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ANIMAL PRODUCTION

Decreased levels of vitamin D_3 and supplementation with 1,25-dihydroxyvitamin D_3 glycoside on performance, carcass yield and bone quality in broilers

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ABSTRACT: *An experiment was performed to evaluate the effect of decreased levels of vitamin D_3 in the premix and 1,25-dyhydroxyvitamin D_3-glycoside (1,25(OH)$_2$D$_3$-glycoside) supplementation on performance, carcass yield and bone quality in 42d old broilers. Seven-d-old male chickens Cobb500® were distributed in a randomized design with six treatments: a control diet with inclusion of vitamin D_3 in the premix, without supplementation of 1,25(OH)$_2$D$_3$-glycoside, and five diets with decreased levels of vitamin D_3 (100%, 75%, 50%, 25% and 0% about the control) plus the addition of 1,25(OH)$_2$D$_3$-glycoside, 50g ton^{-1} of diet. The main results were to reduce the tenor of Vitamin D_3 in the premix when the addition of 1,25(OH)$_2$D$_3$-glycoside did not affect ($P>0.05$) the performance, carcass yield and bone quality variables. However, performance (feed intake, gain weight, feed conversion), yield (warm carcass weight) and bone quality (dry weight, length, mineral matter and breaking strength) of broilers fed with diets without vitamin D_3 in the premix and with addition of 1,25(OH)$_2$D$_3$-glycoside, which was the single source of vitamin D, had as a result very low ($P<0.05$) values comparing to the control. For the purposes of the present research, it was concluded that is possible the reduction of vitamin D_3 tenor in the premix up to 75% when the diet of male broilers is supplemented with 1,25(OH)$_2$D$_3$-glycoside. However, the use of 1,25(OH)$_2$D$_3$-glycoside as a single source of vitamin D, as tested here, is not recommended for broilers diets.*
Key words: *bone growth, bone strength, calcitriol, poultry, Solanum glaucophylum.*

Diminuição dos níveis de vitamina D_3 e suplementação com 1,25-dihidroxivitamina D_3 glicosídeo sobre o desempenho, o rendimento da carcaça e a qualidade dos ossos em frangos de corte

RESUMO: *Um experimento foi realizado para avaliar o efeito da diminuição dos níveis de vitamina D_3 no premix e a suplementação de 1,25-dihidroxivitamina D_3-glicosídeo (1,25(OH)$_2$D$_3$-glicosídeo) sobre o desempenho, rendimento de carcaça e qualidade óssea em frangos de corte de 42 dias. Machos Cobb500® de sete dias de idade foram distribuídos em delineamento inteiramente casualizado com seis tratamentos: uma dieta controle com inclusão de vitamina D_3 no premix, sem suplementação de 1,25(OH)$_2$D$_3$-glicosídeo, e cinco dietas com níveis decrescentes de vitamina D_3 (100%, 75%, 50%, 25% e 0% em relação ao controle) mais a adição de 1,25(OH)$_2$D$_3$-glicosídeo (50g ton^{-1} de dieta). Entre os principais resultados, foi observado que a redução do teor de vitamina D_3 no premix quando adicionado 1,25(OH)$_2$D$_3$-glicosídeo não afetou ($P>0,05$) o desempenho, o rendimento da carcaça e as variáveis da qualidade óssea. No entanto, as variáveis do desempenho e o rendimento (consumo de ração, ganho de peso, conversão alimentar, peso da carcaça quente) e da qualidade dos ossos (peso seco, comprimento, matéria mineral e resistência à ruptura) de frangos alimentados com dietas sem vitamina D_3 no premix, com a adição de 1,25(OH)$_2$D$_3$-glicosídeo como a única fonte de vitamina D, teve como resultado valores muito baixos ($P<0,05$) em comparação ao controle. Para os propósitos da presente pesquisa, concluiu-se que é possível a redução do teor de vitamina D_3 no premix até 75% quando a dieta de frangos de corte machos é suplementada com 1,25(OH)$_2$D$_3$-glicosídeo. No entanto, o uso de 1,25(OH)$_2$D$_3$-glicosídeo como única fonte de vitamina D, conforme testado aqui, não é recomendado para dietas de frangos de corte.*
Palavras-chave: *avicultura, calcitriol, crescimento ósseo, força óssea, Solanum glaucophylum.*

INTRODUCTION

The use of metabolites of vitamin D on broiler diets prevents skeletal problems and promotes bone quality to support gain of muscular mass in broiler strains of rapid growth, especially on birds with bone quality problems, as tibial dyschondroplasia, that affects the performance (BRITO et al., 2010; GARCIA et al., 2013). The more available metabolites to use as additives, are hydroxycholecalciferol (25(OH D_3) and 1,25-dihydroxyvitamin D_3 or calcitriol (1,25(OH)$_2$D$_3$) (APPLEGATE & ANGEL, 2005).

In all animals, the ingested vitamin D_3 is metabolized in 25(OH)D$_3$ on the liver, and then, it is metabolized in 1,25(OH)$_2$D$_3$ (the active metabolite) on the kidneys (WHITEHEAD, 2002). Besides 1,25(OH)$_2$D$_3$ could be obtained from botanical sources, as dry leaves of *Solanum glaucophyllum*

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(BOLAND et al., 2003), also known as *Solanum malacoxylon* or *Solanum glaucum,* a common bush from temperate zone of South America. However, the aqueous extract could be considered toxic due to high levels of calcium, magnesium and phosphorus ions, which leads to depressed of bone resorption. Therefore, desirable bioactive form could be obtained from the salt-free extract of *Solanum glaucophyllum* (LLOYD et al., 1975).

The supplement industry works with a safety margin of two to ten times greater than the actual need of the birds, to guarantee the minimum supplementation of the nutrients after the manufacturing physical processes, transportation and diets storage (COELHO et al, 1995; FÉLIX et al. 2009). However, BRITO et al. (2010) did not recognize the advantage of using high levels of vitamin D_3 on performance of broilers, as well as ALAHYARI-SHAHRASB et al. (2012) suggested that it has been possible to reduce the amount of some vitamins in the premix for finisher's diets by 33% without affecting the performance and metabolic characteristics.

The bone quality is assessed by measuring bone's density, resistance to breakage and percentage of minerals in the bones, through mechanical, physical and chemical features. If the features of bone quality go along with the production rates in the flocks, in satisfactory way, then, the supplementation with metabolites could be positively evaluated.

Studies related with the use of vitamin D_3 metabolites can help companies with integration systems, which would handle mineral and vitamin supplements, to reduce the amount of vitamin D_3 by association with active metabolites; and therefore, enable cost reduction without loss on performance, carcass yield and bone quality of the flocks of chickens. Based on these considerations, this study aimed to evaluate the effect of the vitamin D_3 reduction in the premix and, the $1,25(OH)_2D_3$-glycoside supplementation on performance, bone quality and carcass yield from 7 to 42d broilers.

MATERIALS AND METHODS

Housing, birds, experimental design

An experiment was conducted in the Federal Institute of Rio de Janeiro Campus Pinheiral - RJ. The facility was fitted with heating and pat cooling automatic systems, had been divided in floor pens, 2m x 1.5m with nipple drinkers and manual feeders. Feed and water were provided *ad libitum*. Seven-day old male broiler chickens Cobb500® were distributed in 36 groups with standardized average weight 164±1,6g. Each group was distributed in a randomized design with six experimental treatments and six replications, with 38 birds per replication.

Treatments

Treatments consisted in six experimental diets (Table 1); they were formulated following the nutritional requirements by ROSTAGNO et al. (2011). The control, and five treatments with decreasing levels of Vitamin D_3 (100, 75, 50, 25 and 0%) with additional bioactive metabolite $1,25(OH)_2D_3$ glycoside in the fixed amount 50mg kg^{-1} of diet, as shown in table 2.

The premix of vitamins was manufactured based on the formulation of Nutron® Animal Nutrition, Cargill Company, and the synthetic vitamin D_3 was included in the concentrations as shown in table 2. The additive Panbonis® is from dried leaves of *Solanum glaucophyllum* and has 10mg of $1,25(OH)_2D_3$ glycoside/kg of product.

Performance and carcass yield

To evaluate the broilers performance, the weight at 42 days was recorded and, the feed intake (FI), weight gain (WG), daily WG and feed conversion (FC=FI/WG) of 7-42 days period were calculated. Carcass yield of 42 days old was evaluated using two average weight broilers from each replicate. Body weight in fasting was measured; the relative weight of warm carcass (without viscera, head, neck and feet) had been calculated in relation to the body weight in fasting; and the relative weight of breast, thigh + drumstick and wings have been calculated in relation to the warm carcass weight, and expressed as a percentage.

Bone quality

The bone quality analysis was performed at 42d old on the left tibias of two birds per repetition, for each experimental treatment. Broilers were killed by cervical dislocation. Bones were thawed and, the adhered muscle tissue was removed.

Wet weight (g) and length (mm) of the tibia were measured, and the weight/length ratio (g/mm) was calculated. The breaking strength was determined (MURAKAMI et al., 2009) in a universal testing machine (UMC 300, CAP 30TF, Cotenco®), and the data was expressed in Newton (N) following the recommendations of ANSI / ASAE S459 rules (ASAE Standards, 1998). The analysis was performed in the Wood Technology Lab at Forest Institute and in the Animal Science Institute - UFRRJ, Seropédica-RJ.

The ash and mineral content was determined in the degreased bones (SILVA & QUEIROZ, 2002;

Table 1 - Experimental diet composition (g kg^{-1}) and calculated nutrients concentration.

Ingredients g kg^{-1}	1-7d	------------8-21d------------		-----------22-35d-----------		-----------36-42d-----------	
		Control	Treat	Control	Treat	Control	Treat
Maize meal	517.822	557.029	511.825	580.870	580.804	584.158	617.225
Soybean meal 451.5g/kg CP	405.771	364.624	403.484	330.190	330.177	335.162	305.392
Soybean oil	28.307	34.010	42.728	45.413	45.442	49.157	43.082
Dicalcium Phosphate	19.218	15.719	15.537	16.870	16.870	10.916	11.062
Limestone	10.618	10.702	10.593	10.159	10.159	8.837	8.918
Vitamin & Mineral supplement[1]	4.000	4.000	4.000	4.000	4.000	2.000	2.000
Sodium Bicarbonate	3.651	3.526	3.609	3.702	3.702	3.540	3.541
DL-Methionine	3.609	3.148	2.792	2.570	2.571	2.290	2.568
NaCl	2.577	2.411	2.400	2.287	2.287	2.064	2.062
L-lysine HCl 99%	2.411	2.254	1.000	1.751	1.752	1.010	1.976
L-threonine 98%	1.391	1.226	0.632	0.885	0.886	0.490	0.949
Choline chloride	0.625	0.550	0.550	0.500	0.500	0.375	0.375
Inert	-	0.001	-	0.003	-	0.001	-
Antioxidant	-	0.800	0.800	0.800	0.800	-	0.800
1,25 $(OH)_2D_3$ glycoside	-	-	0.050	-	0.050	-	0.050
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
---Calculated nutritional concentrations g kg^{-1}---							
ME(MJ kg^{-1})	12.39	12.76	12.76	13.18	13.18	13.39	13.39
CP,	228.0	212.0	212.0	197.3	197.3	198.7	198.7
Calcium,	9.2	8.4	8.4	8.4	8.4	6.6	6.6
Phosphorus available	4.7	4.0	4.0	4.2	4.2	3.1	3.1
Sodium	2.2	2.1	2.1	2.1	2.1	1.9	1.9
Potassium	9.8	9.1	9.1	8.4	8.4	8.5	8.5
Chlorine	2.0	1.9	1.9	1.8	1.8	1.7	1.7
Total Lysine	14.6	13.4	13.4	12.1	12.1	11.7	11.7
Digestible lysine	13.5	12.4	12.4	11.1	11.154	10.714	10.714
Digestible Methionine	6.7	6.0	6.0	5.3	5.316	5.075	5.074
Digestible Methionine + cystine	9.7	8.9	8.9	8.0	8.0	7.8	7.8
Digestible Tryptophan	2.5	2.3	2.3	2.1	2.1	2.2	2.2

[1]Guaranteed concentrations per kg of product (1-21d): Iron 13800mg; copper 2775mg; zinc 17925mg; manganese 19275mg; selenium 82.5mg; iodine 277.5mg; vitamin A 618.6mg; vitamin E 7800mg; vitamin K3 412.5mg; thiamine 562.5mg; riboflavin 1387.5mg; pyridoxine 787.5mg; cyanocobalamin 3300mcg; niacin 8250mg; pantothenic acid 2775mg; biotin 19275µg; nicarbazin 31250mg; virginiamycin 4125mg; (22-35d): iron 12525mg; copper 2550mg; zinc 16275mg; manganese 17550mg; selenium 75.0mg; iodine 262.5mg; vitamin A 562.5mg; vitamin E 7050mg; vitamin K3 375mg; thiamine 525mg; riboflavin 1275mg; pyridoxine 712.5mg; cyanocobalamin 3000µg; niacin 7500mg; pantothenic Acid 2550mg; biotin 17550µg; salinomycin 15.0mg; virginiamycin 4125mg. (35-42d): Iron 12525mg; copper 2550mg; zinc 16275mg; manganese 17550mg; selenium 75mg; iodine 262.5mg; vitamin A 562.5mg; vitamin E 7050mg; vitamin K3 375mg; thiamine 525mg; riboflavin 1275mg; pyridoxine 712.5mg; cyanocobalamin 3000µg; niacin 7500mg; pantothenic acid 2550mg; biotin 17550µg. Vitamin D_3 (Table 2).

SILVA et al., 2009); the calcium content was measured by atomic absorption spectrometer (model SpectrAA 55B, Varian) at the Institute of Agronomy- UFRRJ, Seropédica - RJ (ZENEBON et al., 2008).

Statistical analysis

Data were subjected to analysis of variance using the General Linear Models procedure of SAS® software and, in case of statistically significant differences the means were compared by test of Dunnett at 5% of probability.

RESULTS

Performance and carcass yield

Results of performance and carcass yield were shown in the tables 3 and 4, respectively. The performance variables (weight, weight gain, daily weight gain, feed conversion and feed intake) during the 7 - 42 days period, the fasting weight and the warm carcass weight at 42 days were significantly poor ($P<0.05$) when the metabolite $1,25(OH)_2D_3$-glycoside was used as the single source of vitamin D,

Table 2 - Experimental treatments, levels of vitamin D_3[1] (µg of cholecalciferol/kg of diet) in the premix in each phase of growth and supplementation of $1,25(OH)_2D_3$ glycoside.

Treatments	---------------------µg of Vitamin D_3 kg^{-1} of diet-----------------------			µg of $1,25(OH)_2D_3$ glycoside kg^{-1} of diet[2]
	1-21d	22-35d	35-42d	
Control (-)	52.5	48.0	24.0	-
100%VitD_3 (+)	52.5	48.0	24.0	0.5
75% VitD_3 (+)	39.4	36.0	18.0	0.5
50% VitD_3 (+)	26.2	24.0	12.0	0.5
25% VitD_3 (+)	13.1	12.0	6.0	0.5
0% VitD_3 (-)	-	-	-	0.5

[1]Synthetic vitamin D_3; [2]Panbonis® (90% purity) containing 10mg $1,25(OH)_2D_3$ glycoside Kg^{-1} of product, included at 50mg Kg^{-1} of diet.

in comparison with the same variables in broilers fed with control diet. The same variables in the broilers that received treatments 2, 3, 4 and 5 (Table 2) were not affected ($P>0.05$) in comparison to the control. The viability, the carcass yield and parts yield (wing, breast and thigh + drumstick) were not affected by treatments ($P> 0.05$).

Bone quality

Results of bone quality are shown in the table 5. The dry weight, length, mineral matter and breaking strength of the tibia of broilers fed with diet using $1,25(OH)_2D_3$-glycoside as the single source of Vitamin D were poor significantly ($P<0.05$) compared to the control treatment. These variables in broilers from other treatments were not different ($P>0.05$) compared to the control. The variables wet weight, diameter, height, weight/length ratio, and the calcium percentage of the tibia were not influenced ($P>0.05$) by any treatment, compared to the control (Table 5).

DISCUSSION

When $1,25(OH)_2D_3$-glycoside was used as the single source of vitamin D_3, many performance, yield and bone quality variables of broilers Cobb500® at 42 days was extremely impaired. Similar results were previously reported, low values of weight gain associated to moderate hypercalcemia were reported in six weeks broilers supplemented with $1,25(OH)_2D_3$ (RENNIE et al., 1995; RENNIE et al., 1996). In order to justify the occurrence of the poor results, it would be necessary to perform analysis such as the blood and organs metabolite profiles, $1,25(OH)_2D_3$, and others related to the bone formation process, for the purpose to know if the use of fixed quantity (50g of the product per ton of feed), would provide lower levels of vitamin D_3 than required within each stage of development.

The use of 100, 75, 50 or 25% of vitamin D_3, in addition of $1,25(OH)_2D_3$-glycoside, did not

Table 3 - Performance[1] of broilers fed with diets with decreased levels of vitamin D_3 in the premix and supplemented with $1,25(OH)_2D_3$-glycoside (0.5µg Kg^{-1} of feed).

Treatments[2]	BW (Kg)	FI (Kg)	GW (Kg)	DGW(Kg)	FC(Kg Kg^{-1})	V (%)
	42d	-----------------------7-42 d-----------------------				
Control (-)	2883	4125	2719	77.7	1.52	95.29
100%VitD_3 (+)	2888	4237	2727	77.9	1.55	94.49
75% VitD_3 (+)	2923	4129	2758	78.8	1.50	94.05
50% VitD_3 (+)	2946	4189	2782	79.5	1.50	94.41
25% VitD_3 (+)	2881	4370	2716	77.6	1.61	95.46
0% VitD_3 (-)	1636*	3242*	1473*	42.1*	2.23*	93.80
CV (%)	5.15	6.21	5.50	5.50	5.33	1.94

[1]BW: Body weight; FI: feed intake; GW: gain weight; DGW: daily gain weight; FC: feed conversion; V: viability. [2]See table 2. *Significantly difference from the control treatment, Dunnett's test (5%).

Table 4 - Carcass yield at 42d of broilers fed with diets with decreasing levels of vitamin D_3 and supplemented with $1,25(OH)_2D_3$-glycoside (0.5µg Kg^{-1} of feed).

Treatments[1]	FW (Kg)	WCW (Kg)	CY (%)	Wing (%)	Breast (%)	TD (%)
Control (-)	2834	2110	74.49	10.5	39.81	28.58
100%VitD_3 (+)	2880	2136	74.14	10.74	39.6	28.4
75% VitD_3 (+)	3015	2255	74.76	10.43	39.43	28.63
50% VitD_3 (+)	2759	2075	75.31	10.37	39.64	28.76
25% VitD_3 (+)	2987	2200	73.61	10.38	39.23	28.91
0% VitD_3 (-)	1772*	1328*	74.78	11.25	38.83	28.33
CV (%)	11.16	11.76	3.16	8.32	5.04	4.99

FW: Fasting weight; WCW: warm carcass weight; CY: Carcass yield; TD: thigh+drumstick. [1]See table 2. *Significantly difference from the control treatment, Dunnett's test (5%).

affected the performance, and it was close to the Cobb500® standards. In a similar way, vitamins reduction in 33% of the finishing diet premix for broilers rising in floor, did not affect the performance at slaughter age (ALAHYARI-SHAHRASB et al., 2012). Additionally, COLET et al. (2015) studied vitamin reductions in broiler diets and, reported that there was no need for further vitamin supplementation when mineral levels had been balanced. VIEITES et al. (2014) used 1,25 $(OH)_2D_3$-glycoside metabolite to supplement broiler in a quantity five times higher (250 g of Panbonis® ton^{-1}) than that used in the present study and, did not observe significant differences in the performance of 42-d old broiler chickens. According to FÉLIX et al. (2009), the vitamin levels used by the industry are 10 to 25 times higher than the recommended by the NRC (1994), and once the premix used was formulated based on a commercial brand, the vitamin levels were enough to guarantee the standard performance of the broiler strain used on this study. Calcidiol supplementation plus different levels of vitamin D_3 reduces the mortality rate (MICHALCZUK et al., 2010). Still, in the present research, calcitriol supplementation did not affect the viability rate.

The carcass and parts yield (wing, breast and thigh + drumstick) did not differ from control. Similar results were reported by VIEITES et al. (2012). The mechanisms of the 1,25 $(OH)_2D_3$ on muscles of chickens are still to be determined, but is known that vitamin D is necessary for skeletal muscle development and integrity (DOMINGUES-FARIA et al., 2017). In mice, GIRGIS et al. (2015) observed that vitamin D deficiency reduced muscle fiber, found direct effect of vitamin D on the muscle function and corroborated the presence of vitamin D receptors in this tissue. Reduction or withdrawal of some vitamins and minerals in finishing diets did not affect the composition of carcasses of broiler chickens; however, withdrawal of vitamins in the premix during growth

Table 5 - Bone quality of broilers fed with diets with decreasing levels of vitamin D_3 and supplemented with $1,25(OH)_2D_3$-glycoside (0.5µg Kg^{-1} of feed).

Treatment[1]	WW (g)	DW (g)	L (mm)	D (mm)	MM (%)	Ca (%)	W/L	BS (N)
Control (-)	14.4	6.24	110.7	9.21	47.86	32.5	142.6	311.9
100%VitD_3 (+)	14.4	6.29	100.8	8.61	48.46	34.7	143.0	340.9
75% VitD_3 (+)	15.5	6.65	102.6	9.70	47.67	30.9	151.0	335.4
50% VitD_3 (+)	13.8	5.93	100.7	9.03	46.22	31.0	138.3	285.1
25% VitD_3 (+)	15.4	6.50	102.0	9.39	47.78	31.0	151.3	360.3
0% VitD_3 (-)	12.2	4.12*	90.87*	8.64	40.1*	28.4	133.2	146.3*
CV (%)	14.62	13.51	4.16	11.43	6.71	14.98	12.81	24.58

WW: bone wet weight; DW: bone dry weight; L: length; D: diameter; MM: mineral matter; Ca: cálcio; W/L: weight/length ratio (g/mm); BS: breaking strength. [1]See table 2. *Significantly difference from the control treatment, Dunnett's test (5%).

period leads to lower carcass yield (MAIORKA et al., 2002; KHAJALI et al., 2006).

The opposite was observed by KORVER (2005) cited by VIEITES et al. (2014), he reported a better carcass and breast yield in 42-d broilers by vitamin D_3 plus 25(OH)D_3 supplemented diets. Also, SAUNDERS-BLADES & KORVER (2006) observed advantages 25(OH)D_3 metabolite on breast muscle development, yield and meat quality in 42-day-old broilers, similar data were reported by BRITO et al. (2010) because they supplemented with high levels of 25(OH)D_3, and observed higher carcass yield compared to the effect of vitamin D_3.

Bone quality of the tibia was affected and tibia length, the weight were lower when the birds did not receive vitamin D_3 in the diet and the single source of vitamin D was the metabolite 1,25$(OH)_2D_3$ glycoside. Additionally; although, weight/length ratio had not been affected the breaking strength of the bones was lower in those birds, which could be related to the smaller mineral matter of their bones. The stiffness of the bone tissue results from the deposition of calcium and phosphorus, in the form of hydroxyapatite, during the process of bone mineralization. These two minerals make up about 70% of the bone composition; the remaining 30% are composed of organic matter, mainly collagen. Active vitamin D_3 acts on the bone, stimulating protein synthesis by osteoblasts and participating in matrix mineralization (BORGES et al., 2010; SOUZA, 2012). Bones undergo several adaptations throughout development, and mineral content is the main determinant of the bones mechanical properties, in several species, as that, the higher the mineral content the greater the bone strength (CURREY, 2003). This observation explains the effect occurred in the treatment with the use of metabolite as a single source of vitamin D, because the lower percentage of ash was accompanied by the lower mean breaking strength. Conversely, TATARA et al. (2011) studied the effect of 25(OH)D_3 supplementation and did not observe significant differences in turkey tibia length up to 20 weeks of age.

Supplementation of 1,25$(OH)_2D_3$-glycoside as a single source of vitamin D promoted a reduction of the concentration of minerals in the broiler's tibia (from 47.86% to 40.14%). However, it was still within the acceptable percentages of broiler's bone ash (40 - 45%) in birds receiving vitamin D_3 supplementation (AOAC, 1995). Similar results were reported by GARCIA et al. (2013) when they used 1,25 $(OH)_2D_3$ in broiler diets, and obtained a percentage of ash around 44%.

When 1,25$(OH)_2D_3$-glycoside was used as the single source of vitamin D, the percentage of calcium deposited in the bone did not differ from the control treatment, reinforcing the role of 1,25$(OH)_2D_3$ in the physiological regulation of intestinal absorption of Ca, as it was supported by reports of SILVERTHORN (2010), that observed that 1,25$(OH)_2D_3$ could increase active transport of Ca by up to 30%. According to NORMAN & HENRY (2007), when 1,25$(OH)_2D_3$ was given to vitamin D-deficient chicks it was observed that calcium transport reached peak rates between 12 and 14h, maintaining adequate circulating levels and decreasing mobilization of calcium from bones. The 1,25$(OH)_2D_3$ plays a significant role in the regulation of active calcium transport, ensuring maintenance of plasma levels and reducing the mobilization of this mineral in bones when the nutrition and vitamin and mineral supplementation are correct (SILVERTHORN, 2010).

TATARA et al. (2011) studied the effect of 25(OH)D_3 supplementation, nor did they observe differences between tibial mineral volume density (measured by computed tomography and the Somatom Emotion Siemens apparatus) in turkeys up to 20 weeks of age. However, in the study by GARCIA et al. (2013), supplementation with Panbonis® at a concentration of 0.250g/kg diet led to higher weight/length ratio values and greater breaking strength (approximately 336.46N) when compared to the present results.

In the present study, no histological evaluation of the bones was performed to the effect of supplementation with the metabolite on the bone structure. However, the use of decreasing concentrations (100, 75, 50 or 25%) of vitamin D_3, that is, the reduction of up to 75% of the inclusion of vitamin D3 in the premix plus the 1,25$(OH)_2D_3$ glycoside supplementation, did not alter any of bone quality variables of the broilers that were evaluated in the present study.

For the purposes of the present research it was concluded that the performance, carcass weight and bone quality of broiler chickens are affected by the non-inclusion of Vitamin D_3 in the premix; although, supplemented with 50g/ton of 1,25 $(OH)_2D_3$ glycoside. A reduction of vitamin D_3 from the premix up to 75% when the inclusion of 1,25$(OH)_2D_3$-glycoside maintains the performance and bone quality of the male broilers of the Cobb500® lineage. Carcass yield and noble cuts are not influenced by the reduction of vitamin D_3 level of premix and supplementation with 1,25$(OH)_2D_3$ glycoside. However, the use of 1,25$(OH)_2D_3$ glycoside as the single source of D in the amount tested is not recommended for male broilers Cobb500®.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The experiment followed the recommendations of resolution 714 of 06/20/2002 CRMV used by the research ethics committee of the UFRRJ / COMEP process n. 23083.001534 / 2013-17.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

REFERENCES

ALAHYARI-SHAHRASB, M. et al. Decreasing vitamin premix on chicken carcass composition and blood chemistry in floor and battery cage systems. **Italian Journal of Animal Science**, v.11, n.1, e14, 2012. Available from: <https://www.tandfonline.com/doi/full/10.4081/ijas.2012.e14?scroll=top&needAccess=true>. Accessed: Jul 03, 2018. doi: 10.4081/ijas.2012.e14.

AOAC. Vitamins and other nutrients: Official method of determination of vitamin D3 in poultry feed supplements via the chick bioassay, in: **Official Methods of Analysis of AOAC International**, 1995 pp. 57. Arlington, VA.

ASAE STANDARDS. Shear and three-point bending test of animal bone. 1998. *ANSI/ASAE S459 DEC01*.

APPLEGATE, T.J.; ANGEL, R. Los metabolitos de la vitamina D son prometedores para uso en dietas avícolas. **Proceedings of the Vademécum Avícola**. 2005, Santiago.

BOLAND, R. et al. Vitamin D compounds in plants. **Plant Science**, v. 164 n. 3, p. 357-369, 2003. Available from: <https://www.sciencedirect.com/science/article/pii/S016894520200420X?via%3Dihub>. Accessed: Jul 03, 2018. doi: 10.1016/S0168-9452(02)00420-X.

BORGES, L.L. et al. Densidade mineral óssea na produção de frangos de corte. **Revista Científica Eletrônica De Medicina Veterinária**. v. 8, n. 15, 2010. Available from: <http://faef.revista.inf.br/imagens_arquivos/arquivos_destaque/pQ66fD9yF9R73Fx_2013-6-25-16-37-26.pdf>. Accessed: Jul 03, 2018.

BRITO, J. et al. Effect of vitamin D_3 and 25-hidroxicholecalciferol on performance, carcass yield and intestinal morphology in broiler chickens. **Revista Brasileira de Zootecnia** [online]. v. 39, n. 12, p. 2656-2663, 2010. Available from: <http://www.scielo.br/scielo.php?script=sci_abstract&pid=S1516-35982010001200014&lng=en&nrm=iso&tlng=en> Accessed: Jul 03, 2018. doi: 10.1590/S1516-35982010001200014.

COELHO, M.B.; McNAUGHTON, J.L. Effect of composite vitamin supplementation on broilers. **The Journal of Applied Poultry Research**, v. 4, n.3, p. 219-229, 1995. Available from: <https://academic.oup.com/japr/article-abstract/4/3/219/767599?redirectedFrom=fulltext>. Accessed: Jul 03, 2018. doi: 10.1093/japr/4.3.219.

COLET S. et al. Bone characteristics of broilers supplemented with vitamin D. **Brazilian Journal of Poultry Science**, vol. 17, n .3, p. 325-352, 2015. Available from: <http://dx.doi.org/10.1590/1516-635x1703325-332www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-635X2015000300325&lng=en&tlng=en>. Accessed: Jul 03, 2018. doi: 10.1590/1516-635x1703325-332.

CURREY J.D. The many adaptations of bone. **Journal of Biomechanics**, v. 36, n. 10, p. 1487–1495, 2003. Available from: <https://www.jbiomech.com/article/S0021-9290(03)00124-6/fulltext>. Accessed: Jul 03, 2018. doi: 10.1016/S0021-9290(03)00124-6.

DOMINGUES-FARIA, C. et al. Vitamin D and muscle trophicity. **Current Opinion in Clinical Nutritrion and Metabolic Care**, v. 20, n. 3, p. 169–174, 2017. Available from: <https://insights.ovid.com/crossref?an=00075197-201705000-00005>. Accessed: Jul 03, 2018. doi: 10.1097/MCO.0000000000000358.

FÉLIX, A.P. et al. Vitamin levels for broiler. **Ciência Rural**, v. 39, n. 2, p.619-626. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0103-84782009000200050&lng=pt&tlng=pt>. Accessed: Jul 03, 2018. doi: 10.1590/S0103-84782008005000073.

GARCIA, A.F.Q.M., et al. Use of Vitamin D_3 and Its Metabolites in Broiler Chicken Feed on Performance, Bone Parameters and Meat Quality. **Asian-Australasian Journal of Animal Sciences (AJAS)**, v. 26, n. 3, p.408-415, 2013. Available from: <https://www.ajas.info/journal/view.php?number=4663>. Accessed: Jul 03, 2018. doi: 10.5713/ajas.2012.12455.

GIRGIS, C.M. et al. Vitamin D receptor ablation and vitamin D deficiency result in reduced grip strength, altered muscle fibers, and increased myostatin in mice. **Calcified Tissue International**, v. 97, n. 6, p. 602– 610, 2015. Available from: <https://link.springer.com/article/10.1007%2Fs00223-015-0054-x>. Accessed: Jul 03, 2018. doi: 10.1007/s00223-015-0054-x.

KHAJALI, F. et al. Effect of vitamin and trace mineral withdrawal from finisher diets on growth performance and immunocompetence of broiler chickens. **British poultry science**, v. 47, n. 2, p. 159-162, 2006. Available from: <https://www.tandfonline.com/doi/full/10.1080/00071660600610732>. Accessed: Jul 03, 2018. doi: 10.1080/00071660600610732.

LLOYD, W., et al. Stimulation of bone resorption in organ culture by salt-free extracts of solanum glaucophyllum. **Endocrine research communications**, v.2, n.2, p.159-166, 1975. Available from: <https://www.tandfonline.com/doi/pdf/10.3109/07435807509053846>. doi: 10.3109/07435807509053846.

MAIORKA, A. et al. Dietary vitamin or mineral mix removal during the finisher period on broiler chicken performance. **The Journal of Applied Poultry Research**, v. 11, n. 2, p. 121-126, 2002. Available from: <https://academic.oup.com/japr/article/11/2/121/713506>. Accessed: Jul 03, 2018. doi: 10.1093/japr/11.2.121.

MICHALCZUK, M., et al. Effectiveness of vitamin D3 and calcidiol (25-OH-D3) application in feeding broiler chickens-production performance and meat quality. **Polish Journal of Food and Nutrition Sciences**, v. 60, n. 2, p. 121-126, 2010. Available from: <http://journal.pan.olsztyn.pl/?p=rec&s_rok=2010&s_numer=2>. Accessed: Jul 03, 2018.

MURAKAMI, et al. Effect of linseed oil inclusion in diets on the performance and bone parameters of broiler chickens. **Revista Brasileira de Zootecnia**, v. 38, n. 7, p. 1256-1264, 2009. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-35982009000700014>. Accessed: Jul 03, 2018. doi: 10.1590/S1516-35982009000700014.

NORMAN, A.W.; HENRY, H.L. **Vitamin D**. *In: Handbook of vitamins*. 4^{th} ed. 2007. CRC Press.

NRC - National Research Council, Nutrient requirements of poultry. 1994 9^{th} revised ed. National Academy Press, Washington.

RENNIE, J. S. et al. Interaction between dietary 1, 25 dihydroxycholecalciferol and calcium and effects of management on the occurrence of tibial dyschondroplasia, leg abnormalities and performance in broiler chickens. **British poultry science**, v. 36, p. 465-477, 1995. Available from: <https://www.tandfonline.com/doi/abs/10.1080/00071669508417792>. Accessed: Jul 03, 2018 doi: 10.1080/00071669508417792.

RENNIE, J. S.; WHITEHEAD, C. C. Effectiveness of dietary 25- and 1-hydroxy cholecalciferol in combating tibial dyschondroplasia in broiler chickens. **British Poultry Science**, v. 37, n. 2, p 413–421, 1996. Available from: <https://www.tandfonline.com/doi/abs/10.1080/00071669608417872>. Accessed: Jul 03, 2018. doi: 10.1080/00071669608417872.

ROSTAGNO, H. S. et al. **Tabelas brasileiras para aves e suínos; composição de alimentos e exigências nutricionais**. 2011 Viçosa, UFV, Departamento de Zootecnia.

SAUNDERS-BLADES J.; KORVER D. HyD and poultry: bones and beyond. **Proceedings of European Poultry Conference**, 2006. Verona, Italy.

SILVA, D.J.; QUEIROZ, A.C. **Análise de alimentos, métodos químicos e biológicos**. 2002. Viçosa, Editora UFV.

SILVA, R. M. et al. Calcium and phosphorus requirements of finishing meat quail. **Revista Brasileira de Zootecnia**, v.38, n. 8, p. 1509-1517, 2009. Available from: <http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-35982009000800015>. Accessed: Jul 03, 2018. doi: 10.1590/S1516-35982009000800015.

SILVERTHORN, D.U. (Eds) **Fisiologia Humana - Uma Abordagem Integrada** 5^{th} Ed. 2010, pp. 283 (São Paulo, Artmed Press).

SOUZA, A.F.G.O. Tecido ósseo em frangos de corte. **Revista Eletrônica Nutritime**, v. 9, n. p. 1663- 1679, 2012. Available from: <http://www.nutritime.com.br/arquivos_internos/artigos/152v9N1P1663_1679_JAN2012_.pdf>. Accessed: Jul 03, 2018.

TATARA, M. R. et al. Effects of dietary calcium content and vitamin D source on skeletal properties in growing turkeys. **British Poultry Science**, v. 52, p. 718-729, 2011. Available from: <https://www.tandfonline.com/doi/abs/10.1080/00071668.2011.631984?journalCode=cbps20>. Accessed: Jul 03, 2018 doi: 10.1080/00071668.2011.631984.

VIEITES, F.M. et al. Carcass dressing and noble cut yields of male broilers supplemented with 1,25-dihydroxycholecalciferol and reduction of calcium and available phosphorus in the diets. **Proceedings of International conference of agricultural engineering** 2012, Valencia, Spain.

VIEITES, F.M. et al. Performance, carcass and noble cuts yield of broilers fed diets supplemented with Solanum glaucophyllum. **Semina: Ciências Agrárias, Londrina**, v. 35, n. 3, p. 1617-1626, 2014. Available from: <http://www.uel.br/revistas/uel/index.php/semagrarias/article/view/14067>. Accessed: Jul 03, 2018. doi: 10.5433/1679-0359.2014v35n3p1617.

WHITEHEAD, C. Influence of vitamins and minerals on bone formation and quality. **Proceedings of the 11th WPSA European Poultry Conference**, 2002. Bremen, Germany.

ZENEBON, O. et al. **Métodos físico-químicos para análise de alimentos**. (Eds) *Instituto Adolfo Lutz*, 2008, p. 1020. (*São Paulo*, Brasil).