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Non-ruminants

Effects of 1,25-dihydroxycholecalciferol and reduced vitamin D_3 level on broiler performance and bone quality

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ABSTRACT - This study was conducted to evaluate the effect of two levels of vitamin D_3 with or without 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) on live performance and bone quality of broiler chickens. For that, we used a completely randomized design in a 2 × 2 factorial arrangement, with eight replicates of 30 Cobb®500 male broiler chicks each (n = 960). The two levels of vitamin D_3 and the addition or not of 0.5 µg $1,25(OH)_2D_3$/kg were considered as main factors. The vitamin D_3 levels were: 2500/2000 IU/kg and 1250/1000 IU/kg for the starter (1 to 21 days) and grower (22 to 40 days) phases, respectively, with the first representing the levels used in industry (100%) and the second, a reduction in 50% of those levels. The $1,25(OH)_2D_3$ source was *Solanum glaucophyllum*. On days 21 and 40, one broiler per replicate was killed and long bones were removed for analyses of mineral percentage, bone mineral density, biomechanical properties, and morphology. No significant differences were found related to vitamin D_3 levels and the addition or not of $1,25(OH)_2D_3$ for live performance, mineral percentage, strength, stiffness, and morphology. Toughness was lower when $1,25(OH)_2D_3$ was used at 21 days, but this effect was not observed at 40 days of age. Bone mineral density was greater when 100% of vitamin D_3 was used at 40 days of age. The reduction of up to 50% of vitamin D_3 levels is sufficient to ensure performance and bone development of broilers at 21 and 40 days of age. The inclusion of 0.5 µg $1,25(OH)_2D_3$/kg in addition to diets with sufficient levels of vitamin D_3 shows no effect on the improvement of those parameters at the same ages.

Key Words: broilers, growth performance, *Solanum glaucophyllum*

Introduction

To fulfill the growing demand for food, the use of highly specialized broilers with genetic potential for growth has increased. The rapid muscle development is not followed by adequate bone support, which remain immature, burdening the locomotor system. As a result, there is an increase in mortality and fractures due to bone fragility and reduction in welfare, leading to significant economic losses (Silva et al., 2001; Araujo et al., 2012). The formulation of specific diets using vitamin D and its metabolites has been shown as an alternative to reduce these problems.

The vitamin D content available in raw materials used in diets is usually ignored during formulation, and the need for this vitamin is supplied by adding vitamin supplements. As vitamin D_2 (ergocalciferol) potency is about 10 times lower than vitamin D_3 (cholecalciferol) for poultry, the last one is often used. To reach its main metabolically active form, 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$), vitamin D_3 must be hydroxylated first in the liver into 25-hydroxycholecalciferol ($25(OH)D_3$), then in the kidneys (Souza and Vieites, 2014). $1,25(OH)_2D_3$ has an important role in calcium and phosphorus homeostasis, bone growth and remodeling, and in the immune system (Kochupillai, 2008; Muszkat et al., 2010).

According to NRC and Cobb® 500 manual, the recommended vitamin D_3 levels for poultry are 200 and 5000 IU/kg, respectively (NRC, 1994; Cobb, 2015). The deficiency or imbalance of vitamins and minerals is associated with the development of skeletal disorders, such as rickets (Klasing, 2013). However, the companies have been working with a safety margin of about five to ten times higher than the actual need, using supplementation as a preventative tool. The excess of vitamin D can be toxic to tissues by inducing the mineralization of soft tissues,

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has negative effect on leg health, decreases body weight gain, and increases feed cost (Cruickshank and Sim, 1987; Zanuzzi et al., 2012).

The supplementation with different metabolites and sources of vitamin D_3 is a method for maximizing animal performance. The use of metabolites may reduce the energy expenditure, since they are in an advanced form, thus, more available for immediate utilization (Garcia et al., 2013).

The objective was to evaluate the effects of two vitamin D_3 levels with or without $1,25(OH)_2D_3$ supplementation in diets on live performance and bone development of broilers grown under conditions simulating commercial poultry production.

Material and Methods

The experiment was conducted in Igarapé, Brazil (20°04'13"S and 44°18'06" W), under approval of the Ethical Principles in Animal Experimentation Committee (case no. 225/2014).

The experimental design was completely randomized, consisting of a 2 × 2 factorial arrangement: the presence or absence of $1,25(OH)_2D_3$ and two different dietary levels of vitamin D_3, eight replicates, and 30 birds per experimental unit.

One-day-old Cobb® 500 male chicks (n = 960) were obtained from a local hatchery and assigned to floor pens in a poultry house of commercial and experimental design with curtain sidewalls. Thirty chicks were randomly allocated to each of the 32 identical pens (14 birds/m^2). Wood shavings were used as bedding, and each pen was equipped with an automatic water fountain and a tube-type feeder, providing *ad libitum* access to feed and water throughout the study. The light program was 24 h of light in the first 14 days and natural light after this period.

The diets were based on corn and soybean meal and formulated for starter (1 to 21 days) and grower (22 to 40 days) phases. The diets were formulated considering the nutritional values of the raw materials according to Brazilian tables for poultry and swine (Rostagno et al., 2011) (Table 1).

Table 1 - Composition of diets for all phases (1-40 days, as-fed basis; g kg^{-1})

Item	Starter (1 to 21 days)[1]*		Grower (22 to 40 days)[2]**	
Ingredient				
Yellow corn	573.40	573.40	630.00	630.00
Soybean meal (45% crude protein)	350.00	350.00	291.90	291.70
Meat and bone meal (40% crude protein)	36.70	36.70	21.60	21.60
Soybean oil	22.50	22.50	36.60	36.60
Limestone	5.50	5.50	6.60	6.60
Salt	3.90	3.95	4.20	4.25
DL-methionine (98%)	3.20	3.20	3.10	3.10
Vitamin and mineral premix*	2.00	2.00	2.00	2.00
L-lysine HCl (98%)	1.80	1.70	2.70	2.80
Choline chloride (60%)	0.60	0.60	0.50	0.50
L-threonine	0.40	0.40	0.80	0.80
Solanum glaucophyllum ($1,25(OH)_2D_3$)	-	0.05	-	0.05
Nutrient levels				
Metabolizable energy (kcal/kg)	2,997	2,997	3,152	3,152
Crude protein (g/kg)	225.35	225.35	197.92	197.92
Ether extract (g/kg)	53.19	53.19	66.40	66.40
Calcium (g/kg)	9.32	9.32	7.64	7.64
Total P (g/kg)	7.00	7.00	5.80	5.80
Nonphytate P (g/kg)	4.67	4.67	3.63	3.63
Sodium (g/kg)	2.03	2.03	1.99	1.99
Lysine (g/kg)	12.11	12.11	11.29	11.29
Methionine (g/kg)	6.07	6.07	5.67	5.67
Methionine + cystine (g/kg)	8.99	8.99	8.29	8.29
Threonine (g/kg)	7.91	7.91	7.29	7.29
Tryptophan (g/kg)	2.37	2.37	2.03	2.03

* Mineral and vitamin premix (starter period) provided per kg of diet: vitamin A, 9,000 UI; vitamin E, 14 mg; vitamin K_3, 2 mg; vitamin B_1, 2.5 mg; vitamin B_2, 6.2 mg; vitamin B_6, 4 mg; vitamin B_{12}, 14 mcg; nicotinic acid, 40 mg; folic acid, 1 mg; pantothenic acid, 15 mg; Se, 0.2 mg; I, 1.2 mg; Fe, 50 mg; Cu, 10 mg; Mn, 80 mg; Zn, 60 mg; Finase, 500 FTU; Halquinol, 0.03 g; MNGrow, 0.5 g; BHT, 0.1 g.

** Mineral and vitamin premix (grower period) provided per kg of diet: vitamin A, 7,000 UI; vitamin E, 11 mg; vitamin K_3, 1.6 mg; vitamin B_1, 1.6 mg; vitamin B_2, 4.5 mg; vitamin B_6, 2.2 mg; vitamin B_{12}, 10 mcg; nicotinic acid, 32 mg; folic acid, 0.8 mg; pantothenic acid, 12 mg; Se 0.2, mg; I, 1.2 mg; Fe, 50 mg; Cu, 10 mg; Mn, 80 mg; Z, 60 mg; Finase, 500 FTU; Halquinol 0.03 g; Salynomicin, 0.066 g; BHT, 0.1 g.

[1] Vitamin D_3: 2500 (100%) and 1250 (50%) µg/kg.

[2] Vitamin D_3: 2000 (100%) and 1000 (50%) µg/kg.

The vitamin D_3 was provided by two different vitamin supplements, which were the only sources of vitamin D_3 considered during feed formulation. The vitamin supplements contained: 2500 and 2000 IU vitamin D_3/kg of feed for starter and grower phases, respectively (100% according to commercial levels), and 1250 and 1000 IU vitamin D_3/kg of feed for starter and grower phases, respectively (reduction of 50%). The $1,25(OH)_2D_3$ source was a commercial product obtained from dried leaves of *Solanum glaucophyllum* (SG) (10 ppm). The inclusion was of 50 g/ton of feed according to manufacturer's recommendations, resulting in an addition of 0.5 μg $1,25(OH)_2D_3$/kg of feed in a glycosidic form. The presence of metabolite in the plant extract and its activity were characterized by Napoli et al. (1977), Gil et al. (2006), and Bachmann et al. (2013).

Birds and feed were weighed weekly throughout the experimental period to assess performance (average daily feed intake, average daily gain, and feed conversion ratio). These data were used to calculate the accumulated values at 21 and 40 days of the experiment. Mortality was checked daily and used to adjust feed conversion ratio, obtained by average daily feed intake:average daily gain.

At 21 and 40 days, one bird per pen, selected from a range of mean body weight ± 10%, was killed by cervical dislocation. The right and left femora and left and right tibiotarsi were removed, dissected, and cleaned of any adhering tissue. For gross evaluation of long bones, the left femora and right tibiotarsi were sectioned longitudinally to reveal the growth plates (hypertrophy and proliferation zones). This technique allowed the visual evaluation of the cortex thickness and the amount and density of trabecular bone and cartilage in metaphyseal and epiphyseal regions. The right femora were analyzed for ash, calcium, and phosphorus percentage determination on dry fat-free bones, as described by AOAC (1995). The left tibiotarsi were first subjected to the optical densitometry (g/cm^2) analysis using the DPX-ALPHA densitometer model to determine bone mineral density (BMD). Later, they were subjected to a biomechanical assay using a universal machine EMIC® DL 300 model, in a three-point destructive bending test, with a 2000N load cell. The software Instron Series IX recorded the values of breaking strength (determined by maximum load), stiffness, and toughness.

The pen means were the experimental units for broiler performance data and broiler was the experimental unit for the bone parameter data. The means were subjected to ANOVA as a factorial arrangement of treatments with dietary vitamin D_3 levels and presence or absence of $1,25(OH)_2D_3$ as the main effects. All possible interaction among and between the main effects were evaluated using the general linear model procedure of SAS software (Statistical Analysis System, version 9.0). Statements of significance were based on $P \leq 0.05$.

Results

There was no interaction of dietary vitamin D_3 levels with presence or absence of $1,25(OH)_2D_3$ ($P>0.05$) for the performance variables. At 21 and 40 days, the average daily feed intake, average daily gain, and feed conversion ratio were not influenced by the level or additional source of vitamin D_3 ($P>0.05$) (Table 2).

Mortality during the present study was not significantly affected by level of vitamin D_3 or inclusion of the metabolite, with no interaction between the levels and presence or absence of $1,25(OH)_2D_3$ ($P>0.05$) (data not shown).

There was no interaction of dietary vitamin D_3 levels with presence or absence of $1,25(OH)_2D_3$ for ash, calcium, and phosphorus content at 21 and 40 days ($P>0.05$) (Table 3). The mineral content was similar between the treatments at the two ages ($P>0.05$).

There was no interaction of dietary vitamin D_3 levels with or without the use of $1,25(OH)_2D_3$ for breaking strength, stiffness, and toughness at 21 and 40 days neither for BMD at 40 days of age ($P>0.05$) (Table 4). Breaking strength and stiffness were similar between the treatments at 21 and 40 days ($P>0.05$). Toughness was influenced by $1,25(OH)_2D_3$ at 21 days ($P \leq 0.05$). When the metabolite was used, toughness was lower than when the metabolite was not used; however, this difference was not observed at 40 days ($P>0.05$). Bone mineral density was not influenced by the addition or not of $1,25(OH)_2D_3$ at 40 days of age; however, this trait was affected by the vitamin D_3 levels. The use of 100% of vitamin D_3 resulted in greater BMD when compared with 50% of vitamin D_3 ($P \leq 0.05$).

During the gross evaluation of tibiotarsi of broilers at 21 and 40 days, the growth plates in all treatments were regular, with similar thickness, blood vessels, and trabecular bone well distributed, and without the presence of any avascular cartilage plug, which indicates absence of disease (data not shown).

Discussion

The vitamin D_3 deficiency can result in reduction in feed intake and lead to abnormal body development (Andriguetto et al., 2002). This fact was not observed in the present study, which indicates that the reduction in 50% of the vitamin D_3 level, regardless of the addition of 1,25$(OH)_2D_3$, provides the necessary amount of this vitamin for a normal performance of broilers. The body weights found in the present study, for all treatments, were greater than the ones determined by Cobb (2015), of 971 and 2832 g at 21 and 40 days of age, respectively.

These results are contrary to the ones found by Souza et al. (2013). The authors evaluated the performance of broilers supplemented with 1,25$(OH)_2D_3$ levels ranging from 0 to 5 μg/kg, reducing in 20% the levels of available calcium and phosphorus. Feed intake was not influenced by the treatments. However, there was a significant improvement in weight gain and feed conversion ratio when broilers were fed 1 and 2 μg 1,25$(OH)_2D_3$/kg at 42 days.

The results found in the present study are in accordance with Alves (2014). The author compared a control group

Table 2 - Effect of 1,25$(OH)_2D_3$ and vitamin D_3 levels on broiler average daily feed intake (ADFI), average daily weight gain (ADG), and feed conversion ratio (FCR) at 21 and 40 days of age

		Age (days)					
		1-21 days			1-40 days		
		ADFI (g)	ADG (g)	FCR (g/g)	ADFI (g)	ADG (g)	FCR (g/g)
Vitamin D_3	1,25$(OH)_2D_3$						
100% vitamin D_3[1]	0.0 μg/kg	62.12	47.87	1.298	117.50	77.00	1.524
	0.5 μg/kg	62.50	48.75	1.286	120.25	80.00	1.506
50% vitamin D_3[2]	0.0 μg/kg	61.50	47.50	1.294	118.25	78.12	1.515
	0.5 μg/kg	62.50	47.75	1.307	119.25	77.50	1.535
Vitamin D_3							
100% vitamin D_3		62.31	48.31	1.292	118.87	78.50	1.515
50% vitamin D_3		62.00	47.62	1.300	118.75	77.81	1.525
1,25$(OH)_2D_3$							
0.0 μg/kg		61.81	47.68	1.296	117.87	77.56	1.519
0.5 μg/kg		62.50	48.25	1.296	119.75	78.75	1.520
P-value							
Vitamin D_3		0.631	0.279	0.214	0.808	0.447	0.321
1,25$(OH)_2D_3$		0.331	0.322	0.950	0.084	0.170	0.919
Interaction		0.657	0.681	0.059	0.436	0.102	0.062
Standard error		0.0003	0.0002	0.0034	0.0005	0.0004	0.0051

[1] 100% vitamin D_3 corresponds to 2500 and 2000 IU/kg for starter and grower phases, respectively.
[2] 50% vitamin D_3 corresponds to 1250 and 1000 IU/kg for starter and grower phases, respectively.
Significant by F test ($P \leq 0.05$).

Table 3 - Effect of 1,25$(OH)_2D_3$ and vitamin D_3 levels on bone ash (Ash), calcium (Ca), and phosphorus (P) at 21 and 40 days of age

		Age (days)					
		1-21 days			1-40 days		
		Ash (%)	Ca (%)	P (%)	Ash (%)	Ca (%)	P (%)
Vitamin D_3	1,25$(OH)_2D_3$						
100% vitamin D_3[1]	0.0 μg/kg	43.63	16.61	7.21	43.73	15.27	6.66
	0.5 μg/kg	43.76	16.85	6.99	42.81	15.50	6.86
50% vitamin D_3[2]	0.0 μg/kg	43.82	16.34	6.86	43.10	15.06	6.88
	0.5 μg/kg	42.66	16.95	7.14	40.80	14.80	6.94
Vitamin D_3							
100% vitamin D_3		43.69	16.73	7.10	43.27	15.38	6.76
50% vitamin D_3		43.24	16.65	7.00	42.00	14.94	6.91
1,25$(OH)_2D_3$							
0.0 μg/kg		43.72	16.48	7.03	43.41	15.17	6.77
0.5 μg/kg		43.21	16.90	7.07	41.87	15.17	6.90
P- value							
Vitamin D_3		0.567	0.779	0.643	0.304	0.072	0.460
1,25$(OH)_2D_3$		0.516	0.169	0.883	0.193	0.945	0.534
Interaction		0.413	0.539	0.240	0.565	0.307	0.729
Standard error		0.3800	0.1477	0.1047	0.5959	0.1239	0.0987

[1] 100% vitamin D_3 corresponds to 2500 and 2000 IU/kg for starter and grower phases, respectively.
[2] 50% vitamin D_3 corresponds to 1250 and 1000 IU/kg for starter and grower phases, respectively.
Significant by F test ($P \leq 0.05$).

Table 4 - Effect of $1,25(OH)_2D_3$ and vitamin D_3 levels on maximum load (ML), stiffness (S), and toughness (T) at 21 and 40 days of age and bone mineral density (BMD) at 40 days of age

		Age (days)						
		1-21 days			1-40 days			
		ML (N)	S (N/mm)	T (mJ)	ML (N)	S (N/mm)	T (mJ)	BMD (g/cm²)
Vitamin D_3	$1,25(OH)_2D_3$							
100% vitamin D_3[1]	0.0 µg/kg	201.30	106.65	339.87	336.48	163.96	768.00	0.085
	0.5 µg/kg	194.16	119.83	293.50	409.07	179.66	738.75	0.099
50% vitamin D_3[2]	0.0 µg/kg	204.80	105.92	368.15	375.13	173.02	843.37	0.075
	0.5 µg/kg	181.08	115.81	285.00	394.36	177.53	803.62	0.077
Vitamin D_3								
100% vitamin D_3		197.73	113.24	326.56	372.78	171.81	753.38	0.093a
50% vitamin D_3		192.94	110.86	316.69	384.75	175.28	823.50	0.076b
$1,25(OH)_2D_3$								
0.0 µg/kg		203.05	106.29	354.00a	355.81	168.49	805.69	0.080
0.5 µg/kg		187.62	117.82	289.25b	401.72	178.60	771.19	0.088
P-value								
Vitamin D_3		0.611	0.755	0.649	0.640	0.777	0.243	0.020*
$1,25(OH)_2D_3$		0.108	0.138	0.005*	0.080	0.412	0.562	0.249
Interaction		0.381	0.829	0.399	0.301	0.648	0.929	0.404
Standard error		4.7174	3.7511	11.8958	12.9960	5.8737	28.8231	0.0037

[1] 100% vitamin D_3 corresponds to 2500 and 2000 UI/kg for starter and grower phases, respectively.
[2] 50% vitamin D_3 corresponds to 1250 and 1000 UI/kg for starter and grower phases, respectively.
* Significant by F test (P≤0.05).
a,b - Values within a column with different letters differ significantly by F test (P≤0.05).

(100% of vitamin D_3 in premix without $1,25(OH)_2D_3$) to different levels of vitamin D_3 in premix supplemented with the same quantity of $1,25(OH)_2D_3$. Phosphorus and calcium levels were balanced for all treatments. The reduction in 50% of the vitamin level, with the addition of $1,25(OH)_2D_3$, did not differ from the control group for any of the performance parameters at 21 and 42 days.

Likewise, Vieites et al. (2014) studied the inclusion of different levels of $1,25(OH)_2D_3$ (ranging from 0 to 2.5 µg/kg), with calcium and phosphorus levels fixed. The authors concluded that the supplementation with up to 2.5µg $1,25(OH)_2D_3$/kg did not influence performance parameters of broilers at 8 and 42 days.

Those studies, as well as the present research, are in accordance with findings related to vitamin D_3 and balanced mineral levels. These findings show that when calcium and phosphorus levels are adequate, there are no direct effects of vitamin D_3 supplementation on performance of broilers (Edwards Jr. et al., 2002). The addition of more than 1200-1600 IU of vitamin D_3 per kg of feed has little response on these parameters (Baker et al., 1998).

The skeletal status of poultry and the effect of vitamin D on bones are traditionally assessed by histology, estimations of bone ash, calcium and phosphorus percentage, and bone breaking strength (Thorp and Waddington, 1997).

When there is a reduction in vitamin D_3 levels and, consequently, imbalance in calcium and phosphorus plasmatic levels, a mobilization of these minerals is observed from the cortical bone to plasma, as a direct effect of parathyroid hormone (PTH). This response was not observed in the present study, since the bone mineral content, in all treatments, was not affected, reassuring that the reduction in 50% of the vitamin D_3 levels was not severe. The addition of $1,25(OH)_2D_3$ did not improve the mineral deposition in cortical bone.

Compatible findings were found by Alves (2014). The author observed that a reduction in 50% of the vitamin D_3 level with addition of $1,25(OH)_2D_3$ did not differ from the control group for bone ash and calcium content at 21 and 42 days.

Elliot et al. (1995) evaluated two calcium levels (1.00 and 0.65%) and $1,25(OH)_2D_3$ (0 and 5µg/kg) in three-week-old broilers. Both 1.00% calcium and 5µg/kg $1,25(OH)_2D_3$ increased bone ash at this age, which was not observed in the present study with the supplementation of 0.5µg $1,25(OH)_2D_3$ and balanced calcium level. A dietary supplementation of a low Ca diet containing 980 IU vitamin D_3/kg with 10µg $1,25(OH)_2D_3$/kg may increase tibiae bone ash (Edwards Jr., 1989; Edwards Jr., 1990).

Bone mechanical properties are determined primarily by the amounts, arrangement, and molecular structure of collagen and mineral content. Strength and stiffness are closely related to mineralization of bone, which agrees with the present study, since there was no difference between treatments for mineral content and bone strength and stiffness. However, a highly mineralized bone, that is also stiff, will require less energy to fracture than a bone that is more capable of yielding (Turner, 2006).

On the other hand, toughness is mostly improved by collagen, which allows bones to bend without breaking, despite the rigidity provided by the minerals (Wang et al., 2002; Currey, 2003). This biomechanical property indicates the amount of energy needed to cause the material failure. Thus, a tough bone will be more resistant to fracture, even though it may be less resistant to yielding (Turner and Burr, 1993). In the present study, the reduction in toughness observed in animals treated with 0.5 µg/kg $1,25(OH)_2D_3$ might indicate that these bones were less capable of bending, which could be explained by a reduction in the collagen content. According to Artaza and Norris (2009), the addition of $1,25(OH)_2D_3$ in mesenchymal multipotent cell cultures downregulates the expression of collagen I and III; however, we did not measure collagen content to confirm it.

Bachmann et al. (2013) investigated the supplementation of a control diet containing 1000 IU vitamin D_3/kg and imbalanced Ca:P, with a synthetic source of $1,25(OH)_2D_3$ (2.5 µg/kg and 5 µg/kg), purified extract of *Solanum glaucophyllum* (9.5 µg/kg and 37.8 µg/kg), and dried *Solanum glaucophyllum* leaves (10 µg/kg). The authors did not find significant differences among the treatments for tibiae breaking strength and stiffness at 14 days. The present study also used dried *Solanum glaucophyllum* leaves source of $1,25(OH)_2D_3$ and the inclusion of 0.5 µg/kg was not sufficient to improve these variables.

Bone mineral density is the mass of bone material, organic and inorganic, measured in an area (g/cm^2) and depends on the absorption of radiation by the skeleton. Since the inorganic portion is the main component of extracellular bone matrix, BMD is a good indicative of bone mineralization. The measure of BMD through dual-energy x-ray absorptiometry (DEXA) is not largely used in animals, although it is considered a standard method for determination of osteoporosis in humans (Hailey et al., 1996; Silva, 2003).

In the present study, BMD at 40 days of age was greater when broilers received a diet with 100% of vitamin D_3, suggesting that the use of this diet resulted in bones more capable of absorbing radiation, thus more mineralized, than the ones from animals treated with 50% of vitamin D_3. However, despite of this result, the mineral content, stiffness, and breaking strength of all treatments were similar, indicating that the bones of animals fed 50% of vitamin D_3 are as well developed as the bones of animals fed 100% of vitamin D_3 at 40 days of age.

The histological evaluation of the bone growth plate is a method of histopathological diagnosis of bone diseases. When there is a disease in the locomotor system, it is possible to characterize a change in the thickness of the growth plate, a reduction in vascularization, and lower cell differentiation and organization (Thorp and Waddington, 1997).

The most characteristic change in vitamin D_3 deficiency in chicks is the enlargement of the growth plate due to widening of the proliferating and hypertrophic zones. Probably, the deficiency causes delay of chondrocyte hypertrophy. When the deficiency progresses, there is increase of porosity in the cortical bone due to the reabsorption, determining decrease in the mechanical strength of long bones (Klasing, 2013). These changes were not found during the gross evaluation of tibiae and femora of broilers at 21 and 40 days. The growth plates, in all treatments, were regular, with similar thickness, blood vessels, and trabecular bone well distributed and without the presence of any avascular cartilage plug, which indicates absence of disease.

In the present study, the reduction in 50% of the vitamin D_3 levels commonly used in commercial poultry production was not severe enough, which may explain why no differences were observed. These levels (1250 and 1000 IU/kg for starter and grower phases, respectively) in diets with balanced Ca and P were sufficient to ensure the maximum performance and bone development of broilers at 21 and 40 days.

Conclusions

The reduction up to 50% (1250 and 1000 IU/kg in the starter and grower phases, respectively) of vitamin D_3 levels in diets with balanced Ca and P commonly used in commercial poultry production is sufficient to ensure the maximum performance of broilers at 21 and 40 days. The use of 0.5 µg $1,25(OH)_2D_3$/kg, in a glyosidic form, in addition to sufficient levels of dietary vitamin $D_{3,}$ does not improve the parameters measured, even when 50% of vitamin D_3 is used. These results support the claim that an unnecessary excess of vitamin D_3 is used in commercial broiler production. Thus, the reduction in 50% of vitamin D_3 levels used commercially, without supplementation with $1,25(OH)_2D_3$, may represent an important decrease in production costs and row material waste, influencing the industries to narrow the safety margin.

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