Asian-Aust. J. Anim. Sci.
Vol. 26, No. 3 : 408-415 March 2013
http://dx.doi.org/10.5713/ajas.2012.12455

www.ajas.info
pISSN 1011-2367 eISSN 1976-5517

Use of Vitamin D_3 and Its Metabolites in Broiler Chicken Feed on Performance, Bone Parameters and Meat Quality

Ana Flávia Quiles Marques Garcia[1], Alice Eiko Murakami*, Cristiane Regina do Amaral Duarte, Iván Camilo Ospina Rojas, Karla Paola Picoli and Maíra Mangili Puzotti
Animal Science Department, Maringá State University, State of Paraná, Brazil

ABSTRACT: The objective of this experiment was to assess the use of different vitamin D metabolites in the feed of broiler chickens and the effects of the metabolites on performance, bone parameters and meat quality. A total of 952 one-day-old male broiler chicks were distributed in a completely randomised design, with four treatments, seven replicates and 34 birds per experimental unit. The treatments consisted of four different sources of vitamin D included in the diet, D_3, $25(OH)D_3$, $1,25(OH)_2D_3$, and $1\alpha(OH)D_3$, providing 2000 and 1600 IU of vitamin D in the starter (1 to 21 d) and growth phases (22 to 42 d), respectively. Mean weight, feed:gain and weight gain throughout the rearing period were less in animals fed $1\alpha(OH)D_3$ when compared with the other treatments ($p<0.05$). No significant differences were noted among the treatments ($p>0.05$) for various bone parameters. Meat colour differed among the treatments ($p>0.05$). All of the metabolites used in the diets, with the exception of $1\alpha(OH)D_3$, can be used for broiler chickens without problems for performance and bone quality, however, some aspects of meat quality were affected. (**Key Words:** Cholecalciferol, Meat Quality, Bone Strength)

INTRODUCTION

Poultry meat accounts for 30% of global meat consumption (FAO, 2010). This high consumption is associated with the affordable price and high nutritional value of chicken meat. However, with the genetic improvement of birds, bone development has not been proportional to weight gain in these animals, which has resulted in a higher incidence of locomotion problems, this has become a major concern due to reduced performance and increased carcass condemnations in slaughterhouses. Nutritional factors are known to improve locomotion problems. Among these is vitamin D, due to its important role in calcium and phosphorus metabolism.

To be used by the body, vitamin D_3 must be metabolised following ingestion into 25-hydroxycholecalciferol ($25(OH)D_3$) in the liver and subsequently into its active metabolite 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) in the kidneys. These metabolites are currently commercially available, as 1-α-hydroxycholecalciferol ($1\alpha(OH)D_3$), a synthetic analogue of the active metabolite $1,25(OH)_2D_3$, which is converted into its active form in the liver.

Vitamin D supplementation is closely related to a decreased incidence of bone disorders because vitamin D is involved in various physiological processes, including the absorption of calcium and phosphorus, bone mineralisation and mobilisation (Rennie and Whitehead, 1996; Driver et al., 2005; Korver, 2005; Kasim et al., 2006). In the body, vitamin D is required for the absorption of calcium and phosphorus in the intestines, increasing its utilisation efficiency and consequently increasing the bone ash density. Furthermore, vitamin D regulates the secretion of parathyroid hormone (PTH) and stimulates several tissues with vitamin D receptors (Norman, 1985). Therefore, vitamin D deficiency can further aggravate these factors, leading to decreased productivity and emergence of metabolic disorders.

Studies suggest that vitamin D may also affect growth performance (Yarger et al., 1995; Brito et al., 2010) and meat quality (Enright et al., 1999; Han et al., 2012), altering water retention (Montgomery et al., 2000), colour (Wilborn et al., 2004) and shear force (Rider et al., 2004). Vitamin D supplementation increases the intestinal absorption of

* Corresponding Author: Alice Eiko Murakami. Tel: +55-44-3011-8942, Fax: +55-44-3011-8942, E-mail: aemurakami@uem.br
[1] Post-graduation in Animal Science, Maringá State University, State of Paraná, Scholarship of CNPq, Brazil.
Submitted Aug. 22, 2012; Accepted Nov. 19, 2012; Revised Dec. 11, 2012

calcium and phosphorus, stimulating the production of calcium-binding proteins in the mucosa, which activates the calcium activated tenderisation (CAT) complex through the increase in plasma calcium. This complex regulates the enzymatic activity of calpain and other proteases involved in the process of meat tenderisation (Santos, 2006).

The purpose of using these substances is to provide the animals with metabolised forms of vitamin D, increasing its efficiency in the body and decreasing energy expenditure. The absorption rate of $25(OH)D_3$ is approximately 20% higher than that of vitamin D_3 (Applegate and Angel, 2005), and $1,25(OH)_2D_3$ and $1\alpha(OH)D_3$ do not require renal metabolism.

Therefore, the objective of this study was to assess the use of different vitamin D metabolites in the feed of broiler chickens and their effect on performance, bone parameters and meat quality.

MATERIALS AND METHODS

Animals and experimental design

The experiment was conducted in the poultry section of the Iguatemi Experimental Farm, State University of Maringá, under the approval of the Animal Experimentation Ethics Committee (Comitê de Ética de Animais em Experimentação/Universidade Estadual de Maringá-CEEA/UEM-Registration No. 034/2011). The study included 952 Cobb male broiler chicks, distributed into a completely randomised experimental design of four treatments (diets containing different vitamin D_3 metabolites), seven replicates and 34 birds per experimental unit.

Diets

The treatments consisted of four different sources of vitamin D: cholecalciferol (D_3), 25-hydroxycholecalciferol ($25(OH)D_3$), 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) and 1α-hydroxycholecalciferol ($1\alpha(OH)D_3$). The different sources of vitamin D were included into the diet in place of the vitamin D, providing 2,000 IU of vitamin D_3 in the pre-starter and starter phases and 1,600 IU of vitamin D in the growth phase, according to the recommendations described by Rostagno et al. (2005). The metabolites of vitamins D were added to the diet in replacement for the inert component. The vitamin supplement contained no vitamin D.

Diets were formulated using corn and soybean meal, according values based on the food chemical composition and nutritional requirements for male broilers in each phase (Rostagno et al., 2005). Water and feed were provided *ad libitum* in a feeding program divided into three phases: pre-starter, from the first to the seventh day of life; starter, from the eighth to the 21st d; and growth, from the 22nd to the 42nd d. Percentage and calculated compositions of the experimental diets are shown in Table 1.

Performance

Birds and feed were weighed weekly throughout the experimental period to assess performance (weight gain, mean weight, feed intake and feed:gain). Broiler mortality and leftover feed were recorded daily to correct the feed:gain per pen.

Bone sampling and analyses

To assess bone parameters, the left legs of two birds per experimental unit were collected at 7, 21 and 42 d of age and remained frozen (-20°C) until processing.

Once the legs were thawed, the muscle tissue was removed using scissors and tweezers, separating the femur and tibia. The bones were subsequently weighed on an analytical balance (g±0.0001), and the mid-leg length and diameter were measured using digital calipers (mm). The Seedor index (Seedor et al., 1991) was calculated using the formula Seedor index = bone weight (mg)/bone length (mm).

Bone strength analysis was performed using fresh thawed bones. The mechanism consisted of a base that supports the epiphyses of the bone and a force was applied to the central region of the bone. The values were expressed in kilograms-force (kgf).

Following the testing for bone strength, the bones were prepared for the determination of mineral content. The bones were defatted in petroleum ether, dried in a forced air oven, ground and weighed on an analytical balance (0.001 g). They were then dried at 105°C for 12 h, weighed after cooling and calcined in a muffle furnace at 600°C to obtain the ashes, following the methodology described by Oliveira (2006). The ash content of calcium and phosphorus in bone, were performed according to the methodology described by Silva and Queiróz (2004).

Muscle sampling and analyses

Meat quality variables were assessed using samples collected from the breast and thigh of two birds per experimental unit at 42 d of age, with the exception of water-holding capacity, for which samples were collected from only one bird per experimental unit.

The pH was determined directly from the left breast fillet of the birds using a TESTO® pH metre 15 min *post mortem*. Incisions were made and the electrode was inserted into three different regions: the upper, middle and lower breast muscle (pectoralis major). The estimate was obtained from the average of the three measured points.

The colour of the breast and thigh meats was assessed using a KONICA MINOLTA colourimeter model CR300. The characteristics under assessment were L* (lightness-

Table 1. Composition and calculated percentage of the experimental diets

Ingredients (%)	1-7 d	8-21 d	22-42 d
Corn grain	54.81	58.06	62.74
Soybean meal, 45%	37.31	34.54	29.06
Dicalcium phosphate	1.89	1.75	1.54
Limestone	0.84	0.81	0.75
Soybean oil	2.92	2.96	3.99
Salt commom	0.300	0.300	0.300
DL-metionine, 98%	0.356	0.244	0.227
L-lysine HCL, 78%	0.341	0.185	0.222
L-threonine 99%	0.134	0.048	0.054
Mineral vitamin supplement[1,2]	0.400	0.400	0.400
Inert[3]	0.700	0.700	0.700
	100.00	100.00	100.00
Calculated values			
ME (kcal/kg)	2,950	2,959	3,125
Crude protein (%)	22.04	20.79	18.72
Digestible lysine (%)	1.330	1.146	1.045
Digestible met+cys (%)	0.944	0.814	0.753
Digestible tryptophan (%)	0.242	0.228	0.200
Digestible threonine (%)	0.865	0.746	0.679
Digestible valine (%)	0.912	0.870	0.781
Digestible arginine (%)	1.391	1.314	1.156
Calcium (%)	0.939	0.884	0.794
Available phosphorus (%)	0.470	0.442	0.396
Sodium (%)	0.138	0.138	0.138
DEB[4]	210	200	177

[1] Initial vitamin supplement (amount/kg of diet): Vitamin A 2,916,667.00 UI; Vitamin E 8,750.00 mg; Vitamin K_3 433.333 mg; Vitamin B_1 408.333 mg; Vitamin B_2 1,333.334 mg, Vitamin B_{12} 4,166.667 mcg; Niacin 8,983.333 mg; Pantithenic acid 3,166.666 mg; Folic acid 200.00 mg; Antioxidant 1450.00. Growth vitamin supplement (amount/ kg of diet): Vitamin A 2,250,000.00 UI; Vitamin E 7,000.000 mg; Vitamin K_3 455.00 mg; Vitamin B_1 343.000 mg; Vitamin B_2 1,000.00 mg, Vitamin B_{12} 7,000.00 mcg; Niacin 7,105.00 mg; Pantothenic acid 2,612.50 mg; Folic acid 160.00 mg; Antioxidant 1,200.00.

[2] Mineral (amount/ kg of diet): Fe 12,600.000 mg; Cu 3,072.000 mg; I 248.00 mg; Zn 12,600.000 mg; Mn 15,004.000 mg; Se 61.2000 mg; Co 50.400 mg.

[3] The metabolites of vitamins D_3, 25(OH)D_3, 1,25$(OH)_2D_3$ e 1-AlpHa-D_3 were added in replacement for inert: 2,000 UI e 1,600, for the initial and growth period, respectivaly.

[4] Dietary Electrolytic Balance = Na+K+Cl- (mEq/kg).

from dark to light), a* (red/green intensity) and b* (yellow/blue intensity), with three replicates per point in three different regions: the upper, middle and lower breast muscle (pectoralis major) and the inner thigh, at 15 min *post mortem*, following the methodology proposed by Honikel (1998). The hue angle (true colour) and chroma (colour saturation) of the sample were determined at 15 min *post mortem*, according to the methodology described by Harder (2005).

The left breast muscle of the bird was used to determine the weight loss during cooking (WLC). The breast muscle was previously cooled and weighed to obtain the weight before cooking. The muscle was wrapped in aluminium foil and maintained on a commercial hot plate for approximately 10 min at 180°C, until the inside of the breast muscle reached 80°C. After the breast muscle reached room temperature, the sample was weighed again to obtain the weight after cooking.

The analysis of shear force was performed on the same fillets used to determine the weight loss during cooking. The samples were trimmed and cut into three rectangles (1.0×1.0×1.3 cm). The analysis described above was performed using a TAXT2i texturometre coupled to a Warner-Bratzler Shear Force mechanical probe with a 20 kg capacity and crosshead speed of 20 cm/min, providing a measure of shear force (SF) of the sample in kilograms-force (kgf/cm^2).

The analysis of water-holding capacity (WHC) was performed according to the methodology proposed by Nakamura and Katok (1985). The left breast of one bird per experimental unit was collected shortly after slaughter, totalling seven samples per treatment. The method consisted of an initial weighing of 1 g of raw muscle, which was then wrapped in filter paper and centrifuged at 1,500 rpm for four min. The samples were weighed after centrifugation, dried in an oven at 70°C for 12 h and then weighed again.

The percentage WHC was determined by calculating the difference between the weight of the meat sample after centrifugation and the weight of the sample after drying, then dividing this difference by the initial weight of the raw sample and multiplying by 100.

Statistical analysis

The statistical analysis of the data was performed using the Statistics and Genetics Analysis System (SAEG, 2005). Data were subjected to analysis of variance. The means of the studied variables were compared among the different treatments using the Tukey's test for mean comparison, considering $p<0.05$.

RESULTS AND DISCUSSION

Growth performance

The means and standard errors for mean weight, weight gain, feed intake and feed:gain are shown in Table 2. The birds fed diets containing the metabolite $1\alpha(OH)D_3$ presented lower weight gain and feed:gain for all of the evaluated time periods ($p<0.05$). In cases of prolonged toxicity, vitamin D can cause a decrease in feed intake and, consequently, decrease in performance (McCarthy et al., 1984; Zanuzzi et al., 2011).

Feed consumption in this study was affected by the different metabolites. In the time period of 1 to 21 days, the group of birds fed the metabolite $25(OH)D_3$ presented lower feed intake than the groups fed D_3 and $1,25(OH)_2D_3$. However, over the total rearing period (1 to 42 d), the group fed $1\alpha(OH)D_3$ presented the lowest mean feed consumption when compared with the groups fed D_3 and $1,25(OH)_2D_3$. Nevertheless, only the group fed $1\alpha(OH)D_3$ differed from the others ($p<0.05$) in terms of feed:gain for all of the evaluated time periods. Despite being an active analogue of vitamin D, $1\alpha(OH)D_3$ has a lower utilisation efficiency in broilers, thus reducing animal performance (Han et al., 2009).

For the time period of 1 to 21 d, weight gain was greater ($p<0.05$) in animals fed $1,25(OH)_2D_3$ than in those treated with $25(OH)D_3$; however, this difference did not persist across the total rearing period (1 to 42 d). As vitamin D undergoes hydroxylation, it is likely that the molecule tends to become more polar and more easily absorbed in the small intestines (Applegate and Angel, 2005). Furthermore, birds in the starter period do not have a complete enzymatic system with which to perform hydroxylation in the liver, which favours the administration of active metabolites (Swiatkiewiez et al., 2006), thus explaining the increased use of these already hydroxylated metabolites.

In high doses, $1\alpha(OH)D_3$ can become toxic, reducing the absorption of calcium and phosphorus and thus impairing the animal performance (Reddy and Tserng, 1989). In this study, the metabolites were used in the diets to meet the vitamin D_3 requirements for broilers, as recommended by Rostagno et al. (2005), replacing vitamin D_3. This methodology was used to provide equivalent amounts of vitamin D_3 given that each product provides different amounts of vitamin D_3. Therefore, the manufacturers' recommendations of $1\alpha(OH)D_3$ were extrapolated by up to 10 times, which may have caused an overload within the animal bodies, resulting in decreased performance.

The supplementation of the different metabolites together with a source of vitamin D_3 is a method for maximising animal performance because it provides the animals with a storage form of vitamin D. The metabolites will act together with the vitamin D to reduce energy

Table 2. Means and standard errors of the parameters of performance of broilers fed different sources of vitamin D

	D_3	25-OHD_3	$1,25(OH)_2D_3$	$1\alpha(OH)D_3$	CV%
	---- 1-7 d ----				
Average weight (g)	181.69±1.34[a]	181.39±2.24[a]	184.54±1.23[a]	161.29±1.35[b]	2.37
Weight gain (g)	135.60±1.33[a]	135.3±2.24[a]	138.37±1.26[a]	115.13±1.33[b]	3.21
Feed intake (g)	167.15±1.34[a]	163.53±0.99[a]	167.66±1.26[a]	154.04±1.44[b]	2.06
Feed/gain (kg/kg)	1.256±0.02[a]	1.211±0.02[a]	1.212±0.01[a]	1.339±0.02[b]	3.98
	---- 1-21 d ----				
Average weight (kg)	1.017±0.018[ab]	0.996±0.016[a]	1.055±0.011[b]	0.898±0.008[c]	3.70
Weight gain (kg)	0.792±0.015[ab]	0.768±0.015[a]	0.824±0.010[b]	0.692±0.007[c]	4.34
Feed intake (kg)	1.200±0.022[b]	1.119±0.024[a]	1.247±0.013[b]	1.182±0.021[ab]	4.53
Feed/gain (kg/kg)	1.517±0.021a	1.457±0.022[a]	1.515±0.024[a]	1.712±0.031[b]	4.21
	---- 1-42 d ----				
Average weight (kg)	2.672±0.009[a]	2.642±0.020[a]	2.710±0.038[a]	2.315±0,020[b]	2.54
Weight gain (kg)	2.626±0.009[a]	2.596±0.020[a]	2.664±0.038[a]	2.269±0.020[b]	2.59
Feed intake (kg)	4.548±0.055[a]	4.392±0.045[ab]	4.581±0.047[a]	4.283±0.055[b]	3.01
Feed/gain (kg/kg)	1.716±0.011[a]	1.692±0.009[a]	1.716±0.010[a]	1.887±0.017[b]	1.79

[a-b] Values in the same row with different superscripts differ significantly ($p<0.05$). Tukey test 5%.

expenditure, enhancing the results. The use of these compounds as a complete replacement of the vitamin D requirement could be an option with further investigation, especially considering the efficiency of each metabolite, given that the amount provided in this study may have caused toxicity.

Bone analyses

Mean bone parameters are presented in Table 3. Although 1,25$(OH)_2D_3$ increases calcium absorption in the intestines and reduces the formation of calcium complexes and therefore bone disorders (Grudtner et al., 1997; Applegate et al., 2003; Miller et al., 2006), no significant differences (p>0.05) in bone parameters, bone diameter, Seedor index, bone strength, or ash, calcium or phosphorus percentages were noted among the different metabolites used in this study (Table 3). Importantly, the values observed in this study for these characteristics were similar

Table 3. Means and standard errors of the parameters of the tibia and femur bone of broilers fed different sources of vitamin D, at 7, 21 and 42 d

	D_3	25-OHD_3	1.25$(OH)_2D_3$	1α$(OH)D_3$	CV%
	---------- Tibia 7 d ----------				
Seedor index[1,2]	28.56±1.03	29.18±0.74	28.11±0.82	26.34±0.62	6.91
Diameter (mm)[2]	2.916±0.059	2.998±0.062	2.897±0.045	2.812±0.045	4.96
Ash (%)[3]	45.02±3.67	47.95±1.78	49.37±3.63	50.85±1.95	13.29
Calcium (%)[3]	12.04±0.50	11.04±0.99	12.52±0.39	12.38±0.30	9.78
Phosphorous(%)[3]	7.80±0.22	7.24±0.45	7.39±0.52	8.03±0.41	11.74
	---------- Tibia 21 d ----------				
Seedor index	86.42±3.31	91.21±2.27	95.74±2.68	88.31±2.2	7.24
Diameter (mm)	5.414±0.750	5.546±0.138	5.589±0.113	5.432±0.091	6.60
Bone strength (kgf)[2]	16.07±0.91	15.62±1.37	14.35±0.63	14.50±0.39	15.99
Ash (%)	43.14±0.46	45.15±1.16	44.22±7.40	45.14±1.28	20.95
Calcium (%)	13.97±0.61	15.49±0.58	13.83±0.27	15.03±0.19	7.23
Phosphorous (%)	6.68±0.43	7.16±0.27	7.23±0.32	6.22±0.67	12.51
	---------- Tibia 42 d ----------				
Seedor index	196.74±4.02	195.23±2.80	188.40±5.02	183.63±5.15	5.79
Diameter (mm)	8.47±0.212	8.173±0.215	8.239±0.249	7.944±0.199	7.41
Bone strength (kgf)	34.18±6.06	31.66±2.67	34.31±2.68	35.38±2.86	18.92
Ash (%)	34.48±2.34	38.20±4.02	37.79±1.73	393.64±2.32	14.95
Calcium (%)	10.76±0.56	12.19±0.25	14.30±0.88	13.16±0.960	9.33
Phosphorous (%)	7.92±0.83	7.85±0.44	6.70±0.07	10.25±1.17	11.47
	---------- Femur 7 d ----------				
Seedor index	26.38±0.73	26.63±0.54	26.85±1.25	25.50±0.91	9.03
Diameter (mm)	3.111±0.044	3.100±0.083	3.041±0.076	3.092±0.101	6.75
Ash (%)	40.70±0.88	42.59±0.91	43.00±1.58	40.80±1.68	7.11
Calcium (%)	11.37±0.22	10.57±0.35	11.58±0.25	11.85±0.36	6.06
Phosphorous (%)	7.104±0.353	6.431±0.218	7.238±0.281	7.623±0.265	8.46
	---------- Femur 21 d ----------				
Seedor index	86.36±1.76	86.26±1.93	82.47±2.85	87.20±2.16	6.55
Diameter (mm)	6.144±0.103	6.091±0.087	6.085±0.164	6.204±0.148	5.59
Ash (%)	43.14±0.46	45.15±1.16	41.60±0.70	45.14±1.28	4.68
Calcium (%)	13.97±0.61	15.49±0.58	14.10±2.45	15.03±0.19	9.71
Phosphorous (%)	16.53±0.28	16.37±1.08	14.26±1.09	14.43±1.71	8.85
	---------- Femur 42 d ----------				
Seedor index	175.81±6.06	174.2±8.23	187.94±4.95	197.21±15.942	13.72
Diameter (mm)	9.502±0.129	9.492±0.257	9.439±0.119	9.719±0.232	5.55
Ash (%)	34.86±1.45	36.64±1.04	34.00±1.70	32.13±1.13	8.62
Calcium (%)	10.54±0.21	12.47±0.71	11.37±0.67	10.34±0.55	10.02
Phosphorous (%)	7.7l±034	7.38±0.36	7.56±0.74	7.90±0.78	14.43

(p>0.05) * Tukey test 5%. [1] Seedor index = bone weight (mg)/bone length (mm). [2] In the fresh bone. [3] In the dried bone.

Table 4. Means and standard errors of the parameters of meat quality of broilers fed different sources of vitamin D

		D_3	25-OHD$_3$	1,25(OH)2D$_3$	1α(OH)D$_3$	CV%
Breast	L* (15 min *pm*)[1]	43.68±0.42[b]	45.68±0.36[ab]	45.50±0.37[a]	43.99±0.53[ab]	2.49
	a* (15 min *pm*)[2]	5.38±0.41	6.70±0.95	5.07±0.57	5.91±0.47	29.20
	b* (15 min *pm*)[3]	6.93±0.47	7.71±0.55	6.86±0.58	6.06±0.52	20.45
	L* (24 h *pm*)[1]	50.39±0.82	51.20±0.61	51.75±0.40	52.84±0.26	2.94
	a* (24 h *pm*)[2]	8.59±0.41	10.36±0.48	8.92±0.19	10.13±0.47	26.45
	b* (24 h *pm*)[3]	7.33±0.67	8.41±0.26	7.32±0.25	7.05±0.57	15.16
	pH	6.07±0.04	6.07±0.05	6.13±0.06	6.06±0.03	2.01
	WLC(%)[4]	28.71±1.95	29.79±1.58	25.78±4.39	25.87±2.14	32.58
	SF (kgf/cm^{-2})[5]	3.64±0.16	3.87±0.46	3.66±0.37	4.00±0.41	26.96
	WHC (%)[6]	59.48±2.88	62.16±1.58	59.44±2.19	61.60±2.35	10.02
Thigh	L* (15 min *pm*)[1]	51.71±0.81	51.50±0.96	52.97±0.51	51.45±0.64	3.83
	a* (15 min *pm*)[2]	8.59±0.41[b]	10.36±0.48[a]	8.92±0.19[ab]	10.13±0.47[ab]	11.04
	b* (15 min *pm*)[3]	7.33±0.67	8.41±0.26	7.32±0.25	7.05±0.57	15.72
	L* (24 h *pm*)[1]	53.18±0.27	53.76±0.79	54.76±0.81	53.63±0.49	3.16
	a* (24 h *pm*)[2]	11.89±1.00[a]	10.52±0.38[ab]	8.89±0.41[b]	10.21±0.35[ab]	15.42
	b* (24 h *pm*)[3]	7.97±0.52[a]	7.93±0.22[a]	6.77±0.24[ab]	6.20±0.24[b]	12.15

[a-b] values in the same row with different superscripts differ significantly ($p<0.05$). Tukey test 5%.
[1] Lightness. [2] Red/green intensity. [3] Yellow/blue intensity. [4] Weigth loss during cooking. [5] Shear force. [6] Water-holding capacity. *pm: post mortem*.

to those found in the literature (Aslam et al., 1998; Fritts and Waldroup, 2003; Driver et al., 2006; Han et al., 2009). Similar results were reported by Han et al. (2009), who observed that the metabolite 1α(OH)D_3 decreased animal performance, although improvements in bone parameters were observed, indicating that the doses of vitamin D used in this study were inadequate for these bone parameters.

Among the metabolites tested in the present study, 25(OH)D_3 is reported in the literature to be more active than vitamin D_3 and to allow more efficient utilisation (Fritts and Waldroup, 2003). However, the different metabolites used in this study showed similar results for bone parameters. According to Aburto et al. (1998), the effects of D_3, 1,25$(OH)_2D_3$ and 25(OH)D_3 can vary depending on the biological response, with higher or lower efficiency depending on the characteristic assessed, such as greater bone strength or increased meat quality. These authors observed a greater reduction in the incidence of bone disorders with 1,25$(OH)_2D_3$ than with D_3 and 25(OH)D_3.

Moreover, all metabolites were provided in the feed so that equivalent amounts of vitamin D were offered, which may explain the lack of significant differences observed among the various metabolites because vitamin D acts to maintain homeostasis in the body.

Vitamin D_3 deficiency can impair bone formation, considering vitamin D, calcium and phosphorus are extremely important for bone mobilisation (Pereira, 2010). None of the bone parameters under assessment were affected, suggesting that the levels used in the present study were in accordance with the requirements for bone tissue maintenance.

Meat analyses

The variables WHC, SF, WLC and the pH of the meat did not differ ($p<0.05$, Table 4) among the different vitamin D_3 metabolite treatments. Differences among the treatments were expected for the studied characteristics because vitamin D is involved in calcium metabolism and the activation of calpains, calcium-dependent proteases that act in the process of meat tenderisation (Montgomery et al., 2000).

The lightness of breast meat 15 min *post mortem* was greater in animals fed 1,25$(OH)_2D_3$ than in those fed vitamin D_3 ($p<0.05$). Lightness is related to the denaturation of meat protein; therefore, the greater the denaturation, the greater the release of intracellular fluid and the less light transmitted through the fibres. These changes in biochemical reactions result in paler meat and higher values of L* (Duarte et al., 2007). However, the greater lightness in this case did not reach the values characteristic of Pale, Soft and Exudative (PSE) meat (L*<53.0), given that meat considered to be PSE is characterised not only by values of L* but also by a sharp decline in pH. A greater calpain activity and consequently higher values of L* are expected with the use of vitamin D.

The shrinkage of muscle fibres is caused by the decrease in pH, which increases the loss of liquid and the reflectance of the meat. However, despite the differences ($p<0.05$) in lightness (L*) observed among the treatments in this study, the water-holding capacity of breast meat was not affected (p>0.05). In general, higher values of lightness are positively correlated with lower water-holding capacity (Castellini et al., 2002). The latter is related to the formation of lactic acid and decrease in *post mortem* pH, which reduces the enzymatic degradation of the myofibrillar structure, thereby worsening the water-holding capacity (Roque-Specht et al., 2009).

The red/green intensity (a*) of the thigh meat 15 min *post mortem* was greater in birds fed 25(OH)D_3 than in those fed vitamin D_3 ($p<0.05$). The high intensity of a* is related to the formation of the carbon-myoglobin complex, attributed to the reduction of the iron ion present in myoglobin molecules combining with oxygen to form oxy-myoglobin, which confers a darker colour to the meat, influencing the type of muscle fibre.

Vitamin D acts by regulating the concentrations of calcium in animal blood and muscles. Therefore, after the animal is slaughtered, there is a decrease in pH that activates calcium-dependent proteases, with increased activity of calpains and other proteolytic enzymes, causing a consequent effect on colour, texture, tenderness and water-holding capacity. However, in this study, the pH of the meat was similar among animals fed different vitamin D metabolites, indicating that these act in a similar manner on calcium concentrations. Values of pH are negatively correlated with yellow/blue intensity (b*) and lightness (L*). Therefore, a decrease in pH reduces the myoglobin, resulting in meat that is less red and more yellow (Han et al., 2012).

However, at 24 h *post mortem*, the highest values of a* in thigh meat were observed in the treatment containing D_3, and the lowest values were noted for $1,25(OH)_2D_3$, with no significant difference in relation to the other treatments. The yellow/blue intensity (b*) in thigh meat, 24 h *post mortem*, was higher in animals fed vitamin D_3 and 25(OH)D_3 than in those fed 1α(OH)D_3 ($p<0.05$). The metabolite 1α(OH)D_3 is metabolised in the liver to form $1,25(OH)_2D_3$, which has a shorter half-life (6 to 8 h) than 25(OH)D_3 (2 to 3 wks). Therefore, despite having a higher rate of intestinal absorption, its use can be impaired by the lack of body reserves (Silva et al., 2008; Castro, 2011).

Although the different metabolites influence these parameters, the true colour and hue angle of the meat cuts were similar among the treatments because the colour of the meat is a result of the selective absorption of light and depends on numerous factors, such as the concentration of myoglobin, fibre composition, proteins and the presence of liquid in the meat (Gaya and Ferraz, 2006).

CONCLUSION

The results of this study suggest that all of the metabolites used in the diets, with the exception of 1α(OH)D_3, showed similar bone performance and quality. However, meat colour differed among animals fed different vitamin D_3 metabolites.

ACKNOWLEDGEMENTS

Project sponsored by CNPq and Capes, Brazil.

REFERENCES

Aburto, A., H. M. Edwards Jr. and W. M. Britton. 1998. The Influence of vitamin A on the utilization and amelioration of toxicity of cholecalciferol, 25 hydroxycholecalciferol, and 1,25-dihydroxycholecalciferol in young broilerchickens. Poult. Sci. 77:585-593.

Applegate, T. J., R. Angel and H. L. Classen. 2003. Effect of dietary calcium, 25-hydroxycholecalciferol, and bird strain on small intestinal phytase activity in broiler chickens. Poult. Sci. 82:1140-1148.

Applegate, T.J. and R. Angel. 2005. Los metabolitos de la vitamina D son prometedores para uso en dietas avícolas. Vademécum Avícola.

Aslam, S. M., J. D. Garlich and M. A. Qureshi. 1998. Vitamin D deficiency alters the immune responses of broiler chicks. Poult. Sci. 77:842-849.

Brito, J. A. G., A. C. Bertechini, E. J. Fassani, P. B. Rodrigues, E. M. C. Lima and C. Meneghetti. 2010. Efeito da vitamina D_3 e 25-hidroxi-colecalciferol sobre o desempenho, o rendimento de carcaça e a morfologia intestinal de frangos de corte. Rev. Bras. Zootec. 39:2656-2663.

Castellini, C., C. Mugnai and A. Dal Bosco. 2002. Effect of organic production system on broiler carcass and meat quality. Meat Sci. 60:219-225.

Castro, L.C. 2011. The vitamin D endocrine system. Arq. Bras. Endocrinol. Metabol. 55:566-575.

Driver, J. P., G. M. Pesti, R. I Bakalli and H. M. Edwards Jr. 2005. Calcium requirements of the modern broiler chicken as influenced by dietary protein and age. Poult. Sci. 84:1629-1639.

Driver, J. P., A. Atencio, G. M. Pesti, H. M. Edwards Jr and R. I. Bakalli. 2006. The effect of maternal dietary vitamin D_3 supplementation on performance and tibial dyschondroplasia of broiler chicks. Poult. Sci. 85:39-47.

Duarte, K. F., O. M. Junqueira, R. S. Filardi, A. C. Laurentiz, H. B. A. Souza and T. M. F. S. Oliveira. 2007. Efeito dos níveis de energia e programas de alimentação sobre a qualidade de carcaça e desempenho de frangos de corte abatidos tardiamente. Acta Sci. Anim. Sci. 29:39-47.

Enright, K., M. Ellis, F. Mckeith, L. Berger, D. Baker and B. Anderson. 1999. The influence of level of dietary vitamin D_3 supplementation and post-mortem aging time on pork quality. University of Illinois Swine Research Repots.

FAO (Food and Agriculture Organization). 2010. Agrobusiness handbook. Pout. Meat & Eggs.

Fritts, C. A. and P. W. Waldroup. 2003. Effect of source and level ofo vitamin D on live performance and bone development in growing broilers. J. Appl. Poult. Res. 12:45-52.

Gaya, L. G. and J. B. S. Ferraz. 2006. Aspectos genéticos-quantitativos da qualidade de carne em frangos. Ciência Rural, v.36, n.1.

Grüdtner V. S., P. Weingrill and A. L. Fernandes. 1997. Aspectos da absorção no metabolismo do cálcio e vitamina D. Rev. Bras. Reumatologia. 37:143-151.

Han, J. C., X. D. Yang, T. Zhang, H. Li, W. L. Li, Z. Y. Zhang and J. H. Yao. 2009. Effects of 1α-hydroxycholecalciferol on growth performance, parameters of tibia and plasma, meat quality, and type IIb sodium phosphate cotransporter gene expression of one-to-twenty-one-day-old broilers. Poult. Sci. 88:323-329.

Han, J. C., Y. L. Wang, H. X. Qu, F. Liang, J. L. Zhang, C. X. Shi, X. L. Zhang, L. Li and Q. Xie. 2012. One alpha-hydroxycholecalciferol improves growth performance, tibia quality, and meat color of broilers fed calcium- and phosphorus-deficient diets. Asian-Aust. J. Anim. Sci. 25:267-271.

Harder, M. N. C. 2005. Efeito do urucum (*Bixa orellana*) na alteração de características de ovos de galinhas poedeiras. Msc. Dissertação, Universidade de São Paulo, Piracicaba, São Paulo.

Honikel, K. O. 1998. Reference methods for the assessment of physical characteristics of meat. Meat Sci. 49:447-457.

Kasim S., B. L. Blake and X. Fan. 2006. The role of dopamine receptors in the neurobehavioral syndrome provoked by activation of L-type calcium channels in rodents. Dev. Neurosci. 28:505-517.

Korver, D. 2005. Research, analytical techniques and pratical experiences using HyD^{TM}. In: arkansas nutrition conference, Arkansas. p. 12.

Mccarthy, J. T, S. S. Barham and R. Kumar. 1984. 1,25dihydroxyvitamin D, rapidly alters the morphology of the duodenal mucosa of rachitic chicks: evidence for novel effects of 1,25-dihydroxyvitamin D. J. Steroid Eiorhen. 21:253-258.

Miller, E. H. 2006. Vitamin D insufficiency in male osteoporosis. Clin. Cornerstone 8:14-19.

Montgomery, J. L., F. C. Parrish Jr, D. C. Beitz, R. L. Horst, E. J. Huff-Lonergan and A. H. Trenkle. 2000. The use of vitamin D_3 to improve beef tenderness. J. Anim. Sci. 78:2615-2621.

Nakamura, M. and K. Katok. 1981. Influence of thawing methods on several properties of rabbit meat. Bulletin of Ishika Prefecture College of Agriculture, 11:45-49.

Norman A. W. 1985. The vitamin D endocrine system. Physiologist, 28:219-232.

Oliveira, A. F. G. 2006. Estudo do padrão de crescimento ósseo em frangos de corte de diferentes grupos genéticos criados em duas densidades populacionais. Msc. Thesis, Universidade Estadual de Maringá, Maringá, Paraná.

Pereira, R. 2010. Eficiência de uma fitase bacteriana na liberação de fósforo fítico em dietas de frangos de corte. Msc. Dissertação, Universidade de São Paulo, Piracicaba, São Paulo.

Reddy, G. S. and K. Y. Tserng. 1989. Calcitroic acid, end product of renal metabolism of 1,25-dihydroxyvitamin D3 through C-24 oxidation pathway. Biochemistry 28:1763-1769.

Rennie, J. S. and C. C. Whitehead. 1996. Effectiveness of dietary 25-and1-hydroxy colecalciferol in combating tibial dyschondroplasia in broiler chickens. Br. Poult. Sci. 37:413-421.

Rider Sell, N., W. B. Mikel, Y. L. Xiong and J. M. Behrends. 2004. Vitamin D_3 supplementation of cull cows: Effects on longissimus and semitendinosus muscle tenderness. J. Anim. Sci. 82:225-230.

Roque-Specht, V. F., V. Simoni, N. Parise and P. G. Cardoso. 2009. Avaliação da capacidade de retenção de água em peitos de Frango em função do ph final evaluation of water retention capacity in chicken breasts due to final pH. R. Bras. Agrociência, 15:77-81.

Rostagno, H. S., L. F. T. Albino, J. L. Donzele, P. C. Gomes, R. F. Oliveira, D. C. Opes, A. S. Ferreira, S. L. T. Barreto and R. F. Euclides. 2005. Tabelas brasileiras para aves e suínos. Composição de alimentos e exigências nutricionais. Viçosa: UFV, 2ed. 185p.

Santos, E. R. 2006. Caracterização do processo de *rigor mortis*, da maciez dos músculos *Gastrocnemius internus* e *Fibularis longus* e efeito da radiação gama na vida comercial da carne de avestruz (*Struthio camelus*). Ph.D. Thesis, Faculdade de veterinária, Niteroi, Rio de Janeiro.

Seedor, J. G., H. A. Quartuccio and D. D Thompson. 1991. The biophosphonate alendronate (MK - 217) inhibits bone loss due to ovariectomy in rats. J. Bone Miner. Res. 6:339-346.

Silva, B. C. C., B. M. Camargos and J. B. Fuji. 2008. Prevalência de deficiência e insuficiência de vitamina d e sua correlação com PTH, marcadores de remodelação óssea e densidade mineral óssea, em pacientes ambulatoriais. Arq. Bras. Endocrinol. Metab. 52:482-488.

Silva, D. J. and A. C. D. Queiróz. 2004. Análises de alimentos: métodos químicos e biológicos. Viçosa:UFV, p. 235.

Świątkiewicz S., J. Koreleski and J. Kopowski. 2006. Effect of phytase and 25-hydroxycholecalciferol on performance and bone quality in broiler chickens. Medycyna wet. 62:81-84.

SAEG - Sistema de Análises Estatísticas e Genéticas**.** 2005. Universidade Federal de Viçosa - UFV. Versão 5.0. Viçosa, Minas Gerais.

Wilborn B. S., C. R. Kerth, W. F. Owsley, W. R. Jones and L. T. Frobish. 2004. Improving pork quality by feeding supranutritional concentrations of vitamin D3. J. Anim. Sci. 82:218-224.

Yarger, J. G., C. L. Quarles, B. W. Holli and R. W. Gray. 1995. Safety of 25-hydroxycholecalciferol in poultry rations. Poult. Sci. 74:1437-1446.

Zanuzzi, C. N., F. Nishida and P. A. Portiansky. 2012. Effects of *Solanum glaucophyllum* toxicity on cell proliferation and apoptosis in the small and large intestine of rabbits. Res. Vet. Sci. 93:336-342.