,25(OH)_2D_3$ for broilers.

The objective of this research was to determine the best level of feed supplementation with $1,25(OH)_2D_3$ from standardized leaves of *Solanum glaucophyllum* for growing broilers restricted in calcium and available phosphorus.

Materials and Methods

Animals and experimental design

A total of 648 day-old male chicks (Cobb 500) were individually weighed and distributed in 36 concrete floor pens (1.0 m x 1.86 m), covered with rice husk litter. Each pen enclosed 18 birds and was adopted as one replication in a completely randomized design with six dietary treatments and six replications. Feed and water were provided *ad libitum* and animals were raised under thermoneutral condition in a 24 h per day light regimen throughout the experimental period. Treatments and experimental procedures were previously approved by the Committee of Ethics in the Use of Animals of the Federal Institute of Education, Science and Technology of Mato Grosso (# 23197.001804/2013-02).

Treatments consisted of one positive control (PC): corn-soybean-meal diet formulated to reach or exceed Rostagno et al. (2011) nutritional recommendations; one negative control (NC): PC with 15% reduction of Ca and available P; and four NC diets supplemented with 0.5, 1.0, 1.5, or 2.0 µg kg^{-1} of $1,25(OH)_2D_3$. A commercial product composed of dried standardized leaves of *Solanum glaucophyllum* was used as the $1,25(OH)_2D_3$ source (10 µg g^{-1}) and was incorporated into the feed in place of filler. A more detailed description of the experimental diets is presented in Table 1.

Performance and bone development assessment

Birds and feeders were weighed at days 21 and 33 to calculate average body weight and feed consumption. Mortality was checked daily and used to correct feed conversion calculations. After performance assessment, one bird per pen was randomly selected and sacrificed by cervical dislocation. Both tibias were dissected and frozen at -20ºC for further evaluation.

Table 1. Positive-control diet composition (as-fed basis)[1].

Ingredient (%)	1-7 days	8-21 days	22-33 days
Corn (7.88%)	55.59	58.86	61.77
Soybean meal (45%)	39.85	36.63	33.02
Soybean oil	0.00	0.47	1.44
Dicalcium phosphate	1.89	1.54	1.32
Limestone	0.92	0.95	0.89
Salt (NaCl)	0.51	0.48	0.46
Mineral-vitamin premix[2]	0.20	0.20	0.20
Choline chloride (60%)	0.01	0.01	0.01
L-Lys	0.38	0.32	0.33
DL-Met	0.35	0.30	0.28
L-Thr	0.09	0.06	0.05
Antioxidant (BHT)	0.10	0.10	0.10
Virginiamycin (50%)	0.01	0.01	0.01
Salinomicin (12%)	0.01	0.01	0.01
Filler (sand)	0.11	0.07	0.12
Calculated composition (%, unless otherwise indicated)			
Metabolizable energy (kcal kg^{-1})	2982	3050	3150
Crude protein	22.42	21.20	19.80
Calcium (Ca)	0.92	0.84	0.76
Available phosphorus (aP)	0.47	0.40	0.35
Sodium	0.22	0.21	0.20
Digestible Lys	1.33	1.22	1.13
Digestible Met+Cys	0.95	0.88	0.83
Digestible Thr	0.86	0.79	0.74
Digestible Trp	0.26	0.24	0.22

[1] Negative control diets (NC) were obtained by reducing Ca and aP from limestone and dicalcium phosphate, respectively, by 15% (NC for 1-7 days: Ca=0.78%, aP=0.40%; NC for 8-21 days: Ca=0.71%, aP=0.34%; NC for 22-33 days: Ca=0.65%, aP=0.30%). Also, to assess the effects of $1,25(OH)_2D_3$ in Ca- and P-restricted birds, negative control diets were supplemented with 0.5, 1.0, 1.5, and 2.0 µg kg^{-1} of standardized leaves of *Solanum glaucophyllum* (10 µg g^{-1} of $1,25(OH)_2D_3$).
[2] Provided in 1 kg of product: Vit A 5,000,000 UI; Vit D_3 1,000,000 UI; Vit E 15,000 UI; Vit B_1 1 g; Vit B_6 2 g; Pantothenic acid 6 g; Biotin 0.05 g; Vit K_3 1.5 g; Folic acid 0.5 g; Nicotinic acid 25 g; Vit B_{12} 7,500 µg; Se 0.125 g; Mn 8 g; Fe 50 g; Zn 50 g; Cu 10 g; Co 1 g; I 1 g.

After a 30-day storage period, right tibias were defrosted at room temperature, manually cleaned of all adhered soft tissue, and submitted to a breaking strength assay using a universal machine for mechanical testing with 10 t capacity (Contenco®, Brazil). For that, tibias were placed in the same position, with epiphyses supported on hard stands and load applied to the center of the diaphysis, following the recommendations of the American Society of Agricultural Engineering (ASAE, 1992). The distance between stands was 4.5 and 5.4 cm for bones from 21 and 33 day-old broilers, respectively. The load applied to bones at the moment of breakage was automatically recorded as their breaking strength.

The same defrosting and cleaning procedures were used for left tibias, which were then weighed, and their length was measured using a digital caliper. To determine ash content, they were defatted with petroleum ether for 8 h in a *Soxhlet* extractor, dried at 105ºC for 12 h, then burned overnight at 600ºC in a muffle furnace. Seedor index was then calculated by dividing the total ash content of the tibia (mg) by its length (mm) (SEEDOR et al., 2005).

Statistical analysis

Data were initially tested for normality of studentized residuals and homogeneity of variances. Once these assumptions were ensured, one-way ANOVA was performed and, in the case of significant differences, the positive control was contrasted with the other treatments by Dunnett's t-test. Additionally, variables were regressed against supplementary $1,25(OH)_2D_3$ levels using a linear regression model. The positive control treatment was not included in the regression model. All statistical procedures were performed in SAS software, Version 9.0 (SAS Institute Inc, Cary, NC, USA) and statistical significance was set at $p \leq 0.05$.

Results

Negative control did not modify broilers' performance at days 21 or 33 in comparison to PC (Table 2). However, birds fed NC plus 1.0 μg kg^{-1} of $1,25(OH)_2D_3$ showed higher (p=0.01) daily weight gain than PC at day 21. A significant quadratic effect (p=0.03) of $1,25(OH)_2D_3$ level was found for daily weight gain at day 21. The calculated level of $1,25(OH)_2D_3$ for maximum daily weight gain at this age was 0.98 μg kg^{-1} (Figure 1A).

Table 2. Performance of broilers restricted in calcium and available phosphorus and supplemented with $1,25(OH)_2D_3$ from *Solanum glaucophyllum*.

Treatment	1-21 days			1-33 days		
	DWG (g day^{-1})	DFI (g day^{-1})	FCR (g:g)	DWG (g day^{-1})	DFI (g day^{-1})	FCR (g:g)
Positive control (PC)	35.56	55.17	1.55	53.26	84.82	1.59
Negative control (NC)	36.63	55.98	1.53	54.21	84.43	1.56
NC+0.5 μg kg^{-1} of $1,25(OH)_2D_3$	37.38	54.16	1.50	54.36	86.47	1.59
NC+1.0 μg kg^{-1} of $1,25(OH)_2D_3$	38.35*	56.83	1.48	55.26	86.46	1.56
NC+1.5 μg kg^{-1} of $1,25(OH)_2D_3$	36.97	56.37	1.53	54.44	86.30	1.59
NC+2.0 μg kg^{-1} of $1,25(OH)_2D_3$	36.67	53.00	1.45	53.53	82.73	1.55
SEM	0.24	0.58	0.02	0.24	0.70	0.01
Dunnett's t-test probabilities						
PC vs others	0.01	0.39	0.54	0.18	0.60	0.83
Regression probabilities (without PC)						
Linear	0.86	0.33	0.22	0.51	0.51	0.74
Quadratic	0.03	0.17	0.77	0.09	0.07	0.33

DWG = daily weight gain; DFI = daily feed intake; FCR = feed conversion ratio
Means followed by "*" differ significantly ($p \leq 0.05$) from PC by Dunnett's t-test.

On day 21, NC treatment reduced ($p<0.01$) tibia weight, ash, breaking strength, and Seedor index (Table 3). However, irrespective of the level, supplementing NC with $1,25(OH)_2D_3$ brought tibia breaking strength and Seedor index back to similar values as those observed in PC. Tibia weight was not different than PC when NC was supplemented with 1.0 or 1.5 µg kg^{-1} of $1,25(OH)_2D_3$. Nevertheless, tibia ash returned to a similar value as that observed in PC only when NC was supplemented with 0.5 µg kg^{-1} of $1,25(OH)_2D_3$. No effect of treatments was observed on tibia length. A quadratic effect ($p<0.01$) for $1,25(OH)_2D_3$ level was detected for tibia weight, breaking strength, and Seedor index. The calculated levels of $1,25(OH)_2D_3$ for maximum tibia weight, tibia breaking strength, and tibia Seedor index at 21 days were 1.08, 1.22, and 1.15 µg kg^{-1}, respectively (Figures 1B, 1C, and 1D).

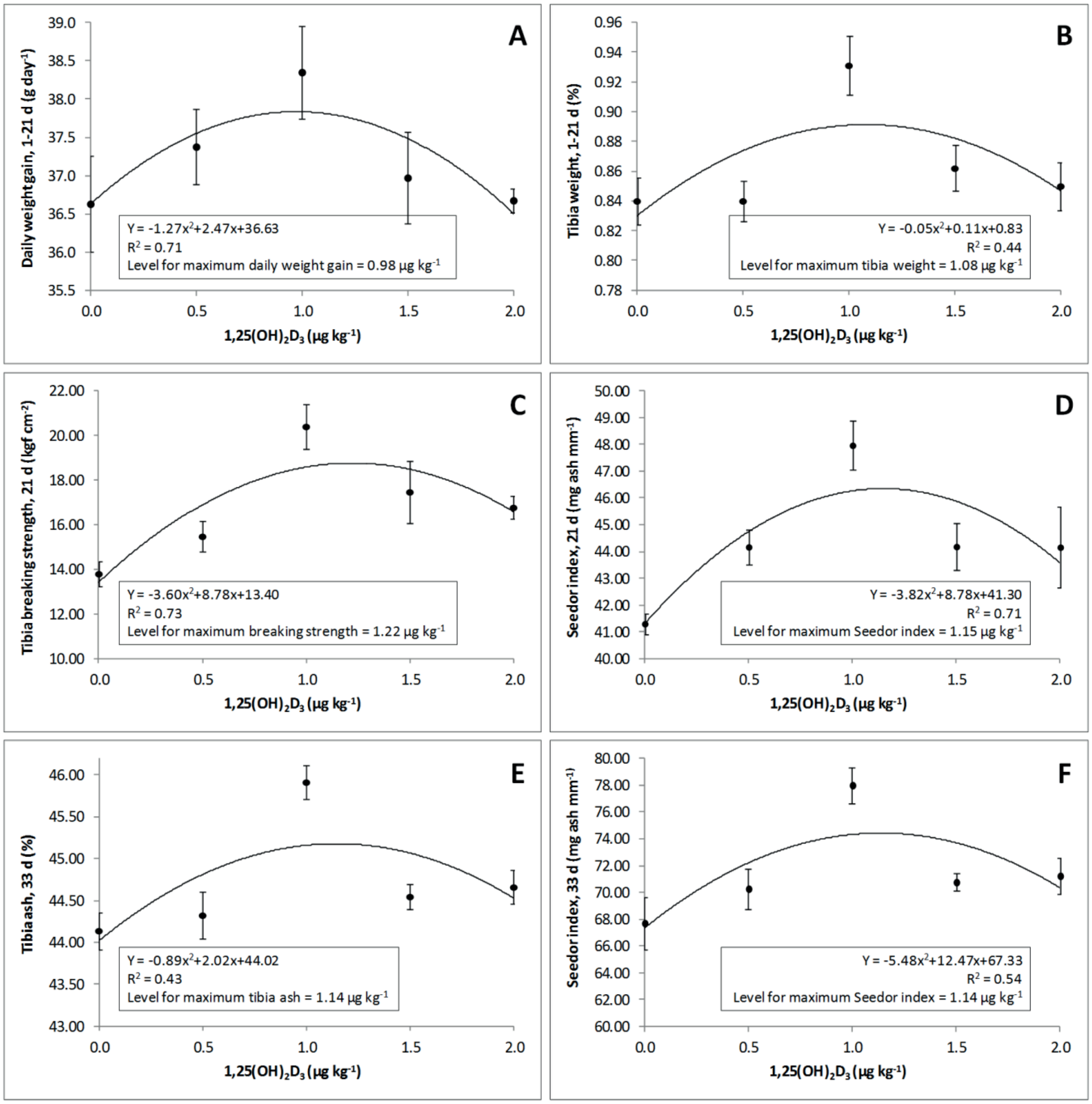
Table 3. Bone development characteristics of broilers restricted in calcium and available phosphorus and supplemented with $1,25(OH)_2D_3$ from *Solanum glaucophyllum*.

Treatment	Tibia characteristics - 21 days					Tibia characteristics - 33 days				
	Weight[1] (%)	Length (mm)	Ash[2] (%)	Breaking strength (kgf cm^{-2})	Seedor[3] (mg mm^{-1})	Weight[1] (%)	Length (mm)	Ash[2] (%)	Breaking strength (kgf cm^{-2})	Seedor[3] (mg mm^{-1})
Positive control (PC)	0.91	71.17	48.37	17.50	46.38	0.89	95.08	47.12	26.95	77.42
Negative control (NC)	0.84*	71.50	46.01*	13.80*	41.30*	0.79*	92.09	44.14*	25.85	67.69*
NC+0.5 µg kg^{-1} of $1,25(OH)_2D_3$	0.84*	70.69	47.30	15.48	44.17	0.83	93.63	44.33*	22.78	70.26*
NC+1.0 µg kg^{-1} of $1,25(OH)_2D_3$	0.93	72.12	46.17*	20.40	47.96	0.86	92.45	45.92*	24.75	77.99
NC+1.5 µg kg^{-1} of $1,25(OH)_2D_3$	0.86	71.62	46.79*	17.47	44.18	0.82*	93.20	44.55*	25.95	70.75*
NC+2.0 µg kg^{-1} of $1,25(OH)_2D_3$	0.85*	69.54	46.89*	16.77	44.16	0.83	91.76	44.66*	25.05	71.22*
SEM	0.01	0.35	0.16	0.47	0.48	0.01	0.36	0.20	0.43	0.81
Dunnett's t-test probabilities										
PC vs others	<0.01	0.36	<0.01	<0.01	<0.01	0.01	0.08	<0.01	0.17	<0.01
Regression probabilities (without PC)										
Linear	0.50	0.31	0.29	0.04	0.15	0.32	0.65	0.23	0.80	0.29
Quadratic	<0.01	0.19	0.17	<0.01	<0.01	0.06	0.16	<0.01	0.46	<0.01

[1] tibia weight (%) = (tibia weight (g) ÷ bird's live weight (g)) × 100
[2] tibia ash (%) = (tibia ash (g) ÷ tibia weight (g)) × 100
[3] Seedor index (mg mm^{-1}) = tibia ash (mg) ÷ tibia lengh (mm)
Means followed by "*" differ significantly ($p \leq 0.05$) from PC by Dunnett's t-test.

On day 33, tibia weight (p=0.01), ash (p<0.01), and Seedor index (p<0.01) were still lower than PC in broilers fed the NC treatment (Table 3). While almost all levels of $1,25(OH)_2D_3$ supplementation increased tibia weight to similar values as that obtained in PC, Seedor index was similar to PC only when NC was supplemented with 1.0 μg kg^{-1} of $1,25(OH)_2D_3$. Nevertheless, tibia ash was persistently lower than PC even after supplementing NC with $1,25(OH)_2D_3$. No effect of treatments was observed on tibia length. A quadratic effect (p<0.01) of $1,25(OH)_2D_3$ level was found for tibia ash and Seedor index at day 33. The calculated level of $1,25(OH)_2D_3$ for maximum tibia ash and Seedor index at this age was 1.14 μg kg^{-1} for both variables (Figures 1E, 1F).

Figure 1. Quadratic regressions of performance and bone characteristics against $1,25(OH)_2D_3$ levels for broilers restricted in calcium and available phosphorus.



Tibia weight (%) = (tibia weight (g) ÷ bird's live weight (g)) × 100
Tibia ash (%) = (tibia ash (g) ÷ tibia weight (g)) × 100
Seedor index (mg ash mm^{-1}) = tibia ash (mg) ÷ tibia lengh (mm)

Discussion

Performance parameters were not affected by the NC treatment, but significant impairment of bone characteristics was observed in NC birds. Scheideler et al. (1995) demonstrated the ability of broilers to sustain normal growth rates even when fed diets marginally unbalanced in Ca and P. In order to maintain blood mineral homeostasis and minimize the impact of Ca and P restriction on growth, metabolic responses induce higher resorption of those minerals from bones (OBA et al., 2012). This mechanism almost certainly contributed to the reduced quality of the tibia observed in NC birds in this trial.

With the exception of tibia ash content at day 33, supplementing NC with 1,25$(OH)_2D_3$ mitigated all the negative effects of Ca and P restriction on bone characteristics in a dose-dependent response model. It is well know that 1,25$(OH)_2D_3$ is the major endogenous regulator of Ca metabolism in birds and, together with parathormone, it increases intestinal Ca absorption, intensifies Ca mobilization from the bones, and decreases renal Ca excretion (VELDURTHY et al., 2016). These activities seem to be mediated by the activation of vitamin D receptor genes, expressed in almost all tissues and cells of the body (HAUSSLER et al., 1997; VERSTUYF et al., 2010; MAESTRO et al., 2016).

On average, 1.15 µg kg^{-1} of 1,25$(OH)_2D_3$ was determined to be the optimal supplementation level for bone characteristics in this trial. Not by chance, treatment NC plus 1.0 µg kg^{-1} of 1,25$(OH)_2D_3$ mitigated the undesirable effects of Ca and P restriction in almost all bone characteristics. Higher levels of 1,25$(OH)_2D_3$ supplementation had negative effects on tibia weight, ash, breaking strength, and Seedor index, responses probably associated with the distorted degree of Ca and P mobilization from bones induced by the excess of 1,25$(OH)_2D_3$ in blood (DELUCA, 2004). According to the European Food Safety Authority (EFSA, 2015), the high level of glycosylation observed in 1,25$(OH)_2D_3$ molecules from *Solanum glaucophyllum* leads to a decrease in its biopotency, which is equivalent to only 25% of the biopotency of vitamin D_3. Despite that, our data showed that dietary supplementation with 1,25$(OH)_2D_3$ from *Solanum glaucophyllum* must be evaluated carefully, as all the absorbed 1,25$(OH)_2D_3$ goes directly into the blood stream, escaping the regulatory mechanisms involved in the control of the endogenous conversion of vitamin D_3 into its active form.

Even though one could assume that the relatively low R^2 values found in our regression models negatively affected the precision of the estimates for best performance and bone characteristics, the high similarity between estimates for all variables and the highly significant *p* values observed for the quadratic models should be considered as proof of their strength. While not atypical, the level of dispersion (SEMs) observed here was higher than what was expected; this probably contributed to the relatively low values of R^2. To the best of our knowledge, all the important sources of variation were properly controlled in both field and laboratory assays, and no apparent reasons for this situation were detected. Possibly, the level of dispersion in our data would be lower had a higher number of replications per treatment been adopted (BLAINEY et al., 2014).

Vitamin D_3 is not nutritionally essential for poultry (SOUZA; VIEITES, 2014); however, its supplementation through the diet is imperative in modern poultry production, because otherwise exposing birds to ultraviolet rays would be mandatory as a way to promote vitamin D_3 synthesis from cholesterol (PIZAURO JUNIOR et al., 2002). While vitamin D_3 has traditionally been supplemented as cholecalciferol, feeding broilers with vitamin D_3 active form (1,25$(OH)_2D_3$) might be an alternative for improving dietary Ca and P utilization. With this approach, it was demonstrated that a 15% reduction in dietary Ca and available P can be sustained by 1,25$(OH)_2D_3$ supplementation without negative effects on broilers' performance and most of their bone characteristics. Nevertheless, vitamin D_3 was present in our experimental diets in both vitamin premix and vegetable feedstuff. Thus, further studies are needed to investigate the effects of $1,25(OH)_2D_3$ supplementation for broilers fed diets with different levels of Ca, P, and vitamin D_3. Liver and kidneys function tests should also be performed in future trials.

Conclusions

Dietary supplementation with $1,25(OH)_2D_3$ from standardized leaves of *Solanum glaucophyllum* could be adopted as a way to support normal growth and reduce the negative effects of a 15% reduction in dietary calcium and available phosphorus on broilers' bone tissue. On average, the optimal dietary level of $1,25(OH)_2D_3$ for bone characteristics is 1.15 µg kg^{-1} of feed. For performance, this level is 0.98 µg kg^{-1} of feed.

Acknowledgements

The second author thanks Capes/Brazil for granting a MSc scholarship.

References

AMERICAN SOCIETY OF AGRICULTURAL ENGINEERS - ASAE. Shear and three-point bending test of animal bone. In: American Society of Agricultural and Biological Engineers. Saint Joseph: ASABE, 1992. 583 p.

ANGEL, R. Metabolic disorders: limitations to growth of and mineral deposition into the broiler skeleton after hatch and potential implications for leg problems. *The Journal of Applied Poultry Research*, Oxford, v. 16, n. 1, p. 138-149, 2007.

BACHMANN, H.; AUTZEN, S.; FREY, U.; WEHR, U.; RAMBECK, W.; MCCORMACK, H.; WHITEHEAD, C. C. The efficacy of a standardised product from dried leaves of *Solanum glaucophyllum* as source of 1,25-dihydroxycholecalciferol for poultry. *British Poultry Science*, Abingdon, v. 54, n. 5, p. 642-652, 2013.

BLAINEY, P.; KRZYWINSKI, M.; ALTMAN, N. Points of significance: replication. *Nature Methods*, New York, v. 11, n. 9, p. 879-880, 2014.

CHENG, Y. H.; GOFF, J. P.; SELL, J. L.; DALLORSO, M. E.; GIL, S.; PAWLAK, S. E.; HORST, R. L. Utilizing *Solanum glaucophyllum* alone or with phytase to improve phosphorus utilization in broilers. *Poultry Science*, Champaign, v. 83, n. 3, p. 406-413, 2004.

DELUCA, H. F. History of the discovery of vitamin D and its active metabolites. *BoneKEy Reports*, London, v. 3, n. 479, p. 1-8, 2014.

DELUCA, H. F. Overview of general physiologic features and functions of vitamin D. *The American Journal of Clinical Nutrition*, Rockvile, v. 80, n. 6, p. 1689S-1696S, 2004.

EUROPEAN COMISSION - EC. The welfare of chickens kept for meat production (broilers). Report of the Scientific Committee on Animal Health and Animal Welfare. Parma: EFSA, 2000. 149 p.

EUROPEAN FOOD SAFETY AUTHORITY - EFSA. Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). Scientific opinion on the safety of solanum glaucophyllum standardised leaves as feed material. *EFSA Journal*, Parma, v. 13, n. 1, p. 1-43, 2015.

FRASER, D. R.; KODICEK, E. Unique biosynthesis by kidney of a biologically active vitamin D metabolite. *Nature*, New York, v. 228, n. 21, p. 764-766, 1970.

GARCIA, A. F. Q. M.; MURAKAMI, A. E.; DUARTE, C. R. do A.; ROJAS, I. C. O.; PICOLI, K. P.; PUZOTTI, M. M. Use of vitamin D3 and its metabolites in broiler chicken feed on performance, bone parameters and meat quality. *Asian-Australasian Journal of Animal Sciences*, Seoul, v. 26, n. 3, p. 408-415, 2013.

HAUSSLER, M. R.; HAUSSLER, C. A.; JURUTKA, P. W.; THOMPSON, P. D.; HSIEH, J.-C.; REMUS, L. S.; SELZNICK, S. H.; WHITFIELD, G. K. The vitamin D hormone and its nuclear receptor: molecular actions and disease states. *Journal of Endocrinology*, Brossard, v. 154, n. 3, p. S57-S73, 1997.

KORELESKI, J.; SWIATKIEWICZ, S. Efficacy of different limestone particle size and 25-hydroxycholecalciferol in broiler diets. *Journal of Animal and Feed Sciences*, Jablonna, v. 14, n. 4, p. 705-714, 2005.

KUMAR, R. The metabolism and mechanism of action of 1,25-dihydroxyvitamin D3. *Kidney International*, Philadelphia, v. 30, n. 6, p. 793-803, 1986.

LI, X.; ZHANG, D.; YANG, T.; BRYDEN, W. Phosphorus bioavailability: a key aspect for conserving this critical animal feed resource with reference to broiler nutrition. *Agriculture*, Basel, v. 6, n. 2, p. 25-39, 2016.

MAESTRO, M. A.; MOLNAR, F.; MOURINO, A.; CARLBERG, C. Vitamin D receptor 2016: novel ligands and structural insights. *Expert Opinion on Therapeutic Patents*, London, v. 26, n. 11, p. 1291-1306, 2016.

MATHIS, G.; BOLAND, R.; BACHMANN, H.; TOGGENBURGER, A.; RAMBECK, W. Safety profile of 1,25-dihydroxyvitamin D3 of herbal origin in broiler chicken. *Schweiz Arch Tierheilkd*, Zurich, v. 158, n. 12, p. 819-826, 2016.

NAPOLI, J. L.; REEVE, L. E.; EISMAN, J. A.; SCHNOES, H. K.; DELUCA, H. F. *Solanum glaucophyllum* as source of 1, 25-dihydroxyvitamin D3. *Journal of Biological Chemistry*, Baltimore, v. 252, n. 8, p. 2580-2583, 1977.

OBA, A.; PINHEIRO, J. W.; SILVA, C. A. da; BALARIN, M. R. S.; BUENO, F. R.; ALMEIDA, M. de; AMARAL, R. V. do; BRUNELLI, S. R. Characteristics productive and meat quality of broiler chickens fed finisher diet without vitamin-mineral supplement or limestone and dicalcium phosphate and submitted to heat stress pre-slaughter. *Semina: Ciências Agrárias*, Londrina, v. 33, n. 2, p. 3371-3378, 2012.

PIZAURO JUNIOR, J. M.; CIANCAGLINI, P.; MACARI, M. Tibial dyschondroplasia: mechanisms of the lesion and control. *Revista Brasileira de Ciência Avícola*, Campinas, v. 4, n. 3, p. 169-186, 2002.

PONCHON, G.; KENNAN, A. L.; DELUCA, H. F. Activation of vitamin D by the liver. *Journal of Clinical Investigation*, Michigan, v. 48, n. 11, p. 2032-2037, 1969.

RATH, N. C.; HUFF, G. R.; HUFF, W. E.; BALOG, J. M. Factors regulating bone maturity and strength in poultry. *Poultry Science*, Champaign, v. 79, n. 7, p. 1024-1032, 2000.

ROSTAGNO, H. S.; ALBINO, L. F. T.; DONZELE, J. L.; GOMES, P. C.; OLIVEIRA, R. de; LOPES, D. C.; FERREIRA, A. S.; BARRETO, S. L. T.; EUCLIDES, R. F. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 3. ed. Viçosa, MG: Rostagno HS, 2011. 252 p.

SCHEIDELER, S. E.; RIVES, D. V.; GARLICH, J. D.; FERKET, P. R. Dietary calcium and phosphorus effects on broiler performance and the incidence of sudden death syndrome mortality. *Poultry Science*, Champaign, v. 74, n. 12, p. 2011-2018, 1995.

SEEDOR, J. G.; QUARTUCCIO, H. A.; THOMPSON, D. D. The biphosphonate aledronate (MK-217) inhibits bone loss due to ovariectomy in rats. *Journal of Bone and Mineral Research*, Washington, v. 20, n. 2, p. 354-362, 2005.

SOUZA, C. S.; VIEITES, F. M. Vitamin D3 and its metabolities for broilers. *Archivos de Zootecnia*, Córdoba, v. 63, n. 241, p. 11-24, 2014.

VELDURTHY, V.; WEI, R.; OZ, L.; DHAWAN, P.; JEON, Y. H.; CHRISTAKOS, S. Vitamin D, calcium homeostasis and aging. *Bone Research*, New York, v. 4, n. 1, p. 16041-16047, 2016.

VERSTUYF, A.; CARMELIET, G.; BOUILLON, R.; MATHIEU, C. Vitamin D: a pleiotropic hormone. *Kidney International*, Philadelphia, v. 78, n. 2, p. 140-145, 2010.

VIEITES, F. M.; DROSGHIC, L. C. A. B.; SOUZA, C. S.; VARGAS JÚNIOR, J. G.; NUNES, R. V.; MORAES, G. H. K. de; CORRÊA, G. S. S.; CARAMORI JÚNIOR, J. G. C. Bone characteristics of broiler chickens fed diets supplemented with *Solanum glaucophyllum*. *Semina: Ciências Agrárias*, Londrina, v. 37, n. 1, p. 381-392, 2016.

VIEITES, F. M.; NALON, R. P.; SANTOS, A. L.; CASTELO BRANCO, P. A.; SOUZA, C. S.; NUNES, R. V.; CALDERANO, A. A.; ARRUDA, N. V. M. de. Performance, carcass and noble cuts yield of broilers fed diets supplemented with *Solanum glaucophyllum*. *Semina: Ciencias Agrarias*, Londrina, v. 35, n. 3, p. 1617-1626, 2014.