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Bone Characteristics of Broilers Supplemented with Vitamin D

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ABSTRACT

Vitamin D is added to broiler diets to supply its physiological requirement for bone formation. The fast growth rate of modern broilers is often associated with poor bone formation. Increasing vitamin D supplementation levels and the use of more available sources have applied to try to prevent leg problems, to increase carcass yield, and to improve the performance of broilers. The present study evaluated three vitamin D supplementation levels (1) 3,500 IU (control); (2) control + 1,954 IU of 25-hydroxycholecalciferol; and (3) control + 3,500 IU of vitamin D in broiler diets supplied up to 21 days of age. The objective was to investigate if the vitamin D levels above the recommendations could reduce leg problems in broilers. In this experiment, a total of 1,296 one-day-old male and female Cobb® 500 broilers were used. A 2 x 3 factorial arrangement was applied, consisting of two sexes and three vitamin D levels. No difference was found between the levels of vitamin D ($p > 0.05$), the performance of males or females, the gait score, the valgus and varus incidence, the tibial dyschondroplasia incidence, the occurance of femoral degeneration, the bone colorimetric, and the carcass yield. Parts yield differences were found ($p > 0.05$), except for liver and intestine yields. We concluded that the lowest tested vitamin D level (3,500 IU per kilogram of feed) added to the diet was the best choice in terms of cost/benefit to help minimizing leg problems in broilers.

INTRODUCTION

Cholecalciferol (vitamin D) plays an essential role in calcium (Ca) and phosphorus (P) absorption, and, therefore, influences the utilization of these minerals. Studies suggest that vitamin D and dietary phytase activity may have synergistic actions (Edwards, 2002; Whitehead *et al*., 2004; Afsharmanesh & Pourreza, 2005), such as enhancing phytate-phosphorus utilization, and mobilizing Ca from the bone to the extracellular fluid.

Vitamin D has been studied for the prevention of bone conditions in broilers (Fritts & Waldroup, 2005; Rao *et al*., 2008). Until recently, tibial dyschondroplasia was the main leg problem affecting broilers (Almeida Paz *et al*., 2008). However, this issue does not seem to be as severe because vitamin D supplementation has become increasingly frequent, which together with genetic selection, has prevented that disease (Khan *et al*., 2010).

Vitamin D is added to broiler diets to provide Ca balance, and to improve Ca intestinal absorption (Waldenstedt, 2006). Studies indicates that broiler body weight, weight gain, and bone strength are enhanced with increasing dietary vitamin D levels (Whitehead *et al*., 2004; Rao *et al*., 2008; Khan *et al*., 2010). Rostagno *et al*. (2011) recommend the use of 1,290 IU of vitamin D for broilers reared under Brazilian conditions; however, most commercial farms use 3,500 IU to minimize leg problems. The severity of tibial dyschondroplasia was higher in broilers fed diets containing 200 IU of vitamin D3 /kg than those fed with a higher level of vitamin D3 (Khan *et al.*, 2010). The authors also verified that feeding levels of 1,500 or 3,500 IU of vitamin D3 positively affected the birds' immune system.

This study aimed at investigating the recommended vitamin D level with the addition of 25-hydroxycholecalciferol in broiler diet supplement, and broiler performance variables, carcass and bone characteristics at 42 days of growth.

METHODOLOGY

This experiment was approved by the Ethics Committee on Animal Use (CEUA) of that university (009/2013).

Location

The present study was carried out in an experimental poultry house of the School of Agricultural Sciences of the Federal University of Grande Dourados, Brazil, located at latitude 22°13'18" S, longitude 54°48'23" W, and altitude of 430 m. The regional climate is characterized by humid and hot summers and dry winters.

Birds, management, and diets

A total of 1,296 one-day-old male and female Cobb® 500 chicks was randomly distributed according to an experimental design in a 2 x 3 factorial arrangement (two sexes and three dietary vitamin D levels), totaling six treatments with four replicates of 54 birds each. The house was equipped with evaporative cooling equipment and exhaustion fans for internal temperature control. The cooling equipment was automatically activated to meet the birds' thermal comfort temperature. Tube feeders and bell drinkers were used to supply feed and water *ad libitum*.

Birds were fed a diet with identical nutritional levels, except for vitamin D, between d1 and 21. The following dietary treatments were applied: (T1) 3,500 IU of vitamin D (control); (T2) Control + 1,954 IU of 25-hydroxycholecalciferol, and (T3) Control + 3,500 IU (7,000) of vitamin D. The feeding program was divided four phases (a) pre-starter (0-7 days): diet with 22.5% crude protein (CP), 1.4% lysine, 0.6% methionine, 0.92% calcium, and 0.51% phosphorus; (b) starter (8 to 18 days): diet with 21% CP, 1.1% lysine, 0.51% methionine, 0.84% calcium, and 0.52% phosphorus; (c) grower (19 to 36 days): diet with 18% CP, 1% lysine, 0.45% methionine, 0.68% calcium, and 0.45% phosphorus; (d) finisher, with 18% CP, 1% lysine, 0.45% methionine, 0.68% calcium, and 0.4% phosphorus. All other nutrients were supplied according to the recommendations of Rostagno *et al*. (2011). All birds received single diet after 21 days of thee grow out.

Parameter measured

Birds, feed offer, and feed residues were weighed weekly to evaluate their live performance (weight gain, average weight, feed intake, feed conversion).

On d 42 of the grow-out, gait score (GS) was evaluated according to the number of steps that the bird walked along 1 m. A 0 to 5 scale was applied. Score 0 was given to birds walking 10 steps; score 1 was given to birds walking 6-9 steps; score 2 was given to birds walking 4-5 steps; score 3 was given to birds that walked up to 3 steps; score 4 was given to lame birds; and score 5 was given to severely lame birds that were not able not walk (Kestin *et al*., 1992). Valgus and varus (VG and VR) deformities and footpad dermatitis (FD) were assessed in 50% of the birds on the same day. The VG and VR deformities were assessed using a caliper and a protractor to measure the angle between the tibia and the third finger of birds' right and left legs. Valgus was characterized by an angle greater than 10° and positive, and varus when the angle was lower than 10° and negative (Almeida Paz *et al*., 2010).

Footpad dermatities was scored according to lesion severity according to a 0 to 2 scale. Score 0 was given to birds with no footpad lesions; score 1 was given to birds with lesions up to 5 mm in diameter; and score 2 for birds with lesions larger than 5 mm in diameter. Both feet were scored.

On d 42 of the grow-out period, 144 broilers were slaughtered according to the standard slaughter method. Birds were selected according to gait score and identified. Four birds with each gait score (0 to 5) were randomly selected, totaling 24 birds per treatment. During slaughter, old scratches (which occurred during the grow-out) and new scratches (which possibly occurred during harvesting and transportation) were recorded. After evisceration, carcasses were weighed to determine carcass yield (carcass weight relative to live weight). Breast, leg (thigh and drumstick), wing, back, heart, liver, and gizzard yields (parts weight relative to carcass weight) were evaluated, as well as intestines, neck, and head yieldss.

The right and left femur edge were examined for femoral degeneration (FDE). The integrity of the femoral head was scored according to a 1 to 5 scale under visual examination. Score 1 was given when the femur head and joint capsule were intact; score 2 was given when the bone was still intact, but the joint capsule was partially or completely detached; score 3 was given when the femoral head presented no cartilage and was partly broken; score 4 was given when the femoral head was detached and the bone tissue was exposed and reddish, but it still maintained its anatomical shape; and score 5 was given when the femoral head lost its anatomical shape (Almeida Paz *et al.*, 2008).

Tibial dyschondroplasia (TD) was evaluated by visually examining the growth plate of the left and right tibiae. Tibiae were scored according to a 0 to 4 scale, where score 0 indicates no thickening of the growth plate (absence of lesion); score 1 shows the accumulation of 3 mm of cartilage outside the range of average growth; score 2 shows the development of lesions between 3-6 mm; score 3 shows lesion larger than 6 mm; and score 4 is given to cases where the length of the lesion is in a large area or almost the entire tibia epiphysis is damaged (Almeida Paz *et al.*, 2010).

Colorimetric analysis was applied to the raw bones of the right drumsticks and thighs. Forty-four samples of the tibia and the femur were randomly selected and analyzed using a colorimeter (Minolta® 400R), operating with D65 illuminant and viewing angle of 10° in the CIELAB system positioned on the proximal epiphysis. Bone lightness (L*), redness (a*), and yellowness (b*) were determined. Triplicate readings were made on each epiphysis, and their average was used for statistical analysis.

The same number of thighs and drumsticks (44) was used for the evaluation of the black bone syndrome. These parts were baked in an oven until the internal temperature reached 90 °C, which took approximately 30 min. After baking, meat samples were macroscopically examined for the darkening of the meat around the bone. Meat samples were scored for its apparent color on a 0 to 2 scale. Score 0 was assigned when the meat was not dark; score 1 was given when the meat was slightly darkened, and score 2 was given when the meat was dark.

Statistical analysis

A one-way analysis of variance was applied to determine the main effects of dietary vitamin D levels on broiler performance, carcass and parts yields and bone parameters. The ANOVA procedure of SAS® statistical package, version 9.2 (SAS Institute, Cary, NC, USA) was used to process data. Means were compared by Fisher's and Chi-square test at 5% probability level. Spearman's correlation test was applied to determine possible correlations among treatments.

RESULTS AND DISCUSSION

Performance parameters were not affected by dietary vitamin D levels or bird sex (p> 0.05). These results are consistent with previous studies (Fritts & Waldroup, 2005; Rao *et al.*, 2008), whose authors did not find any effect of different dietary levels of vitamin D on broiler performance (p>0.05).

The prevalence of leg problems was not different among treatments (p<0.05) leading to similar performance among the studied groups. Probably, all birds were equally able to reach the drinkers and feeders. The lowest vitamin D level added to the experimental diets was high when compared with the Brazilian recommendations (Rostagno *et al.*, 2011). Different weight gain may have been obtained if dietary Ca and P levels were unbalanced. It is reported in current literature that vitamin D could increase the intestinal absorption and decrease the renal excretion to ensure homeostasis (Waldenstedt, 2006). Khan *et al.* (2010) found a better body weight and feed conversion ratio in 28 and 42-d-old broilers when a high vitamin D level was fed. However, the vitamin D levels used in that study were lower than those applied in the present study. Whitehead *et al.* (2004) found that body weight and bone strength increased as vitamin D supplementation levels incrased, and obtained the best results in the group fed 125 mg of vitamin D. The authors evaluated different vitamin D levels in the diet of broilers up to 14 days of age (5, 20, 125, 250 mg/kg feed) associated with Ca (8 and 13 g/kg feed), and P (3.5 and 5 g/kg of feed) levels.

Broilers fed with low dietary Ca and P levels presented lower weight gain, feed conversion ratio, tibial ash content, and Ca and P serum levels (p<0.05) than those fed a balanced diet (Rao *et al.*, 2008). Waldenstedt (2006) also found that broiler vitamin D requirements increased when dietary Ca or P contents were below optimal levels. In the present study, the lack of effect of dietary vitamin D may be explained by the adequate dietary Ca and P balance, and consequently, no vitamin D supplementation was required. This result indicates that when broilers are fed diets with balanced Ca and P levels, the dietary supplementation of high vitamin D levels does not necessarily promote better performance or bone characteristics.

No influence of treatments (p> 0.05) was found on the whole carcass, breast, thigh and drumstick (leg), wing, or back yields (Table 1). The carcass yield obtained in the present trial did not differ from a previous study (Angel *et al.*, 2006) using different dietary P levels of P and supplemental vitamin D sources, including the 25-hydroxycholecalciferol. Brito *et al*. (2010) obtained higher carcass yield (p<0.05) with the dietary addition of 25-hydroxycholecalciferol compared with cholecalciferol.

Results from the Fisher's test applied to mean results showed that dietary vitamin D supplementation had different effects on tibial dyschondroplasia according to sex. The incidence of DT was low (3.0%, 2.8%, and 3.2% corresponding to vitamin D levels in T1, T2, and T3, respectively) in the present study. Applying the tested levels of vitamin D might have prevented DT. Whitehead (2009) also detected lower incidence of DT in broilers fed diet containing 0.80% Ca and 0.50% P; and vitamin D levels between 5,000 IU to 10,000 IU/kg; compared with vitamin D levels lower than 5000 IU.

Joint deviation (left and right), gait score, FDE, and the fresh (NS) and old scratch (OS) results are presented in Table 2. There was no effect of treatment on FDE (p > 0.05). Both male and female birds showed a higher prevalence of valgus in the left leg (LVG) and varus in the right leg (RVR). Regarding GS results obtained with all treatments, 80% of females and 75% of the males presented scores between 0 and 2. In birds with more severe walking disability (scores 4 and 5), the percentage was approximately 3% for females and 5% for males. This higher percentage of males with gait problems is probably due to their higher body weight compared with females. Leg angle deviation and gait scores were evaluated by nonparametric tests and were correlated (Table 3).

Table 1 – Carcass and parts yields of female and male broilers fed different dietary vitamin D levels.

Yield (%)	Vit. D levels	Sex		Average	CV%	P-value
		Female	Male			
Carcass	T1	73.23	73.19	73.21		
	T2	72.94	74.01	73.45	3.01	0.45
	T3	73.21	74.00	73.65		
	Average	73.11	73.73			
Breast	T1	41.28	40.90	41.08		
	T2	40.82	40.91	40.86	6.34	0.46
	T3	39.62	40.74	40.22		
	Average	40.06	40.85			
Leg	T1	28.64	29.43	29.04		
	T2	29.52	29.05	29.29	6.39	0.22
	T3	28.32	29.42	28.91		
	Average	28.88	29.30			
Wing	T1	10.39	10.44	10.41		
	T2	10.53	10.29	10.41	8.03	0.95
	T3	10.46	10.36	10.41		
	Average	10.46	10.36			
Feet	T1	4.33	5.17	4.78		
	T2	4.32	4.96	4.64	11.18	0.0001
	T3	4.46	5.06	4.79		
	Average	4.36	5.06			
Back	T1	19.24	18.24	18.73		
	T2	18.16	18.95	18.55	9.54	0.11
	T3	19.46	18.84	19.12		
	Average	18.89	18.68			

The absence of letters in rows and columns indicates no difference (p> 0.05) between sex and dietary vitamin D level. (T1) 3,500 IU of vitamin D (control); (T2) Control + 1,954 IU of 25-hydroxycholecalciferol, and (T3) Control + 3,500 IU (7,000) of vitamin D.

Table 2 – Percentage of right leg valgus (RVG), left leg valgus (LVG), right leg varus (RVR), left leg varus (LVR), new scratches (NS), old scratches (OS), femoral degeneration (FDE) and gait score (GS) of female and male broilers fed different levels of dietary vitamin D.

	Female			Male		
	T1 (%)	T2 (%)	T3 (%)	T1 (%)	T2 (%)	T3 (%)
RVG	17.88	25.80	17.39	26.5	43.51	45.00
LVG	21.85	43.55	20.65	29,00	51.56	58.75
RVR	14.60	8.87	9.78	9.40	0.78	3.75
LVR	7.28	5.64	7.60	8.55	0.78	3.75
NS	7.92	2.97	8.91	5.94	9.90	7.92
OS	4.95	2.97	3.96	4.95	4.95	11.88
FDE	1.64	1.58	1.47	1.88	1.79	1.70
GS						
0	18.50	23.36	27.83	26.83	15.24	14.83
1	24.00	34.60	39.15	26.83	30.00	24.40
2	42.98	32,1	24.53	23.41	30.00	28.59
3	8.69	7.50	3.30	16.58	19.06	23.00
4	1.42	1.40	2.83	3.41	1.90	3.83
5	1.42	0.93	2.36	1.95	3.80	3.35

The absence of letters in rows and columns indicates no difference between numbers (p> 0.05). T1 (3,500 IU of vitamin D), T2 (3,500 IU of vitamin D + 1,954 IU of 25-hydroxycholecalciferol), and T3 (7,000 IU of vitamin D).

A positive correlation between GS and RVG and LVG was found (Table 4). Walking problems in chickens are commonly associated with valgus deviation. A positive correlation was also found between valgus of the right leg with the valgus of the left leg. When one of the legs presents a problem, it is accepted that the birds overload the other limb, which causes pain or discomfort while walking, and, consequently affecting their gait. Positive correlations were found between the variables VG (right and left feet), FD (right and left feet), and VR (right and left feet). The positive correlation between RVR and LVR is probably the result of the bird's attempt to maintain its body balance. When varus is present in one leg (negative angle), the bird tries to compensate this deviation by placing most of the body weight on the other leg, overloading the limb. Fernandes *et al*. (2012) analyzed the correlation between gait score and valgus deformity and found that the displacement of the hock joint on its axis was a source of valgus deformity of both right and left legs. Therefore, leg disorders are not always related to femoral degeneration or tibial dyschondroplasia. Birds with or without these lesions may present the same gait scores.

Table 3 – Spearman's correlation coefficients between gait score (GS), right-leg valgus (RVG), left-leg valgus (LVG), right-leg varus (RVR), left-leg varus (LVR), right-foot pad dermatitis (RFD), left-foot pad dermatitis (LFD), new scratches (NS), and old scratches (OS) of female and male broilers fed different dietary vitamin D levels.

	GS	RVG	LVG	RVR	LVR	RFD	LFD	NS	OS
GS	1.00	0.24***	0.17**	ns	ns	Ns	ns	Ns	ns
RVR	-	1.00	0.74***	ns	ns	0.65***	0.60***	Ns	ns
LVG	-	-	1.00	ns	ns	0.54***	0.57***	Ns	ns
RVR	-	-	-	1.00	0.57***	Ns	ns	Ns	ns
LVR	-	-	-	-	1.00	Ns	0.21*	Ns	ns
RFD	-	-	-	-	-	1.00	0.87***	Ns	ns
LFD	-	-	-	-	-	-	1.00	Ns	ns
NS	-	-	-	-	-	-	-	1.00	ns
OS	-	-	-	-	-	-	-	-	1.00

ns: not significant. * p <0.05 ** p <0.01 *** p <0.001.

Bone color was not affected (p>0.05) by the treatments (Table 4). Mean values of L* both of the femur and tibia were within the acceptable range, according to the scale of Mota (2013) (acceptable L*≥40, intermediate = between 35 and 40, and unacceptable ≤ 35).

The results of the Chi-square test showed that the effects of treatments and sex on the incidence of the black bone syndrome in the femurs and tibiae were not statistically dependent (p > 0.05). The incidence of black bone syndrome was 33% when considering the sum of the scores 1 and 2 in the 0 to 2 scale applied. Dietary vitamin D supplementation level may have reduced black bone syndrome severity. This syndrome is associated with how chicken meat is stored, and it is possibly caused by the presence of ice crystals within the cells at the time of cooking (Baldo *et al*., 2013).

CONCLUSION

Different dietary levels of vitamin D had no effect on broiler performance, carcass and parts yields, or on the bone variables measured.

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Table 4 – Lightness value (L*), redness (a*), and yellowness (b*) values of the femur and tibia of female and male broilers fed different dietary vitamin D levels.

Bone	Color	Vit. D level	Sex		Average	CV%	P-value
			Female	Male			
Femur	L*	T1	45.08	40.27	42.89		
		T2	43.99	47.84	45.91	9.60	0.11
		T3	45.24	43.83	44.42		
		Average	44.70	44.37			
	a*	T1	7.13	7.65	7.37		
		T2	7.24	6.85	7.04	17.09	0.52
		T3	6.18	6.97	6.64		
		Average	6.91	7.10			
	b*	T1	11.93	11.69	11.82		
		T2	10.98	11.29	11.16	16.89	0.22
		T3	17.36	9.71	13.54		
		Average	13.78	10.81			
Tibia	L*	T1	41.07	40.40	40.77		
		T2	41.55	40.96	41.25	6.49	0.98
		T3	41.68	41.24	41.42		
		Average	41.42	40.91			
	a*	T1	8.84	10.44	9.57		
		T2	9.10	8.79	8.95	18.68	0.25
		T3	7.84	9.69	8.92		
		Average	8.66	9.56			
	b*	T1	11.94	12.54	12.21		
		T2	11.93	10.77	11.35	9.17	0.08
		T3	11.70	12.42	12.12		
		Average	11.87	11.85			

The absence of letters in rows and columns indicates no difference (p>0.05) between the levels and variables. T1 (3,500 IU of vitamin D). T2 (3,500 IU of vitamin D + 1,954 IU of 25-hydroxycholecalciferol), and T3 (7,000 IU of vitamin D).

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