REVISTA ENERGIA NA AGRICULTURA

DOI: http://dx.doi.org/10.17224/EnergAgric.2017v32n4p364-371

ISSN: 1808-8759 (cd-rom)
2359-6562 (on-line)

PRODUCTIVITY AND BONE QUALITY OF BROILERS SUPPLEMENTED WITH VITAMIN D (25-OHD$_3$)

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ABSTRACT: Problems with broilers bone quality are becoming more frequent and alternatives have been sought to reduce them without altering poultry performance. The experiment was conducted to assess the productivity and bone quality of broilers supplemented with 25-OHD$_3$. A total of 2400 broilers were distributed in randomized block design with 2x2x2 factorial arrangement (gender, Cobb and Ross strains, supplemented or not with 25-OHD$_3$), totaling eight treatments with six repetitions of 50 birds each. Birds' performance, gait score and bone quality (Seedor index, bone resistance, bone ash, minerals levels (Ca, P and Mg) and black bone syndrome) were evaluated. There was interaction between gender and strains for feed intake and weight gain; the feed conversion ratio was better for males; there was an interaction between strain and supplementation for viability. There was a difference in gait score for gender and strain. Males femur had a higher Seedor index, bone resistance and lower ashes level. Ross strain showed higher Seedor index. Regarding tibia ash levels in and femur Mg content, there was strain and supplementation interaction. Black bone syndrome was not influenced by the studied variables. Some of the assessed characteristics had positive responses and others characteristics discreetly responded to the treatments.

KEYWORDS: Animal performance, black bone syndrome, bone resistance, gait score, Seedor index.

PRODUTIVIDADE E QUALIDADE ÓSSEA DE FRANGOS DE CORTE SUPLEMENTADOS COM VITAMINA D (25-OHD$_3$)

RESUMO: Problemas com a qualidade do osso de frangos de corte estão cada vez mais frequentes e tem se buscado alternativas para redução dos mesmos sem alteração do desempenho das aves. O experimento foi conduzido com o objetivo de avaliar a produtividade e a qualidade óssea de frangos de corte suplementados com 25-OHD$_3$. Foram utilizados 2.400 frangos distribuídos em blocos casualizados com esquema factorial 2x2x2 (gêneros, linhagens Cobb e Ross, suplementadas ou não com 25-OHD$_3$), totalizando oito tratamentos com seis repetições de 50 aves cada. O desempenho, *gait score* e qualidade óssea (índice Seedor, resistência, cinzas, níveis de minerais (Ca, P e Mg) e síndrome do osso negro) foram avaliados. Houve interação sexo linhagem para o consumo de ração e o ganho de peso; a conversão alimentar foi melhor para os machos; houve interação linhagem suplementação para viabilidade. O *gait score* foi diferente entre sexos e linhagens. Para o fêmur os machos apresentaram maior índice Seedor, resistência óssea e cinzas. A linhagem Ross apresentou maior índice Seedor e houve interação linhagem suplementação para cinzas na tíbia, bem como para o teor de Mg no femur. A síndrome do osso negro não foi influenciada pelas variáveis estudadas. Conclui-se que algumas características avaliadas apresentaram respostas positivas e outras responderam discretamente aos tratamentos.

PALAVRAS-CHAVE: Desempenho animal, gait score, índice Seedor, resistência óssea, síndrome do osso negro.

1 INTRODUCTION

The animal welfare has become the key to build customer loyalty by food industry, making the use of methods that evaluate broilers welfare (Mendes et al., 2012). The poultry industry has used the gait score, which method consists in subjective evaluation of bird walking ability and estimates its welfare (KESTIN et al., 1992).

The lameness as well as bone disorders can be related to fast growing strains developmente, because these strains show higher final weight and lower slaughter age, what increase the locomotion problems incidence, increase bird mortality, carcass damnation in the slaughterhouse and meat quality. However, locomotion disorders are rarely observed in semi-intensive system (KESTIN et al., 1992).

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Among bone disorders that affect broilers of current strains is the black bone syndrome. This syndrome affects about 30% of bones (DUTCH STATE MINES, 2008) and is characterized by darkening the adjacent meat to the bone due to extravasation of blood from the bone marrow and prejudice the acceptance of consumers.

The occurrence of bone disorders can be related to the quality of the bone, which can be evaluate by characteristics like bone mineral composition, Seedor index, bone resistance and ashes levels. The characteristics of bone quality can be influenced by bird gender, strains, vitamin D supplementation and other nutrients supplementation.

Vitamin D supplementation has been widely researched in recent years due to its important role in bone mineralization (JONES; TWOMEY, 2008). The biologically assailable form of vitamin D_3 is 1,25-OHD_3. Some researchers suggest that vitamin D supplementation could improve the performance (FRITTS; WALDROUP, 2005), and other researchers indicate that this does not occur (CHOU et al., 2009). Vitamin D (25-OHD_3) supplementation can positively influence the bone quality (SAUNDERS-BLADES; KORVER, 2006; WHITEHEAD, 2010; ABBASI et al., 2017) and gait score (NÄÄS et al., 2012).

This experiment was conducted to assess bone quality and productivity of male and female broilers from two strains, supplemented or not with vitamin D (25-OHD_3).

2 MATERIAL AND METHODS

The adopted procedures were approved by the Ethics Commission on Animal Use of FMVZ/UNESP (096/2013). A total of 2400 broilers of two commercial strains with 1-day-old were distributed equally among the treatments. The experimental design was a randomized block with 2x2x2 factorial arrangement (gender, Cobb® 500 and Ross® 308 strains, supplemented or not with 25-OHD_3), totalizing eight treatments with six repetitions of 50 birds each. The treatments were: T1 - Cobb male not supplemented; T2 - Cobb female not supplemented; T3 - Cobb male supplemented with 25-OHD_3; T4 - Cobb female supplemented with 25-OHD_3; T5 – Ross male not supplemented; T6 – Ross female not supplemented; T7 – Ross male supplemented with 25-OHD_3; T8 – Ross female supplemented with 25-OHD_3. This design was chosen to demonstrate the influence of each factors involved in the evaluated characteristics, once gender, strain and vitamin D supplementation can affect the broilers performance and bone quality.

The feed base was corn and soybean meal, with nutritional requirements adapted from Rostagno et al. (2011). In addition to the recommended levels of vitamin D_3, were added 69 mg were added (equivalent to 2,760 IU / kg of diet) of 25-OHD_3 / t to the feed of supplemented treatments (T3, T4, T7 and T8), following the commercial recommendation. The feeding was divided into three phases (starter - 1 to 21 days, grower - 22 to 35 days, and finisher - 36 to 42 days) and the birds received 24L:0D from the first to third day-old, posteriorly 20L:4D.

Broilers' performance were evaluated by feed intake (g $birds^{-1}$ $period^{-1}$), weight gain (g $birds^{-1}$ $period^{-1}$), feed conversion ratio and viability. The gait score assessment was carried out on 100% of the broilers at 41^{st} day-old and was based on subjective observations of how the birds walked for a distance of 1 linear meter, delimited within the pens. The scores ranged from 0 to 2 (WEBSTER et al., 2008).

Afetr 43 days, 182 broilers were taken to the experimental slaughter, stunned by electro narcosis and then slaughtered by cutting the jugular vein and carotid arteries as commercial form. These birds tibias and femurs of these birds were removed, identified and stored at a temperature of -10°C for 48 hours. The right legs were boneless and the bones were weighed and dried at 105°C for 24 h, then weighed again. The bones were weighed and the length measured with a digital pachymeter. Seedor index was calculated from bone weight and length as [Seedor Index = bone weight (mg) / bone length (mm)] (SEEDOR, 1995). These same bones were used to determine bone breaking force in bending test with a texture analyzer (TA.XTPlus, Texture Technologies, Hamilton, MA, United States) and blade set probe (HDP/BS Blade Set, Stable Micro Systems, UK) at 10 mm down and 4 $mm/minute^{-1}$. This device was adapted to allow a clearance free of the bone diaphysis in 6.0 cm for the tibia and 4.0 cm for the femur. The bone ash was (CHOWDHURY et al., 2009), percentages of calcium (Ca), phosphorus (P) And magnesium (Mg) (SUÁREZ et al., 2015) were calculated.

All left tibias and femurs were baked in a kiln for firing until they reach internal temperature of 95°C. Then, the samples were submitted to macroscopic evaluation of black bone syndrome, assigning scores related to the appearance, classifying them in acceptable (region close to the bone without browning), intermediate (region near the bone slightly darkened) or unacceptable (region near the bone with severe darkening) [Figures 1A, B and C].

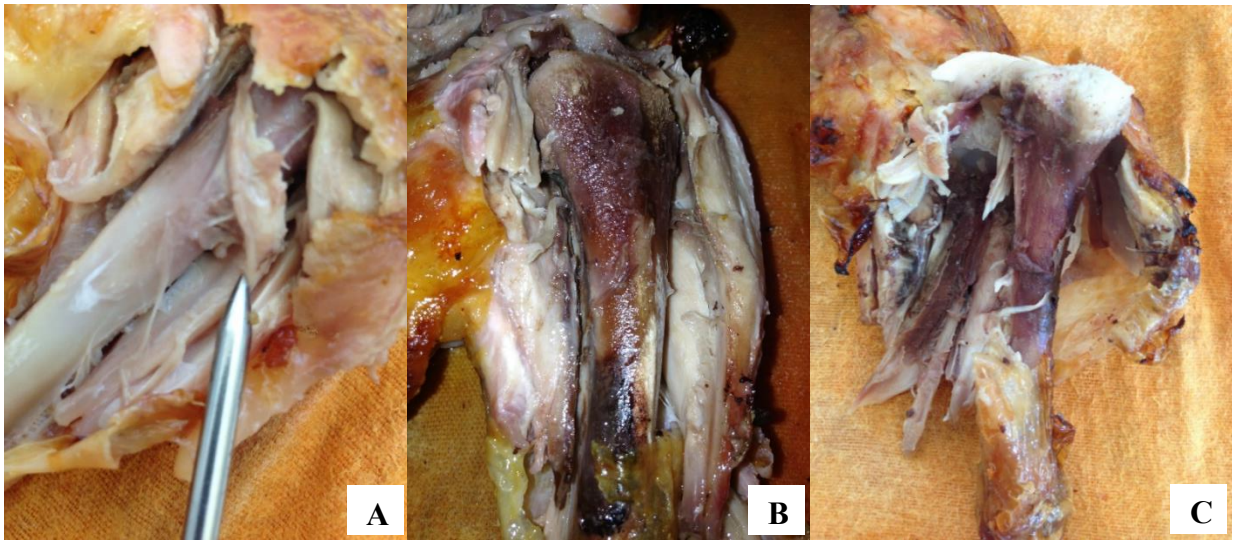

Figure 1 - *Macroscopic evaluation of the darkening of the flesh (black bone syndrome). In A: acceptable (region near the bone without darkening). In B: intermediate (region near the bone slightly darkened). In C: unacceptable (region near the bone with severe darkening).*

The data were analyzed using MIXED procedure on SAS (Statistical Analysis System, version 9.2.). The performance, carcass and cuts yield, bone strength, ashes and minerals levels were submitted to variance analysis (ANOVA) and the averages compared by Tukey test ($p < 0.05$). The nonparametric data (gait score and black bone syndrome), did not met the presuppositions of the statistical model (normality and homogeneity). Therefore, due to frequency, the Chi-square test was applied ($p < 0.05$) to black bone syndrome in drumsticks. The Fisher exact test was applied ($p < 0.05$) to the gait score and black bone syndrome data in thigs.

3 RESULTS AND DISCUSSION

There was a statistically significant interaction between gender and strain interaction ($p = 0.0120$). Males from the Ross strain showed a higher feed intake and thus were heavier (Table 1). Feed conversion ratio was better only for males ($p = 0.0036$). Broilers' viability was influenced by strain and supplementation interaction ($p = 0.0242$), with better results for birds from Ross strain supplemented with 25-OHD_3 and from Cobb strain not supplemented. Then, the strains may respond differently to supplementation with this vitamin D metabolite.

Table 1 *- Performance of broilers from the Cobb® 500 and Ross® 308 strains, supplemented with vitamin D (25-OHD_3) in the period from 1st to 42nd days old.*

Strain	Sex		Average	Supplementation		Average
	Male	Female		0 mg^{-1} t	69 mg^{-1} t	
	Feed Intake			Feed Intake		
Cobb® 500	4.940 Ba	4.201 Bb	4.570	4.600	4.542	4.571
Ross® 308	5.081 Aa	4.663 Ab	4.872	4.822	4.908	4.865
Average	5.010	4.432		4.711	4.725	
	Weight Gain			Weight Gain		
Cobb® 500	2.877 Ba	2.385 Bb	2.631	2.655	2.607	2.631
Ross® 308	2.903 Aa	2.579 Ab	2.741	2.706	2.765	2.735
Average	2.890	2.482		2.680	2.686	
	Feed Conversion Ratio			Feed Conversion Ratio		
Cobb® 500	1.73	1.76	1.74	1.74	1.75	1.74
Ross® 308	1.73	1.80	1.76	1.77	1.78	1.77
Average	1.73 b	1.78 a		1.75	1.76	
	Viability			Viability		
Cobb® 500	98.15	98.67	98.41	99.00 Aa	97.82 Ba	98.41
Ross® 308	98.17	99.17	98.67	97.82 Ab	99.64 Aa	98.73
Average	98.16	98.92		98.41	98.73	

	Probability							
	Sex	Strain	Sup	Sex*Strain	Sex*Sup	Strain*Sup	Sex*Strain*Sup	VC (%)
FI	< 0.0001	< 0.0001	0.5920	0.0012	0.6908	0.0749	0.7633	7.85
WG	< 0.0001	0.0006	0.6265	0.0120	0.0790	0.4557	0.2419	9.03
FC	0.0036	0.1347	0.9237	0.7014	0.0883	0.6184	0.3036	3.66
VB	0.2653	0.6335	0.6411	0.7572	0.7490	0.0242	0.6222	2.21

Sup = suplementation; FI = feed intake (g / poultry / period); WG = weight gain (g / poultry / period); FC = feed conversion ratio; VB = viability (%); VC = variation coefficient (%). For each source of variation, the measured values followed by uppercase letters in the columns and lowercase letters in the rows differ in Tukey test ($p < 0.05$).

The males performance superiority to females is known (DELEZIE et al., 2010). The females present lower feed intake and weight gain, tending to show worse feed conversion, a fact that is related to higher fat deposition (DELEZIE et al., 2010). Regarding bird viability, the supplementation with vitamin D could be an alternative to reduce mortality due to the potential role in the immune system (KAMEN; TANGPRICHA, 2010), as was found for Ross strain in this study. The commercial broiler strains are constantly changing; in a study conducted 16 years ago (FLEMMING et al., 1999) Ross strain was top in feed intake and weight gain. In contrast to these results, other author (DELEZIE et al., 2010) found that these performance characteristics were higher to Cobb strain. Thus, the genetic potential of the strains can be affect by nutrition and environmental conditions.

Regardless of supplementation or not with vitamin D (25-OHD_3), the strain ($p = 0.0329$) and the gender ($p < 0.0001$) affected the gait score frequency, and this characteristic was fairly affected by the gender (Table 2). Females showed higher frequency of gait score 0, which is attributed to birds that walk normally and males showed higher frequency of gait score 1, attributed to birds that walked with some difficulty. Birds of Cobb strain showed higher incidence of gait score 0 when compared to the Ross strain.

The great development of male broilers results in walking ability impairment (BRICKETT et al., 2007), and worse gait score. In addition, commercial strains frequently shows susceptibility of lameness (TALATY et al., 2010), probably due to the difference in weight gain. The genetic improvement for weight gain imposes pressure on the skeletal system, resulting in less walking ability and a higher incidence of lameness (VENÄLÄINEN et al., 2006). So, there is an interest for improvements in bone structure with the use of nutrients such as vitamin D in the feed, which is important in the metabolization of calcium and phosphorus. Thus, it better gait score was expected to broilers supplemented with 25-OHD_3, as observed in a previous study (NÄÄS et al., 2012). In the present study, gait score was not influenced by vitamin D supplementation probably due to bone damage that may occurred during hatching (HULET, 2007). However, is worth mentioning that the purpose of the gait score is to estimate the welfare (KESTIN et al., 1992) and not to assess bone quality.

Among tibia bone quality evaluations, Seedor index for the tibias was affected by gender, strain and supplementation with vitamin D (25-OHD_3), but there was no interaction between them. The best results were found for males ($p > 0.0001$), Cobb strain ($p = 0.0011$) and without supplementation ($p = 0.0241$). However, the bone resistance was affected only by the gender ($p < 0.0001$). Males presented superior values (Table 3). Tibia ash levels analyses resulted on strain and vitamin D (25-OHD_3) supplementation interaction ($p = 0.0173$, $p = 0.0232$, respectively), with lower ash levels from Cobb strain when not supplemented (Table 3).

Table 2 *- Gait score frequency in broilers from Cobb® 500 and Ross® 308 strains, supplemented with vitamin D (25-OHD_3).*

	Sex		Strain		Supplementation	
GS	Male	Female	Cobb® 500	Ross® 308	0 mg^{-1} t	69 mg^{-1} t
0	81.12 b	88.41 a	87.79 a	83.10 b	85.91	84.96
1	16.26 a	10.85 b	11.19	14.91	12.76	13.37
2	2.62	0.73	1.02	1.99	1.34	1.67

GS = gait score; 0 - Bird walked normally and made at least 10 uninterrupted steps, 1 - Bird presented difficulty to walk and made between six and ten uninterrupted steps and 2 - Bird walked with great difficulty and made less than six uninterrupted steps or did not walk. Values followed by lowercase letters in the rows differ in Fisher exact test ($p < 0.05$).

Males showed denser bones (higher Seedor index), more resistant to breakage than females. Considering that the Seedor index and bone resistance are closely related (ALMEIDA PAZ et al., 2009), males showed better bone quality. Greater bone strength in males was also previously reported (GOMES et al., 2004). Higher Seedor index and greater bone strength did not result in higher ashes percentage in males when compared to females have already been reported by other authors (BRICKETT et al., 2007).

Table 3 *- Cobb® 500 and Ross® 308 strains, supplemented with vitamin D (25-OHD$_3$), Seedor index, bone strength, percentage of ash and tibia minerals.*

	Sex		Strain		Supplementation	
	Male	Female	Cobb® 500	Ross® 308	0 mg^{-1} t	69 mg^{-1} t
SI	0.22 a	0.18 b	0.19 b	0.21 a	0.20 a	0.19 b
BS	32.51 a	27.50 b	28.99	31.02	29.42	30.59
%A	23.48	22.73	23.23	24.23	22.63	24.83
%Ca	36.41	36.18	36.35	36.25	36.60	36.00
%P	15.87	15.44	15.35	15.97	16.01	15.31
%Mg	0.72	0.70	0.71	0.70	0.71	0.70

	Probability							
	Sex	Strain	Sup	Sex*Strain	Sex*Sup	Strain*Sup	Sex*Strain*Sup	CV (%)
SI	< 0.0001	0.0011	0.0241	0.2277	0.1914	0.2243	0.8748	11.51
BS	< 0.0001	0.0651	0.2876	0.5630	0.9917	0.8576	0.0640	11.04
%A	0.5778	0.2589	0.0171	0.40191	0.8117	0.0173	0.2634	7.94
%Ca	0.8060	0.9164	0.5736	0.4843	0.8566	0.5154	0.1746	2.66
%P	0.9829	0.3404	0.2841	0.5029	0.5699	0.3560	0.4688	4.53
%Mg	0.1183	0.5860	0.6082	0.6462	0.3560	0.8236	0.9033	2.39

Sup = supplementation; SI = Seedor Index; BS = bone strength (kgf); %A = ash percentage; %Ca = calcium percentage; %P = phosphorus percentage; %Mg = magnesium percentage; VC = variation coefficient (%). The measured values followed by different letters in the rows differ in Tukey test ($p< 0.05$).

When analyzing the femurs, Seedor index was affected by gender ($p < 0.0001$) and strain ($p = 0.0337$), with better results for males and Ross strain (Table 4). There was difference in bone resistance ($p < 0.0001$) and ashes levels ($p = 0.0532$) only when assessing the bird gender of the bird, the males showed greater bone resistance and the females larger ashes levels.

Ashes levels is a point that indicates bone mineralization because it is composed by mineral matter. The higher ashes amount, the most minerals are present in the bone. The ashes levels shows little variation between genders and strains (GOUS et al., 1999), however in this study, this femur characteristic was higher in females, corroborating with the findings from BRICKETT et al. (2007). Moreover, tibias ashes levels of not supplemented broilers from the Cobb strain waere lower than in others, making 25-OHD$_3$ supplementation for this strain birds advantageous.

Ross strain presented denser bones (greater Seedor index) than Cobb strain. This difference between strains may be related to the genetic bird potential, what influences the growth of long bones such as the tibia and the femur. Birds supplemented with vitamin D (25-OHD$_3$) tibias presented worse Seedor index. Other study conducted to evaluate bone quality parameters of Cobb strain supplemented with vitamin D sources (D$_3$, 25-OHD$_3$, 1.25-(OH$_2$) D$_3$ and 1α (OH)D$_3$) male broilers showed that these vitamin sources were not a determining factor for in Seedor index changes (GARCIA et al., 2013).

Table 4 *- Cobb® 500 and Ross® 308 strains supplemented with vitamin D (25-OHD$_3$) Seedor index, bone strength, percentage of ash and femur minerals.*

	Sex		Strain		Supplementation	
	Male	Female	Cobb® 500	Ross® 308	0 mg^{-1} t	69 mg^{-1} t
SI	0.28 a	0.23 b	0.25 b	0.26 a	0.25	0.25
BS	39.24 a	31.92 b	35.23	35.93	35.78	35.38
%C	22.58 b	24.25 a	23.63	23.19	23.81	23.01
%Ca	36.92	36.32	35.89	36.85	36.88	35.86
%P	15.77	15.86	15.62	16.00	16.27	15.35
%Mg	0.73	0.73	0.73	0.74	0.73	0.73

	Probability							
	Sex	Strain	Sup	Sex*Strain	Sex*Sup	Strain*Sup	Sex*Strain*Sup	VC (%)
SI	< 0.0001	0.0337	0.5761	0.2122	0.3390	0.7976	0.0690	11.27
BS	< 0.0001	0.5117	0.7034	0.5202	0.1538	0.1967	0.4137	11.04
%C	0.0532	0.6005	0.3469	0.5520	0.2783	0.7123	0.7313	5.08
%Ca	0.4756	0.5821	0.5332	0.0728	0.1000	0.0660	0.3919	4.18
%P	0.8902	0.5500	0.1511	0.4823	0.3918	0.4372	0.5013	4.67
%Mg	0.6700	0.4872	0.7159	0.7629	0.2208	0.0232	0.4618	3.40

Sup = supplementation; SI = Seedor Index; BS = bone strength (kgf); %A = ash percentage; %Ca = calcium percentage; %P = phosphorus percentage; %Mg = magnesium percentage; VC = variation coefficient (%). The measured values followed by different letters in the rows differ in the Tukey test ($p< 0.05$).

In this study, strains and supplementation with 25-OHD_3 did not affected tibia and femur bone strength. In previous studies, some authors reported the same results from three strains (Hybro® PG, Ross® 308 and Isa Label JA® 57) of male broilers (OLIVEIRA et al., 2014). The supplementation with 25-OHD_3 seems to have a limit to improve bone quality, since when calcium levels in feed is adequate, the 25-OHD_3 is not effective (ROBERSON et al., 2005).

The Cobb strain femur magnesium content improved with vitamin supplementation. Calcium and Phosphorus levels almost did not change in all treatments, perhaps due to the large percentage of these minerals in the bone content what guarantees levels relatively constant.

The black bone syndrome frequency was not affect by gender, strains and vitamin D (25-OHD_3) supplementation (Table 5, $p > 0.05$).

Table 5 *- Frequency of the black bone syndrome (macroscopic evaluation) in thigs and drumsticks of broilers from Cobb® 500 and Ross® 308 strains, supplemented with vitamin D (25-OHD_3).*

	Sex		Strain		Supplementation	
SON	Male	Female	Cobb® 500	Ross® 308	0 mg^{-1} t	69 mg^{-1} t
			Thigh Meat			
ACE	44.33	42.35	50.56	36.56	39.77	46.81
INT	51.55	51.76	47.19	55.91	54.55	48.94
UNA	4.12	5.88	2.25	7.53	5.68	4.26
			Drumstick Meat			
ACE	23.96	16.67	17.24	23.66	19.77	21.28
INT	59.38	69.05	67.82	60.22	62.79	64.89
UNA	16.67	14.29	14.94	16.13	17.44	13.83

ACE = acceptable; INT = intermediate; UNA = unacceptable. Thigh Meat: $p > 0.05$ in the Fisher exact test. Drumstick Meat: $p > 0.05$ in the Chi-square test.

The incidence of black bone syndrome is related to birds' gender and strain (KORVER, 2010). However, the results obtained in this study did not confirm this. Another factor that could be related to the incidence of the black bone syndrome is nutrition, specifically vitamin D due to its role in bone formation. Even so, vitamin D (25-OHD_3) supplementation was not efficient to decrease the bone porosity and to improve the bone resistance. Other authors reported a reduction in blood diffusion and consequently better aspect of meat with the addition of vitamin D_3 (SAUNDERS-BLADES; KORVER, 2006). Whitehead (2010) found similar results with reduced incidence of black bone syndrome in tibias after supplementing with 69 mg 25-OHD_3/t. Is important to consider that the black bone syndrome assessment is subjective and thus may differ between researches.

The results regarding productivity, bone quality and the use of vitamin D in addition to the basal levels are still contradictory, probably because the metabolism of minerals and vitamins is influenced by many factors that are not always consider in studies.

4 CONCLUSIONS

The use of 25-OHD_3 in broiler feed must be analyzed in function of strain, gender and each attribute object to improvement, because not all characteristics evaluated in this study had positive responses and some of them responded subtly to the addition of this metabolite.

5 ACKNOWLEDGMENTS

To Sao Paulo research fundation – FAPESP for financial support research (process 2013/16554-5) and to Coordination for the Improvement of Higher Level – CAPES for the scholarship.

6 REFERENCES

ABBASI, T.; SHAKERI, M;. ZAGHARI, M.; KOHRAM, H. Growth performance parameters, bone calcification and immune response of in ovo injection of 25-hydroxycholecalciferol and vitamin K3 in male ross 308 broilers. **Theriogenology**, Philadelphia, v. 90, p. 260-265, 2017.

ALMEIDA PAZ, I. C. L.; MENDES, A. A.; BALOG, A.; KOMIYAMA, C. M.; TAKAHASHI, S. E.; ALMEIDA, I. C. L.; GARCIA, E. A.; VULCANO, L. C.; BALLARIN, A. W.; SILVA, M. C.; CARDOSO, K. F. G. Efeito do cálcio na qualidade óssea e de ovos de poedeiras. **Archivos de Zootecnia**, Cordoba, v. 58, n. 222, p. 173-83, 2009.

BRICKETT, K. E.; DAHIYA, J. P.; CLASSEN, H. L.; ANNETT, C. B.; GOMIS, S. The impact of nutrient density, feed form, and photoperiod on the walking ability and skeletal quality of broiler chickens. **Poultry Science**, Champaign, v. 86, p. 2117-2125, 2007.

CHOU, S. H.; CHUNG, T. K.; YU, B. Effects of supplemental 25-hydroxycholecalciferol on growth performance, small intestinal morphology, and imune response of broiler chickens. **Poultry Science**, Champaign, v. 88, p. 2333-2341, 2009.

CHOWDHURY, R. K.; ISLAM, M. S.; KHAN, M. J.; KARIM, M. R.; HAQUE, M. N.; KHATUN, M. AND PESTI, G. M. Effect of citric acid, avilamycin, and their combination on the performance, tibia ash, and immune status of broilers. **Poultry Science**, Champaign, v. 88, p. 1616–1622, 2009.

DUTCH STATE MINES. **Nutrictional products**. 2008. Available in:

<http://www.dsm.com/en_US/downloads/dnp/HyDFolder.pdf+academic+black+bone+syndrome+in+chicken>. Accessed: August 28, 2011.

FLEMMING, J. S.; JANZEN, S. A.; ENDO, M. A. Teste com linhagens comerciais de frango de corte - avaliação dos parâmetros zootécnicos. **Archives of Veterinary Science**, Curitiba, v. 4, n. 1, p. 57-59, 1999.

FRITTS, C. A.; WALDROUP, P. W. Comparison of cholecalciferol and 25-hydroxychloecalciferol in broilers diets designed to minimize phosphorus excretion. **Journal Applied Poultry Research**, Cary, v. 14, n. 1, p. 156-166, 2005.

GARCIA, A. F. Q. M.; MURAKAMI, A. E.; DUARTE, C. R. A.; ROJAS, I. C. O.; PICOLI, K. P.; PUZOTTI, M. M. Use of vitamin D_3 and its metabolites in broiler chicken feed on performance, bone parameters and meat quality. **Asian-Australasian Journal of Animal Science**, Gawanak-gu, v. 26, p. 408-415, 2013.

GOMES, P. C.; RUNHO, R. C.; D'AGOSTINI, P.; ROSTAGNO, H. S.; ALBINO, L. F. T. LOPES, P. S. Exigência de fósforo disponível para frangos de corte machos e fêmeas de 22 a 42 e de 43 a 53 dias de idade. **Revista Brasileira de Zootecnia**, Viçosa, v. 33, p. 1734-1746, 2004.

GOUS, R. M.; MORAN JR., E. T.; STILBORN, H. R.; BRADFORD, G. D.; EMMANS, G. C. Evaluation of the parameters needed to describe the overall growth, the chemical growth, and the growth of feathers and breast muscles of broilers. **Poultry Science**, Champaign, v. 78, p. 812-821, 1999.

HULET, R. M. Managing incubation: where are we and why? **Poultry Science**, Champaign, v. 86, p. 1017–1019, 2007.

JONES, B. J.; TWOMEY, P. J. Issues with vitamin D in routine clinical practice. **Rheumatology**, Basel, v. 47, p. 1267-1268, 2008.

KAMEN, D. L.; TANGPRICHA, V. Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. **Journal of Molecular Medicine**, Heidelberg, v. 88, p. 441–450. 2010.

KESTIN, S. C.; KNOWLES, T. G.; TINCH, A. E.; GREGORY, N. G. Prevalence of leg weakness in broiler chickens and its relationship with genotype. **Veterinary Record**, London, v. 131, p. 190-194, 1992.

KORVER, D. Reducing the incidence of black bone. **World Poultry**, v. 26, p. 36-38, 623 2010.

MENDES, A. S.; PAIXÃO, S. J.; MAROSTEGA, J.; RESTELATTO, R.; OLIVEIRA, P. A. V.; POSSENTI, J. C. Mensuração de problemas locomotores e de lesões no coxim plantar em frangos de corte. **Archivos de Zootecnia**, Cordoba, v. 61, n. 234, p. 217-228, 2012.

NÄÄS, I. A.; BARACHO, M. S.; BUENO, L. G. F.; MOURA, D. J.; VERCELINO, R. A.; SALGADO, D. D. Use of Vitamin D to Reduce Lameness in Broilers Reared in Harsh Environments. **Brazilian Journal of Poultry Science**, Campinas, v. 14, n. 3, p. 159-232, 2012.

OLIVEIRA, A. F. G.; BRUNO, L. D. G.; MARTINS, E. N.; GARCIA, E. R. M.; MONTEIRO, A. C.; LEITE, M. C. P.; POZZA, P. C.; SANGALI, C. P. Efeito da densidade de criação e do grupo genético sobre a composição mineral e desenvolvimento de ossos longos de frangos de corte. **Ciências Agrárias**, Teresina, v. 35, n. 2, p. 1023-1034, 2014.

ROBERSON, K. D.; LEDWABA, M. F.; CHARBENEAU, R. A. Studies on the efficacy of twenty-five hydroxycholecalciferol to prevent tibial dysnchondroplasia in ross broilers fed marginal calcium to Market age. **International Journal of Poultry Science**, Faisalabad, v. 4, n. 2, p. 85-90, 2005.

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

SAUNDERS-BLADES, J.; KORVER, D. Hyd and poultry: bones and beyond. In: EUROPEAN POULTRY CONFERENCE, 2006, Verona. **Proceedings…** Verona, 2006.

SEEDOR, J. G. The biophosphanate alen-dronate (MK-217) inhibit bone loss due to ovariectomy in rats. **Journal of Bone and Mineral Research**, Hoboken, v. 4, p. 265-270, 1995.

SUÁREZ, M. H.; DOPAZO, G. A.; LÓPEZ, D. L.; ESPINOSA, F. Identification of Relevant Phytochemical Constituents for Characterization and Authentication of Tomatoes by General Linear Model Linked to Automatic Interaction Detection (GLM-AID) and Artificial Neural Network Models (ANNs). **PLoS ONE**, San Francisco, v. 10, n. 6, p. e0128566, 2015.

TALATY, P. N.; KATANBAF, M. N.; HESTER, P. Y. Bone mineralization in male commercial broilers and its relationship to gait score. **Poultry Science**, Champaign, v. 89, p. 342–348, 2010.

VENÄLÄINEN, E.; VALAJA, J.; JALAVA, T. Effects of dietary metabolisable energy, calcium and phosphorus on bone mineralization, leg weakness and performance of broiler chickens. **British Poultry Science**, Oxfordshire, v. 47, p. 301-310, 2006.

WEBSTER, A. B.; FAIRCHILD, B. D.; CUMMINGS, T. S.; STAYER, P. A. Validation or a three. Point gait scoring system for field assessment de walking ability of commercial broilers. **Journal of Applied Poultry Research**, Cary, v. 17, p. 529-539, 2008.

WHITEHEAD, C. C. Update on current european broiler bone problems. **Australian Poultry Science Symposium**, Camden, v. 21, p. 22-25, 2010.