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Supplementation of minerals and vitamins in broiler diets: effect on performance and bone quality

Suplementação de minerais e vitaminas em dietas de frangos de corte: efeito sobre desempenho e qualidade óssea

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Abstract
Differences in growth rate and carcass composition of broilers may require higher levels of vitamins and minerals with higher bioavailability. The aim of this trial was to supplement commercial diets for broilers with optimized levels of vitamins and with different mineral sources to assess the effect on performance, carcass yield and bone quality. A total of 1,800 Cobb Slow male broiler chicks were distributed into a 2 x 2 factorial completely randomized design (vitamin programs - optimized and commercial versus mineral sources - inorganic [sulfates] and carbo-amino-phospho-chelate, CAPC). The vitamin D_3 metabolite, 25 (OH) D_3, was included in the optimized vitamin premix. Birds and feed leftovers were weekly weighed. Data was subjected to statistical analysis using the GLM procedure of SAS software. Supplementing diets with a combination of optimized vitamin programs and CAPC as mineral source resulted in better feed conversion at 42 days of age (P<0.05). Supplementation with CAPC minerals resulted in a lower red index (*a) (P<0.05) in the adjacent thigh muscles and drumstick bones of broilers when thawed and roasted, indicating less bone porosity. The inclusion rates of mineral sources with higher bioavailability can be reduced, decreasing the negative impact of mineral excretion and the polluting effect on the environment.
Keywords: 25(OH)D_3, black bone, organic minerals, feed conversion.

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Resumo
As diferenças na velocidade de crescimento e na composição de carcaça dos frangos podem exigir níveis mais altos de vitaminas e de minerais com maior biodisponibilidade. O objetivo deste trabalho foi suplementar dietas comerciais para frangos de corte com níveis otimizados de vitaminas e com diferentes fontes minerais para avaliar o efeito sobre o

desempenho produtivo, rendimento de carcaça e qualidade óssea. Foram utilizados 1800 pintos de corte machos da linhagem Cobb Slow, divididos em um delineamento inteiramente casualizado em esquema fatorial 2 x 2 (programas vitamínicos – otimizado e comercial Vs fontes de minerais – inorgânicos (sulfatos) e carbo-amino-phospo-chelate, CAPC). No premix vitamínico otimizado foi incluído o metabólito da vitamina D_3, 25(OH)D_3. As aves e as sobras de ração foram pesadas semanalmente. A análise estatística dos dados foi realizada pelo procedimento GLM do software SAS. A suplementação de dietas com associação de programas vitamínicos otimizados e fonte mineral CAPC resultou em melhor conversão alimentar aos 42 dias de idade (P<0,05). A suplementação com minerais CAPC resultou em menor índice de vermelho (*a) (P<0,05) na musculatura adjacente aos ossos das coxas e sobrecoxas dos frangos de corte quando descongeladas e assadas, indicando menor porosidade óssea. Fontes mais biodisponíveis de minerais permitem reduzir a sua inclusão nas dietas e diminuem o impacto negativo da excreção de minerais e o efeito poluidor sobre o meio ambiente.
Palavras-chave: 25(OH)D_3; *black bone*; conversão alimentar; minerais orgânicos.

Introduction

Poultry industry is under a constant challenge to achieve a more efficient production in order to reduce costs. In addition to this, constant changes in consumption habits and the growing search for food safety, has forced the industry to introduce continuous changes in order to meet the demand and sensitivity of these consumers who are searching for products with added high quality and nutritional value products[(1)].

In addition, poultry production has undergone changes during the last few years. Genotypes have improved in weight gain and feed conversion, carcass composition has evolved towards leaner birds, and especially selected lines have been developed for breast yield. In this context, new studies on the nutritional requirements of vitamins and minerals have aroused particular interest of researchers because the knowledge of the requirements of these nutrients is based on information obtained in research conducted in the 1950s and 1960s[(2)].

These differences in growth rate and carcass composition of broilers may require more elevated levels of vitamins and minerals with higher bioavailability. Higher levels of vitamins in broiler diets can compensate for changes in intake due to health or environmental problems, bioavailability of the sources used, and problems that compromise the quality of the diets processing [(3)].

Although representing a small portion in the formulation of a diet, vitamins are essential nutrients that act in more than 30 cellular metabolic reactions and are associated with higher efficiency of synthesis systems in the animal organism [4,5].

Besides the functions of vitamin D, linked to skeletal growth, mineralization and bone tissue maintenance [6,7], Hutton et al.[8] showed that 25(OH)D_3 supplementation stimulates satellite cell activity in the *pectoralis major* muscle of the bird, thus 25(OH)D_3 increases muscle yield through muscle fiber hyperplasia.

Vignale et al [9] pointed out another way in which the vitamin acts on muscle growth. 25(OH)D_3 stimulates protein synthesis through an increase in the rates of translation initiation, a process that involves several initiation factors, kinases and phosphatases, whose activities are regulated by phosphorylation. This activation occurs through signaling pathways dependent on the mTOR protein kinase (sensitive to rapamycin).

Supplementation with 25(OH)D_3 provides more active vitamin D, so that there is a decrease in energy expenditure for its metabolization and an increase in its efficiency in the body[10], in addition to the absorption rate, which is approximately 20% higher than that of D_3[11].

Although vitamin supplementation in diets is indispensable, not all experiments demonstrate the need for each one of them. Most nutritionists add this element as a precaution, aiming to reduce stressors and the risk of subclinical diseases[12].

The presence of minerals in the diet, such as Cu, Fe, Mn, Zn and Se, are essential for the growth of broilers due to their involvement in many physiological and biosynthesis processes, functioning mainly as intracellular enzyme catalysts or as parts of enzymes. These microelements are part of various proteins involved in intermediate metabolism, in hormone secretion pathways, and in the immune system[13].

Traditionally, these microminerals are added to diets as inorganic salts, such as sulfates, oxides, and carbonates, in order to address clinical deficiencies that prevent the bird from reaching its genetic growth potential[14].

Due to the low bioavailability and even the lack of knowledge related to the absorption and utilization of these minerals, the levels supplied in the diets are often higher than required by the bird, resulting in oversupply and, consequently, higher excretion into the environment[15]. Another form of mineral supplementation is through the supply of inorganic minerals bound to a molecule, the ligand. The binding can be of a mineral with an amino acid, forming the metal-amino acid complex, bound to two or three amino acid molecules, forming the chelate, or to polysaccharides.

Favorable results of including organic minerals in the diet as a replacement for inorganic sources have already been demonstrated, such as improved performance, bone ash deposition[14], serum antioxidant status[15], as well as reduction of minerals excreted in the litter[13, 14].

The more bioavailable minerals offer greater stability and benefit from biochemical protection against the different chemical reactions that can occur when added to the diet itself, expecting a better bird performance and higher absorption and utilization of these minerals when supplemented[16], as well as less environmental impact[13, 17]. However, regardless of the form in which the micromineral element is presented, its functions are maintained, so the form of presentation interferes only with bioavailability.

The objective of the present study was to evaluate the supplementation of commercial broiler diets with optimized levels of vitamins and with different mineral sources on productive performance and bone quality.

Material and methods

The study was performed in the experimental poultry house of the Federal University of Paraná - Setor Palotina. All procedures using animals in this study were submitted for evaluation and approved by the Ethics Committee on Animal Experimentation of the Setor Palotina of UFPR, under protocol number 51/2014.

A total of 1,800 male Cobb Slow broiler chicks were assigned to a 2 × 2 factorial completely randomized design (vitamin programs - optimized and commercial versus mineral sources - inorganic and carbo-amino-phospho-chelate, CAPC) totaling four treatments, nine replicates, and 36 experimental units with 50 birds each.

The vitamin programs used were: Commercial Vitamin Program and Optimized Vitamin Program, following the recommendations of the Optimum Vitamin Nutrition program (OVN™, DSM). As for mineral sources, the following were used: inorganic mineral source, minerals in the form of sulfate and Carbo-Amino-Phospho-Chelate (CAPC) source (Minerais Tortuga). The treatments were:

1 - Commercial Vitamin Program + inorganic mineral source

2 - Optimized Vitamin Program + inorganic mineral source

3 - Commercial Vitamin Program + CAPC mineral source

4 - Optimized Vitamin Program + CAPC mineral source

Corn and soybean meal-based diets were formulated according to the chemical composition of the food and the nutritional recommendations adopted by the poultry agroindustries in the region (Table 1).

Table 1. Nutritional composition of the diets fed to broilers

Ingredients, %	**Initial**	**Grow-out**	**Finishing**
Corn	53.07	55.12	56.98
Soybean meal	34.00	29.90	28.20
Meat meal	5.20	4.50	4.00
Soybean oil	4.10	5.50	5.90
Viscera meal	1.50	3.00	3.00
Limestone	0.340	0.420	0.420
Regular salt	0.310	0.310	0.290
Sodium bicarbonate	0.103	-	-
Vitamin and mineral premix	0.500	0.500	0.500
Choline 60%	0.086	0.052	0.042
L-Lysine 70%	0.316	0.284	0.280
L-Threonine 98%	0.092	0.084	0.089
L-Valine	0.021	0.012	-
Nutritional composition			
CP, %	23.30	22.12	21.25
ME, Kcal/kg	3.100	3.230	3.278
CF, %	7.16	8.69	9.08
CF, %	3.08	2.89	2.82
Calcium, %	1.03	1.02	0.95
Available P , %	0.46	0.45	0.42
Dig. Lys, %	1.250	1.160	1.111
Dig. AA,%	0.761	0.779	0.789
Dig Thr, %	0.813	0.766	0.745
Dig Trp, %	0.225	0.209	0.200
Dig Leuc, %	1.670	1.599	1.552
Dig Ile, %	0.692	0.698	0.699
Dig Val, %	0.963	0.905	0.860
Dig Arg, %	1.411	1.325	1.266
Choline, g/kg	1.850	1.651	1.553
Na, %	0.212	0.176	0.161
Cl, %	0.273	0.260	0.238
K, %	0.900	0.826	0.793
Na+K+Cl, Meq/100g	246	215	206

[1]Levels as described in Table 2.

The commercial and optimized vitamin premix and mineral premix in the form CAPC and sulfates were included according to the treatments and recommendations at a dose of 5 kg/ton (Table 2).

The vitamin D_3 metabolite, 25(OH)D_3, was included only in the treatments with the Optimized Vitamin Program. The feeding program was divided into three phases: starter (1 to 18 days), growing (19 to 35 days) and finishing (36 to 42 days). Feed was provided *ad libitum* and as mash.

Table 2. Vitamin and mineral programs per ton of feed

		Optimized vitamin programs			Commercial vitamin programs		
Vitamins		Initial	Grow out	Finishing	Initial	Grow out	Finishing
Vitamin A	IU	12,500	11,000	10,000	12,000	9,000	7,000
Vitamin D_3	IU	3,000	3,000	3,000	3,500	3000	2,500
25(OH)D_3	IU	2,760	2,760	-	-	-	-
Vitamin E	mg	150	100	100	30	25	20
Vitamin K	mg	4	4	4	3	3	3
Vitamin B1	mg	4.0	3.0	2.0	3.0	2.4	1.8
Vitamin B2	mg	8.0	7.0	6.0	8.0	6.5	5.0
Vitamin B6	mg	5.0	5.0	4.0	5.0	4.2	3.5
Vitamin B12	mg	0.030	0.020	0.020	0.020	0.015	0.012
Niacin	mg	60	60	50	40	35	30
Pantothenic ac.	mg	20	17	14	18	15	12
Folic acid	mg	2.5	2.0	2.0	2.5	1.5	1.0
Biotin	mg	0.30	0.25	0.20	0.24	0.21	0.20
Microminerals		**Microminerals CAPC***			**Inorganic Microminerals ****		
Manganese	mg	56	56	56	120	100	100
Zinc	mg	44	44	44	100	80	80
Iron	mg	44	44	44	70	60	60
Copper	mg	8.6	8.6	8.6	8.0	8.0	8.0
Selenium	mg	0.340	0.340	0.340	0.240	0.240	0.240
Iodine	mg	1	1	1	1	1	1

*Microminerals CAPC: copper carbo-amino-phospho-chelate; iron carbo-amino-phospho-chelate; manganese carbo-amino-phospho-chelate; selenium carbo-amino-phospho-chelate; zinc carbo-amino-phospho-chelate and calcium iodate.
**Inorganic microminerals: iron sulfate; manganese sulfate; zinc sulfate; copper sulfate; sodium selenite and calcium iodate.

Birds were placed in climate-controlled poultry houses (hoods, evaporative plates and heating by electric brooders), divided into 36 pens (38 square feet), the floor covered by reused wood shavings (6th flock). Thermal comfort temperature was maintained according to age. As a function of the heating system (300W halogen light bulb), the birds received 24 hours of light until they were 14 days of age, and after this period, 16 hours of light and 8 hours of darkness. Litter management was performed from 14 to 28 days by turning it on alternate days.

Birds were weighed at the beginning and at the end of the experiment, as well as the feed leftovers, to evaluate average weight, weight gain, average daily gain, feed intake and feed conversion. Feed conversion was corrected by the weekly bird mortality, according to the methodology written by Sakomura and Rostagno[18].

At 42 days of age, after a six-hour fasting, 30 birds/treatment were stunned by electroshock and slaughtered by bleeding by cutting the jugular vein. Subsequently, they scalded, plucked, and eviscerated, according to CONCEA Normative Resolution 37 of February 15, 2018 (Guidelines on Euthanasia Practice of the National Council for Animal Experimentation Control – CONCEA)

After removing all adherent tissue from the left leg, the tibiae of 30 birds/treatment were weighed, and the length and diameter were measured, using a digital caliper (mm). The Seedor index[19] was obtained by dividing the bone weight (mg) by their length (mm). They were then subjected to bending tests at a constant strain rate for viscoelastic material, using Texture Analyzer universal testing equipment, with a 500 kg load cell, and a head speed of 10 mm/sec. After the strength to breakage test, the thickness of the cortical bone wall was measured in the medial portion of the diaphysis with the aid of a digital caliper (mm) in the lateral phase adjacent to the osseous fibula and in the opposite region, medial phase, obtaining two measurements from which the average value of cortical bone thickness was calculated.

To evaluate the occurrence of black bone syndrome, the right tibias of 30 birds/treatment were cooled down immediately after slaughter and opened lengthwise to expose the muscle tissue adjacent to the tibia bone for 30 minutes. The readings were performed at three different points per sample. Values of luminosity (L*), red index (a*) and yellow index (b*) were expressed in the CIELAB color system using a HunterLab® colorimeter. Two intact legs from 30 more birds/treatment were collected and frozen for 60 days. After thawing and exposure of the tissue adjacent to the femur and tibia bones, the color was evaluated (L* value and a* and b* indexes) and then the legs were placed in a single roasting pan and roasted for ± 2 hours at 200º C. After roasting, legs were kept on a surface to cool down to room temperature, and then the color was read again, as recommended by Whitehead and Fleming [20].

For statistical analysis, data were checked for outliers and the assumptions of normality of studentized errors (Cramer Von Mises test) and variance homogeneity (Brown-Forsythe test). After c, the data were tested by analysis of variance using the GLM procedure of the SAS software (SAS Institute, 2002). Data on viability did not meet the normality assumption; the methodology of generalized linear models[21] was used for the analysis, assuming a gamma distribution.

Results and Discussion

There was no statistical difference ($P<0.05$) for the productive performance of broilers. However, at 42 days of age, the interaction between vitamin programs and mineral sources influenced the feed conversion of the birds (Table 3). In the interaction breakdown, it is possible to observe that the birds fed diets prepared with optimized vitamin programs and CAPC mineral source showed better feed conversion ($P<0.05$), as well as when commercial vitamin programs and inorganic mineral source are combined

(Table 4).

Table 3. Productive performance at 42 days of broilers receiving diets supplemented with different vitamin programs and mineral sources

	Body weight,g	Weight gain, g	Feed intake, g	Feed conversion
Vitamin program				
Commercial	2926.31	2850.11	4262.03	1.501
Optimized	2937.82	2885.68	4356.86	1.500
Mineral source				
Inorganic	2918.85	2857.53	4255.29	1.494
CAPC	2945.28	2857.26	4364.00	1.507
CV, %	4.33	4.25	3.77	1.70
P value				
Vitamins (V)	0.5374	0.6141	0.0551	0.1561
Minerals (M)	0.7875	0.3888	0.0904	0.9832
V x M	0.9400	0.9141	0.2705	0.0026

CAPC: Carbo-Amino-Phospho-Chelate. In the same column, averages followed by different letters are statistically different.

Table 4. Breakdown of the interaction between vitamin programs and mineral sources on broiler feed conversion at 42 days of age

	Inorganic mineral source	CAPC mineral source	P value
Commercial Vitamin Programs	1.480^{Ab}	1.521^{Aa}	0.0108
Optimized Vitamin Programs	1.508^{Aa}	1.492^{Ba}	0.1372
P value	0.0916	0.0015	

CAPC: Carbo-Amino-Phospho-Chelate. Averages followed by different capital letters in the column and lower-case letters in the row are statistically different.

The results obtained for feed conversion indicate an interdependence between mineral and vitamin sources. Nutritional recommendations for vitamin levels in poultry diets are very old and few current studies discuss these requirements taking into consideration the genetic evolution to weight gain. Supplementation of diets for fast-growing broiler breeds with different sources and higher levels of vitamins and minerals can improve intestinal integrity and morphometry, as well as intestinal mucosal development, and protect enterocytes from pro-apoptotic oxidative stress, resulting in higher efficiency of nutrient uptake [22].

Additional supplementation of vitamins and minerals has been a technique adopted to formulate feed for birds subjected to different stress situations: thermal, immunological or high stocking density. Studies have shown improved productive performance of birds supplemented with higher levels of vitamins only in a situation of heat stress [15, 23, 24].

Studies on mineral supplementation of broiler diets do not always show consistent results, especially in relation to performance indices [25,26]. However, most results indicate that minerals supplied in more bioavailable forms are effective and can advantageously replace inorganic forms. One of the main benefits is inclusion at lower levels, which can reduce the mineral content in poultry excreta [27].

On the other hand, Gai et al. (28) considered that the higher supplementation levels of vitamin A and E resulted in a better feed conversion of broilers in thermoneutral temperatures, similar to the results reported in the present study.

The evaluation of tibia bone measurements of broilers showed no statistical difference (P>0.05) for both vitamin supplementation and/or mineral source (Table 5).

Table 5. Tibial bone measurements of broilers at 42 days of age receiving diets supplemented with different vitamin programs and mineral sources

	Weight (g)	Length (mm)	Diameter (mm)	Bone resistance (kg)	Seedor	Thickness (mm)
Vitamin Program						
Commercial	37.04	113.64	8.24	40.62	326.0	1.50
Optimized	37.17	113.09	8.19	41.83	328.4	1.53
Mineral source						
Inorganic	36.66	112.98	8.28	41.15	324.5	1.54
CAPC	37.52	113.75	8.16	41.26	329.7	1.50
CV, %	10.55	2.83	8.54	25.58	10.05	16.89
Variance analysis (P value)						
Vitamins (V)	0.8884	0.3865	0.7280	0.5378	0.7270	0.5734
Minerals (M)	0.2570	0.2230	0.4198	0.9525	0.4012	0.5167
V x M	0.5478	0.4082	0.2630	0.7591	0.3854	0.1129

CAPC: Carbo-Amino-Phospho-Chelate.

There was a significant effect ($p<0.05$) of mineral source supplementation on luminosity (L*) and yellow indices (b*). Supplementation of diets with CAPC sources resulted in higher L* and b* indices when compared to diets with inorganic mineral sources, i.e., the muscle tissue showed a lighter color than that observed for birds supplemented with diets containing inorganic mineral sources (Table 6), which demonstrates that supplementation of diets with CAPC minerals results in a more compact bone, although bone measurements were not different due to mineral supplementation. Bone porosity was not measured in this study, however, the lower leakage of blood into the adjacent muscle tissue of the leg, as measured by the *b color spectrum, indirectly indicates a lighter color of the fresh meat.

Table 6. Post-slaughter color analysis in muscle tissue adjacent to the tibia bone of 42-day-old broilers fed diets supplemented with different vitamin programs and mineral sources

	L* value	a* index	b* index
Vitamin Program			
Commercial	47.32	4.918	10.77
Optimized	48.01	4.797	11.03
Mineral source			
Inorganic	46.16^{B}	4.638	10.21^{B}
CAPC	49.14^{A}	5.072	11.59^{A}
CV, %	12.78	58.38	33.63
Variance analysis (P value)			
Vitamins (V)	0.5317	0.8160	0.6986
Minerals (M)	0.0088	0.4127	0.0414
V x M	0.3709	0.4538	0.8481

L*: luminosity index, *a: red index, b*: yellow index (b*).
CAPC: Carbo-Amino-Phospho-Chelate. CV: Coefficient of variation. In the same column, averages followed by different letters are statistically different.

Since broilers have a fast growth rate, their bones have low levels of mineralization and high porosity. These characteristics make birds more prone to injuries and disorders, such as black bone [29]. Deficiency or suboptimal levels of some minerals can contribute to bone porosity because they interfere with the expression of genes related to bone remodeling activity. The gene for the *receptor activator* of *nuclear factor-kB ligand* (RANKL), which has a stimulatory action on osteoclastic activity in bone, and osteoprotegerin (OPG), which has an inhibitory action, was altered in Mn deficiency [30]. These authors demonstrated that the RANKL/OPG ratio was increased with Mn deficiency, leading to greater differentiation of osteoclasts and an increase in their activity, thus increasing resorption and, consequently, bone porosity.

High degrees of porosity compromise the bone structure, allowing the medullary content to leak into the bone surface and then dissipate into the adjacent meat. This effect is visible after cooking, and because of the rather dark appearance of the bone, this disorder has been named *black bone*.

Sixty days after slaughter, it was shown that the treatments did not modify the evaluation of the color indices of thawed and raw legs (tibia and femur) (Table 7), except for the L* index, which was higher for tibias of birds supplemented with commercial vitamin levels, regardless of processing. The comparison of processing, frozen only or frozen and roasted, showed statistical differences ($P<0.05$) for the color indices, red (a*) and yellow (b*), demonstrating that there was blood extravasation after heat processing.

These results are better understood when the interaction ($P<0.05$) between processing and mineral supplementation in the diets is broken down (Table 8). When compared to thighs from birds supplemented with CAPC mineral source, the inorganic mineral supplementation resulted in a higher red index (*a) when thighs were thawed and roasted.

It can be inferred that mineral supplementation in the CAPC form acted favorably on bone mineralization, which limited blood extravasation into the muscle tissue adjacent to the tibia bone. The preservation of thighs and drumsticks by cold, more specifically by freezing, leads to the formation of ice crystals that disrupt bone marrow cells when thawed and cooked, causing extravasation of hemoglobin present in the bone marrow, especially in fragile or porous bones.

Table 7. Color analysis in muscle tissue adjacent to thawed and processed tibia bone of 42-day-old broilers fed diets supplemented with different vitamin programs and mineral sources

	Femur			Tibia		
	L* value	**a* index**	**b* index**	**L* value**	**a* index**	**b* index**
Vitamin Program						
Commercial	48.74	2.697	12.84	50.12[a]	1.758	12.14
Optimized	47.86	2.746	12.85	47.72[b]	1.537	11.77
Mineral source						
Inorganic	48.23	2.823	12.94	48.39	1.705	11.61
CAPC	48.35	2.616	12.74	49.41	1.581	12.31
Processing						
Frozen	49.47	2.109[b]	9.900[b]	49.75	1.177	8.311[b]
Frozen and roasted	47.11	3.334[a]	15.78[a]	48.02	2.120	15.66[a]
CV, %	19.72	81.12	25.03	18.93	108.70	28.87
Variance analysis (P value)						
Vitamins (V)	0.4751	0.8373	0.9691	0.0403	0.3771	0.3098
Minerals (M)	0.9066	0.4555	0.6209	0.3419	0.5783	0.0849
Processing (P)	0.5770	<0.0001	<0.0001	0.1941	0.0001	<0.0001
V x M	0.7623	0.1918	0.5932	0.1414	0.1212	0.8107
V x P	0.8828	0.8743	0.4304	0.1496	0.5650	0.4456
M x P	0.2914	0.4926	0.6516	0.5435	0.0030	0.7369
V x M x P	0.7680	0.3349	0.7264	0.2090	0.3154	0.2929

CAPC: Carbo-Amino-Phospho-Chelate. CV: Coefficient of variation. In the same column, averages followed by different letters are statistically different.

Table 8. Breaking down the interaction between mineral source and processing on the red color index (a*) of broiler tibias

	Mineral source		
Processing	Inorganic	CAPC	P value
Frozen	0.8980[Ba]	1.4667[Aa]	0.0713
Frozen and roasted	2.5137[Aa]	1.6995[Ab]	0.0216
P value	<0.001	0.5010	

CAPC: Carbo-Amino-Phospho-Chelate. Averages followed by different capital letters in the column and lower-case letters in the row are statistically different by Tukey's test at 5%.

Black bone syndrome occurs in about 30% broiler thighs and drumsticks. Bone darkening and diffusion of the coloration into the adjacent meat, especially after cooking, can be intensified by freezing and thawing the meat. After these processes, bones have a dark appearance before cooking and after, the red color changes to brown or gray and, in severe cases, to black[29, 31]. The appearance of meat is one of the first characteristics observed by consumers, color being an attribute influencing consumer acceptance in the purchase of chicken meat.

In the long term, the solution may lie in genetic selection of breeders, with emphasis on the bird bone structure. Quick solutions are related to nutrition and the most obvious nutrients to be considered for improvement of black bone syndrome are Ca, P and vitamin D, with research being focused on vitamin D levels and forms [31,32]. Whitehead [31] has already shown that vitamin D (25-OHD$_3$) supplementation reduced the incidence of this syndrome.

It is necessary to find nutritional, genetic and/or management strategies in order to quantitatively and qualitatively improve the production of animal products, such as meat, eggs and milk[8].

Conclusion

Compared to conventional programs, the supplementation of diets with a combination of optimized vitamin programs and CAPC mineral source, resulted in similar productive performance. However, supplementation with CAPC mineral source resulted in a lighter color of the thawed and roasted thighs, indicating less blood leakage through the pores of cortical bones. More bioavailable sources of minerals make it possible to reduce their inclusion in diets and decrease the negative impact of mineral excretion and its polluting effect on the environment.

Conflict of interest

The authors declare no conflict of interest.

References

1. UBABEF - União Brasileira de Avicultura. Carne de frango, uma unanimidade que vai do norte ao sul do Brasil. Revista Avicultura Brasil. 2012; 1:8-14.

2. National Research Council - NRC. Nutrient requirements of poultry. 9.ed. Washington: National Academy of Sciences; 1994.

3. Mota MM, Hermes RG, Araújo CSS, Pereira ASC, Ultimi NBP, Leite BGS, Araújo LF. Effects on meat quality and black bone incidence of elevated dietary vitamin levels in broiler diets challenged with aflatoxin. Animal. 2019; 13(12): 2932-2938.

4. Marks HL. Growth rate and feed intake of selected and nonselected broilers. Growth. 1979; 43: 80-90.

5. Jensen LS. Fat soluble vitamin problems in biochemical diagnosis. Athens, University of Georgia. Georgia Nutrition Council. 1974: 14.

6. Anderson JJB, Toverud SU. Diet and vitamin D: a review an emphasis on human function. The Journal of Nutritional Biochemistry. 1994; 5(2): 58-65.

7. Champe PC, Harvey RA, Ferrier DR. Bioquímica Ilustrada. Porto Alegre: Artmed; 2006

8. Hutton KC, Vaughn MA, Litta G, Turner J, Starkey JD. Effect of vitamin d status improvement with 25-hydroxycholecalciferol on skeletal muscle growth characteristics and satellite cell activity in broiler chickens. Journal of Animal Science. 2014; 92(8): 3291-3299.

9. Vignale K, Greene ES, Caldas JV, England JA, Boonsinchai N, Sodsee P, et al. 25-hydroxycholecalciferol enhances male broiler breast meat yield through the mtor pathway. The Journal of Nutrition. 2015; 145: 855–863.

10. Garcia AFQM, Murakami A E, Duarte CRA, Rojas ICO, Picoli KP, Puzotti M M. Use of vitamin D3 and its metabolites in broiler chicken feed on performance, bone parameters and meat quality. Asian-Australasian Journal of Animal Sciences. 2013; 26: 408-415.

11. Applegate TJ, Angel TJ, Angel R. Los metabolites de la vitamin D son prometedores para uso en dietas avícolas. In: Vademécum avícola. Santiago: Centrovet; 2005.

12. Sanda ME, Ezeibe COM, Anene B.M. Effects of Vitamins A, C and E and Selenium on Immune Response of Broilers to Newcastle Disease (ND) Vaccine. Journal of Agriculture and Veterinary Science. 2015; 8:13-15.

13. Vieira R, Ferket P, Malheiros R, Hannas M, Crivellari R, Moraes V, Elliott S. Feeding low dietary levels of organic trace minerals improves broiler performance and reduces excretion of minerals in litter. British Poultry Science. 2020; 61(5): 574-582.

14. Bao YM, Choct M, IJI P. A effect of organically complexed copper, iron, manganese, and zinc on broiler performance, mineral excretion, and ac- cumulation in tissues. Journal of

Applied Poultry Resesearch. 2007: 16448-455.

15. . Savaram Venkata RR, Bhukya P, Raju MVLN, Ullengala R. Effect of Dietary Supplementation of Organic Trace Minerals at Reduced Concentrations on Performance, Bone Mineralization, and Antioxidant Variables in Broiler Chicken Reared in Two Different Seasons in a Tropical Region. Biological trace element research. 2020; doi: 10.1007/s12011-020-02481. PMID: 33216320.

16. Khatun A, Chowdhury SD, Roy BC, Dey B, Haque A, Chandran B. Comparative effects of inorganic and three forms of organic trace minerals on growth performance, carcass traits, immunity, and profitability of broilers. Journal of Advanced Veterinary and Animal Research. 2019; 6(1): 66-73.

17. Baloch Z, Yasmeen N, Pasha TN. Effect of replacing inorganic with organic trace minerals on growth performance, carcass characteristics and chemical composition of broiler thigh meat. African Journal of. Agricultural Research. 2017; 12(18):1570–1575.

18. Sakomura NK, Rostagno HS. Métodos de pesquisa em nutrição de monogástricos. Jaboticabal: Funep; 2007.

19. Seedor JG, Quarraccio HH, Thompson DD. The biophosphonate alendronate (mk-217) inhibits bone loss due to ovariectomy in rats. Journal of Bone and Mineral Research. 1991; 6(4): 339-346.

20. Whitehead CC, Mccormack HA, Mcteir I, Fleming R H. The maximum legal limit for vitamin D3 in broiler diets may need to be increased. British Poultry Science. 2004; 45: 24-26.

21. Nelder J, Wedderman R. Generalized linear models. Journal of the Royal Statistical Society: Series A (General).1972; 135: 370–384.

22. Sugiharto S, Isroli I, Yudiarti T, Widiastuti E. The effect of supplementation of multistrain probiotic preparation in combination with vitamins and minerals to the basal diet on the growth performance, carcass traits, and physiological response of broilers. Veterinary World. 2018; 11(2): 240-24.

23. Vaz RGMV, Oliveira RF, Donzele JL, Albino LFT, Sirqueira JC, Oliveira WP, et al. 2014. Níveis de vitamin em rações para frangos de corte mantidos em ambiente de alta temperatura no período de 1 a 42 dias de idade. Bioscience Journal. 2014; 30(5): 1522-1528.

24. Habibian M, Ghazi S, Mohammad MM. Effects of dietary selenium and vitamin e on growth performance, meat yield, and selenium content and lipid oxidation of breast meat of broilers reared under heat stress. Biological Trace Element Research. 2016; 169: 142–152.

25. Funari Júnior P, Albuquerque R, Alves FR, Murarolli MDA, Trindade Neto MA, Silva EM. Diferentes fontes e níveis de selênio sobre o desempenho de frangos de corte. Brazilian Journal of Veterinary Research and Animal Science. 2010; 47(5): 380-384.

26. Carvalho GB, Lopes JB, Silva SRG, Dourado lRB, Miranda DFH, Costa FAL. Desempenho, morfometria duodenal e histopatologia do fígado de frangos de corte alimentados com dietas contendo diferentes níveis de selênio orgânico em condições de estresse calórico. Revista

Brasileira de Saúde e Produção Animal. 2015; 16(2): 365-376.

27. Świątkiewicz S, Arczewska-Włosek A, & Jozefiak D. The efficacy of organic minerals in poultry nutrition: review and implications of recent studies. World's Poultry Science Journal. 2014; 70(3): 475-486.

28. Gai ZT, Toledo GSP, Costa PC, Lopes JM, Visentini P, Kloeckner PE. Efeito de níveis alto, médio e baixo das vitamins A, E, piridoxina (B6), ácido fólico e biotina no desempenho de frangos de corte de 1-42 dias. Revista Brasileira de Zootecnia. 1997; 26(2): 304-309.

29. Baldo GAA, Almeida Paz ICL, Alves MCF, Naas IA, Garcia RG, Caldara FR, Gavilan CWS. Black bone syndrome in chicken meat. Brazilian Journal of Poultry Science. 2013; 15(2): 317–322.

30. Liu R, Jin C, Wang Z, Wang Z, Wang J, Wang L. Effects of manganese deficiency on the microstructure of proximal tibia and OPG/RANKL gene expression in chicks. Veterinary Research Communications. 2015; 39(1): 31-37, 2015.

31. Whitehead C. The black bone syndrome in broilers. International Hatchery Practice. 2009; 23(8): 7-9.

32. Korver D. Reducing the incidence of black bone. World Poultry Science. 2010; 26: 36-38.