Mineral Source and Vitamin Level in Broiler Diets: Effects on Performance, Yield, and Meat Quality

ABSTRACT

The purpose of this trial was to supplement commercial broiler diets with optimum vitamin programs and higher availability of mineral sources, and to evaluate the effect on performance, yield and meat quality of broilers. The study used 1800 male broiler chicks randomly distributed in a 2 x 2 factorial design (vitamin programs – optimum and commercial vs. mineral sources – inorganic (sulfates) and carbo-amino-phospho-chelate (CAPC)). Supplementation associating optimum vitamin levels and mineral source CAPC resulted in better feed conversion and higher carcass weight at 42 days of age ($p<0.05$). Supplementation of diets with optimum vitamin levels resulted in higher absolute and relative breast weight, lower abdominal fat deposition, and reduction ($p<0.05$) of broiler breast water loss by dripping. Supplementation with CAPC minerals resulted in higher breast weight, lower abdominal fat deposition, less elastic muscle tissue, that is, a higher level of tenderness resulting in less resistance of muscle fibers and skin with higher tear strength than the skin of birds fed inorganic sources. Associating optimum vitamin programs and CAPC mineral source resulted in lower ($p<0.05$) lipid peroxidation levels in thighs and drumsticks after 10 and 40 days freezing. No difference ($p>0.05$) was found in the association of vitamin programs and mineral sources on the occurrence of white striping and dorsal myopathy. Supplementing the diets with optimized vitamin programs associated with a more bioavailable mineral source resulted in a positive contribution to the meat quality of broilers.

INTRODUCTION

Commercial broiler is today one of the animals with higher nutritional efficiency and fast growth. However, broiler production still faces challenges as the activity reaches new and higher productivity thresholds.

The nutritional composition of broiler diets can still influence the physiology and expression of several genes, making the animals more or less efficient in converting food into body growth.

Several factors occurring before and after slaughter are responsible for the final quality of broiler carcass. The diet can modify meat quality, altering its protein value, amino-acids profile, and amount of vitamins and minerals.

Conventional supplementation using vitamin and mineral additives is essentially based on the role of these nutrients in metabolism as cofactors in metabolic reactions and participation in energy synthesis and obtainment processes in the animal body. Recent studies, however, have proven the participation of some vitamins in major metabolic pathways mainly related to broiler growth and muscle development by modulating cell signaling and gene transcription (Yarger et al., 1995a; Li et al., 2009; Brito et al., 2010; Hutton et al., 2014; Vignale et al., 2015).
Besides the relationship between the functions of vitamin D and skeletal growth, mineralization and maintenance of bone tissue (Anderson & Toverud, 1994; Champe et al., 2006), Hutton et al. (2014) demonstrated that 25(OH)D3 supplementation stimulates the activity of satellite cells in the bird’s pectoralis major muscle. Thus, 25(OH)D3 increases protein synthesis by increasing the translational initiation rates, a process which involves several initiation factors, kinases and phosphatases, activities regulated by phosphorylation. This activation occurs by means of mTOR (rapamycin sensitive) protein kinase-dependent signaling pathways.

Berri et al. (2013) and Hutton et al. (2014) showed a greater proportion of proliferative satellite cells and of number of vitamin D receptors (VDRs) in the breast muscles of the birds fed with a combination of vitamin D3 and 25-OH-D3, compared with the broilers fed only with vitamin D3. These preliminary results may help to explain the enhancement of broiler breast meat yields at the processing plant.

Recent studies have also shown that vitamin D supplementation modifies body composition and fat deposition (Bhat et al., 2014).

As a result of the high polyunsaturated fatty acids (PUFA) proportion, poultry meat is more susceptible than pork and beef to oxidative processes, especially lipid oxidation (Delles et al., 2014). Lipid peroxidation is the main cause of fatty acids breakdown that modifies the original flavor and leads to characteristic rancid odor and flavor, besides limiting shelf-life. This is an important cause for rejection or depreciation by consumers and processors (Pedroso & Madrid, 2009; Habibian et al., 2016).

Failures in the antioxidant system can also contribute to myopathies that occur in current broiler lines, but research results are not conclusive (Bailey et al., 2015). Recently, nutrigenomic studies associated with proteomic research have indicated a potential link between the diet nutrients and the expression of specific enzymes and metabolites in the muscle (Li et al., 2009).

Mineral sources with higher bioavailability can be added to diets at lower concentrations, with no negative effects on the birds’ performance and the environment. The inclusion of these sources will allow a better understanding of minerals complex participation in a large number of enzyme and metabolite systems (Richards et al., 2010; Świątkiewicz et al., 2014; Elkhairey et al., 2015).

In commercial poultry diets, minerals are traditionally supplemented as inorganic salts (carbonates, oxides or sulfates), to provide the mineral levels to meet the birds’ nutritional requirements. However, inorganic trace minerals (ITM) can suffer high rates losses due to food antagonism, resulting in a significant reduction of their bioavailability reduction (Saripinar-Aksu et al., 2012). As a consequence, many nutritionists use higher mineral levels, often exceeding the birds’ requirements (Świątkiewicz et al., 2014).

The continuous use of these inorganic mineral salts as animal feed additives have been implicated in environmental pollution resulting from the accumulation of residues in birds’ excreta. Abdallah et al. (2009) reported that broilers fed diets with 100% minerals with higher availability (Zn, Cu, Mn, and Fe) had higher weight gain and better feed conversion compared to diets with inorganic minerals. Similar results were obtained by El-Husseiny et al. (2012). On the other hand, Nollet et al. (2007) and Perić et al. (2009) did not observe significant performance differences in birds fed different mineral sources (Sirri et al., 2016).

These concepts are very important as modern poultry production is increasingly committed to environmental issues, food security, animal well-being, and sustainability.

The purpose of this trial was to supplement commercial broiler diets with optimum vitamin programs and higher availability mineral sources, and to evaluate the effect on performance, yield and meat quality of broilers.

**MATERIALS AND METHODS**

The trial was performed in the experimental poultry house of the Universidade Federal do Paraná (UFPR) – Sector Palotina – localized in the west of Parana State, Brazil. All procedures using animals in this experiment were evaluated and approved by the Animal Experimentation Ethics Committee of Sector Palotina, protocol number 51/2014.

**Birds and Experimental Diets**

The study used a completely randomized design, 2 x 2 factorial (vitamin programs – optimum and commercial vs. mineral sources – inorganic and carbo-amino-phospho-chelate, CAPC). A total of 1800 male broiler chicks Cobb Slow were allocated to four treatments, nine replicates and 36 experimental units with 50 birds each.
The vitamin programs used were: Commercial Vitamin Program and Optimum Vitamin Program, following the Optimum Vitamin Nutrition (OVN™, DSM) program recommendations. The mineral sources used were: minerals as sulfate and Carbo-Amino-Phospho-Chelate (CAPC) (Tortuga Minerals). The treatments were as follows:

1. Commercial vitamin programs + source of inorganic minerals
2. Optimum vitamin programs + source of inorganic minerals
3. Commercial vitamin programs + CAPC
4. Optimum vitamin programs + CAPC

Based on corn and soybean meal, the diets were formulated based on the chemical composition ingredients and the nutritional recommendations adopted by the local poultry integrations (Table 1).

### Table 1 – Nutritional composition of diets provided to the broilers.

| Ingredients, %     | Starter   | Grower   | Finisher  |
|--------------------|-----------|----------|-----------|
| 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      |
| Offal meal         | 1.50      | 3.00     | 3.00      |
| Limestone          | 0.340     | 0.420    | 0.420     |
| Salt               | 0.310     | 0.310    | 0.290     |
| Sodium bicarbonate | 0.103     | -        | -         |
| Vitamin and mineral premix<sup>1</sup> | 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: 3100, 3230, 3278
- CF, %: 7.16, 8.69, 9.08
- Calcium, %: 3.08, 2.89, 2.82
- Available P, %: 1.03, 1.02, 0.95
- Dig Lys, %: 0.46, 0.45, 0.42
- Dig AAS, %: 1.250, 1.160, 1.111
- Dig Thr, %: 0.761, 0.779, 0.789
- Dig Trp, %: 0.813, 0.766, 0.745
- Dig Leuc, %: 0.225, 0.209, 0.200
- Dig Ile, %: 1.670, 1.599, 1.552
- Dig Val, %: 0.692, 0.698, 0.699
- Dig Arg, %: 0.963, 0.905, 0.860
- Choline, mg/kg: 1580.87, 1651.35, 1553.94
- 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

<sup>1</sup>Levels are described on Table 2.

The commercial and optimum vitamin premix and the CAPC and sulfate mineral premix were added according to the treatments and recommendations, 5 kg/ton (Table 2).

25(OH)D₃, the vitamin D₃ metabolite, was included only in the treatments with optimum vitamin program. The feeding program was divided into three phases: starter (1 to 18 days), grower (19 to 35 days) and finisher (36 to 42 days). The mash feed was provided ad libitum.

Birds were housed in an environmentally controlled poultry barn (exhaustion system, evaporative pads and electrical brooders), divided into 36 pens (3.52 m²), with reused wood shavings (6th flock) covering the floor. The thermal comfort temperature was maintained according to age. Birds had 24 hours of
light until day 14, due to the heating system (300 W halogen lamp), and 16 hours of light and 8 hours of dark from then on. The litter was revolved every other day from day 14 to 28.

**Productive Performance and Yield Carcass**

Birds and leftover feed were weighed every week to monitor weight gain, average daily gain, feed intake, and feed conversion. Feed conversion was corrected by the weekly mortality according to the method described by Sakomura & Rostagno (2007). The daily mortality records were also used to calculate the viability rate.

At 42 days, carcass yield was determined in 5 birds per replicate pen (sixty birds/treatment). Birds with body weight closest to the average body weight of the pen were submitted to pre-slaughter fasting for 6 hours, duly identified, and euthanized by electric stunning (11 V and 11 mA for 11 seconds) and killed manually. This included cutting the carotid artery and jugular vein, followed by scalding (53.8 °C for 2 min), feathering, and eviscerating, following the MAPA normative instruction (BRASIL, 2000).

For carcass yield calculation, the weight of the warm eviscerated carcass, without feet, head and abdominal fat was considered, relative to live weight that was obtained individually before slaughter of the birds. The premium cuts yield was calculated considering the whole breast (with skin and bones) yield, legs (thigh and drumstick with bones and skin), back and wings with skin, calculated in relation to the weight of the eviscerated carcass. Abdominal fat present around the cloaca, cloacal bursa, gizzard, proventriculus, and adjacent abdominal muscles was removed as described by Smith (1993). It was then weighed and also calculated based on the eviscerated carcass weight.

**Meat Quality Analyses**

Thirty breasts samples per treatment were used in the analysis of meat quality and functional properties. The water loss by dripping was monitored according to Boccard et al. (1981). The right *Pectoralis minor* muscle was weighed, placed in polyethylene plastic bags and suspended in galvanized steel hooks, and maintained under refrigeration for 48 hours. They were then weighed to determine the percentage of water loss by dripping.

Water loss by pressure was determined using a sample (approximately 2 grams) of the cranial portion of the left *Pectoralis major* muscle (breast fillet). The samples were placed between two filter papers and pressed by two acrylic plates weighing 10 kg for ten minutes. The samples are then weighed again to

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**Table 2 – Vitamin and mineral programs per kg of feed.**

| Vitamins       | Optimum vitamin programs | Commercial vitamin programs |
|----------------|--------------------------|-----------------------------|
|                | Starter                  | Grower                     | Finisher                  | Starter                  | Grower                     | Finisher                  |
| Vitamin A IU  | 12,500                   | 11,000                     | 10,000                    | 12,000                   | 9,000                      | 7,000                     |
| Vitamin D<sub>3</sub> IU | 3,000                   | 3,000                      | 3,000                     | 3,500                    | 3,000                      | 2,500                     |
| 25(OH)D<sub>3</sub> mg | 0,069                   | 0,069                      | -                         | 30                       | 25                         | 20                        |
| Vitamin E mg  | 150                      | 100                        | 100                       | 30                       | 25                         | 20                        |
| Vitamin K Mg  | 4.0                      | 4.0                        | 4.0                       | 3.0                      | 3.0                        | 3.0                       |
| Vitamin B<sub>1</sub> Mg | 4.0                    | 3.0                        | 2.0                       | 3.0                      | 2.4                        | 1.8                       |
| Vitamin B<sub>2</sub> Mg | 8.0                    | 7.0                        | 6.0                       | 8.0                      | 6.5                        | 5.0                       |
| Vitamin B<sub>6</sub> Mg | 5.0                    | 5.0                        | 4.0                       | 5.0                      | 4.2                        | 3.5                       |
| Vitamin B<sub>12</sub> Mg | 0.030                   | 0.020                      | 0.020                     | 0.020                    | 0.015                      | 0.012                     |
| Niacin Mg     | 60                       | 60                         | 50                        | 40                       | 35                         | 30                        |
| Pantothentic acid 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                      |
| **Trace minerals** | **CAPC trace minerals** | **Inorganic trace minerals** |
| 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                         |

*CAPC trace minerals: 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 trace minerals: iron sulfate, manganese sulfate, zinc sulfate, copper sulfate, sodium selenite and calcium iodate.**
determine the percentage of water loss by pressure (Bridi & Silva, 2009).

The caudal portion of the left Pectoralis major muscle was used to test the water loss by freezing. Samples were weighed, frozen for 24 hours, thawed and weighed (Bridi & Silva, 2009).

To analyze water loss by cooking, the medial portions of the left Pectoralis major were placed in polyethylene bags and cooked in a water bath at 180°C for 60 minutes. The samples were then refrigerated for 24 hours and weighed to obtain the percentage of water loss by cooking. Both techniques were performed according to the modified methodology of Silva Sobrinho (1999).

pH was measured in the cranial portion of the right Pectoralis major muscle/treatment, 1 hour (initial pH) and 24 hours (final pH) after slaughter under refrigeration at 0 ± 2°C, one reading in each point in time (Olivo, 2006).

The procedures described by Whipple et al. (1990) were followed to measure the shear force. The breast samples used in the cooking water loss test were refrigerated for 24 hours after cooking. Shear force measurement was made perpendicular to the muscle fibers orientation with the Warner-Bratzler blade adapted to the texturometer (TA-XT2i Model, Stable MycroSystems Ltd., Goldalming, UK). The parameters used were 2.5 mm/s speed, 10 g shot and 20 mm tension.

Lipid oxidation of the drumstick meat was evaluated on fresh meat and after freezing (10, 20, 40, and 50 days after slaughter) by measuring the concentration of thiobarbituric acid reactive substances (TBARS), according to the method described by Vyncke (1970). The result was expressed as malondialdehyde (MDA) milligrams per kilogram of sample. Butylated hydroxytoluene (BHT) was used as antioxidant in all subsamples.

**Skin Resistance and Elasticity Analyses**

Skin resistance and elasticity were measured in the drumstick skin samples of 30 birds/treatment. The samples were submitted to flexion assay at constant deformation rate for viscoelastic material, with the aid of a fixation device for the perforation test adapted to the texturometer (TA-XT2i Model, Stable MycroSystems Ltd., Goldalming, UK). The skin tearing force (kg) and the skin elasticity, which corresponds to the distance covered by the testing tip before reaching the peak, were obtained. The parameters used were 1 mm/s speed, 10 g shot and 15 mm tension.

**Myopathy Analyses**

To evaluate the dorsal myopathy, a lesion score of the anterior Latissimus dorsi muscle was adopted immediately after slaughter, as proposed by Zimermann (2008). According to the method of Kuttappan et al. (2012), severity of the white striping lesion was initially classified. After the classification described, samples from the Pectoralis major muscle were collected and underwent fixation in 10% buffered formalin. The segments were placed in such a way as to obtain longitudinal sections of the fibers, eight microns thick, and then were stained with Mallory Trichrome. Images were captured using a high-resolution digital camera PRO SERIES from Midia Cibertecnics, connected to an Olympus Bx 40 microscope, 4 x magnification. A computerized image analyzer, IMAGE PROPLUS 5.2 (Midia Cibertecnics), was used to read the images. Based on the differences in muscle tissue, connective tissue and fat colors, a color contrast was established using the image analyzer tools, these structures were then quantified.

**Statistical Analyses**

For the statistical analysis, data were verified as to the presence of outlier values and the assumption of studentized errors normality (Cramer-von Mises test) and homogeneity of variance (Brown-Forsythe test) were tested. After the non-violation of these assumptions was found, data underwent analysis of variance using the GLM procedure of the SAS program (SAS Institute, 2002). The Kruskal-Wallis test was used to evaluate the results obtained in the analysis of white striping (macroscopic) and dorsal myopathy occurrence score.

**RESULTS AND DISCUSSION**

At seven days of age, the birds fed the diets with inorganic mineral source were heavier (p<0.05) than those fed diets with CAPC mineral source (Table 3). The same effect was found for body weight gain, as birds fed the diets with inorganic mineral source had a higher body weight gain (p<0.05) when compared to birds fed diets with CAPC mineral source, but the effect was not maintained in the following weeks. There was no statistical difference (p>0.05) in body weight gain at other ages.

Regarding feed intake, it was found that birds from 1 to 7 days of age that were fed the diet with commercial vitamin levels had a lower feed consumption (p<0.05) than the birds that were fed the diet with optimum
levels (Table 3). There were no statistical differences \( (p>0.05) \) in the other age groups.

Feed conversion was similar among the treatments at the different ages that were evaluated. At 42 days of age, however, there was a significant interaction between the vitamin levels and the mineral sources. In the interaction analysis (Table 4) it is shown that the birds fed diets with optimum vitamin levels and CAPC mineral source had a better feed conversion \( (p<0.05) \), as well as when commercial vitamin levels and inorganic mineral sources are associated.

### Table 3 – Production performance of broilers fed diets supplemented with different vitamin levels and mineral sources.

|                      | Body weight, g | Body weight gain, g | Feed intake, g | Feed conversion |
|----------------------|----------------|---------------------|----------------|-----------------|
| **Vitamin programs** |                |                     |                |                 |
| Commercial           | 157.57         | 111.957             | 146.02         | 1.306           |
| Optimum              | 158.96         | 113.859             | 151.47         | 1.332           |
| **Mineral source**   |                |                     |                |                 |
| Inorganic            | 160.49         | 115.668             | 150.28         | 1.301           |
| CAPC*                | 156.05         | 110.148             | 147.21         | 1.337           |
| CV, %                | 3.76           | 5.30                | 4.36           | 4.66            |
| Vitamins (V)         | 0.4894         | 0.3483              | 0.0169         | 0.2059          |
| Minerals (M)         | 0.0326         | 0.0094              | 0.1648         | 0.0839          |
| V x M                | 0.2801         | 0.3845              | 0.6802         | 0.1713          |

### Table 4 – Unfolded interaction to feed conversion of broilers fed diets supplemented with different vitamin levels and mineral sources at 42 days of age.

|                      | Inorganic Mineral | CAPC* | \( p \) value |
|----------------------|------------------|-------|--------------|
| Commercial Vitamin Program | 1.480 \( {a} \) | 1.521 \( {a} \) | 0.0108 |
| Optimum Vitamin Program    | 1.508 \( {a} \) | 1.492 \( {a} \) | 0.1372 |
| \( p \) value          | 0.0916            | 0.0015 |              |

\( * \)CAPC: Carbo-Amino-Phospho-Chelate. CV: Coefficient of variation. Averages followed by different letters in the column are statistically different.

Data related to absolute and relative carcass, breast, legs, wings and fat yields are shown in Table 5. There was a significant interaction \( (p<0.05) \) between the vitamin levels and mineral sources in relation to
carcass weight. Birds fed diets supplemented with commercial levels of vitamins and CAPC mineral source or diets supplemented with optimum vitamin levels associated with CAPC or inorganic mineral source had higher carcass weight (p<0.05) (Table 6).

These results suggest the role of vitamin supplementation on carcass weight. Independent of the mineral supplementation source, optimum vitamin levels contributed positively to meat deposition on broilers carcass. Vitamins are essential nutrients for animal development as they participate as cofactors in metabolic reactions and allow a higher efficiency of synthesis systems in the animal body (Rutz et al., 2002).

Vitamin B6, or pyridoxine, is an important component of enzymes that regulate the body’s protein metabolism. It acts on the amino acids non-oxidative degradation reactions and its requirement is proportional to the protein level in the diet (McDowell, 2000). Deposition of lean tissue will only be efficient if the intake of energy and vitamins involved in protein metabolism is sufficient to allow the animal’s genetic expression. Thus, besides the knowledge about the relationship between nutrients intake and energy intake in high muscle deposition breeds, further research is necessary to ensure a correct vitamin level, compatible with the other nutrients.

In relation to the breast weight, the inclusion of CAPC minerals had a positive effect (p<0.05) when compared to inorganic minerals (Table 5), and of the optimum vitamin levels (p<0.05) when compared to diets supplemented with commercial vitamin levels. This effect was also found in breast yield, demonstrating again the role of vitamins as cofactors in a large number of metabolic reactions.

### Table 5 – Absolute weights and carcass yield at 42 days of age of broilers fed diets supplemented with different vitamin levels and mineral sources.

| Vitamin programs | Carcass, g | Breast, g | Thighs, g | Wings, g | Fat, g |
|------------------|-----------|------------|------------|----------|--------|
| Commercial       | 2334.61   | 933.58b    | 719.41     | 223.81   | 45.19  |
| Optimum          | 2354.11   | 964.87a    | 721.16     | 226.65   | 43.74  |
| Mineral source   |           |            |            |          |        |
| Inorganic        | 2332.00   | 937.26b    | 717.09     | 222.56a  | 45.41  |
| CAPC*            | 2357.05   | 961.42a    | 723.57     | 227.93a  | 43.52  |
| CV, %            | 7.52      | 9.79       | 8.67       | 7.56     | 27.45  |
| Vitamins (V)     | 0.216     | 0.011      | 0.773      | 0.2038   | 0.3569 |
| Minerals (M)     | 0.102     | 0.050      | 0.230      | 0.0158   | 0.2319 |
| V x M            | 0.026     | 0.382      | 0.075      | 0.6751   | 0.0752 |
| CV, %            |           |            |            |          |        |
| Carcass, %       | 74.85     | 40.22a     | 30.84      | 9.663    | 1.972a |
| Breast, %        | 75.49     | 40.81a     | 30.66      | 9.591    | 1.859a |
| Thighs, %        | 75.22     | 40.65      | 30.79      | 9.61     | 1.971a |
| Wings, %         | 75.12     | 40.40      | 30.72      | 9.63     | 1.859a |
| Fat, %           | 5.43      | 5.46       | 5.86       | 5.27     | 28.07  |
| CV, %            | 5.43      | 5.46       | 5.86       | 5.27     | 28.07  |

| Vitamin programs | Carcass, g | Breast, g | Thighs, g | Wings, g | Fat, g |
|------------------|-----------|------------|------------|----------|--------|
| Commercial       | 2,305.38b | 0.0437     | 0.2618     | 0.2359   | 0.0332 |
| Optimum          | 2,358.62a | 0.3959     | 0.6605     | 0.9514   | 0.0174 |
| Mineral source   |           |            |            |          |        |
| Inorganic        | 75.22     | 40.65      | 30.79      | 9.61     | 1.971a |
| CAPC*            | 75.12     | 40.40      | 30.72      | 9.63     | 1.859a |
| CV, %            | 5.43      | 5.46       | 5.86       | 5.27     | 28.07  |
| Vitamins (V)     | 0.0685    | 0.9127     | 0.6898     | 0.1685   | 0.0896 |
| Minerals (M)     | 0.4915    | 0.9127     | 0.6898     | 0.1685   | 0.0896 |
| V x M            | 0.0873    | 0.0143     | 0.0437     | 0.2359   | 0.0332 |

*CAPC: Carbo-Amino-Phospho-Chelate. CV: Coefficient of variation.
Averages followed by different letters in the column are statistically different.
The role of vitamins in myogenesis processes (Hutton et al., 2014) may explain the higher vitamin requirements for the breast muscle yield. These authors demonstrated that 25(OH)D3 supplementation stimulates the activity of satellite cells in the Pectoralis major muscle. Thus, 25(OH)D3 increases the muscle yield by means of muscle fiber hyperplasia. The myofibrils increase in post-hatching is a result of the skeletal muscle satellite cells activity, myogenic precursors, that initiate their development during the final embryonic stage, and are able to proliferate, differentiate, and join the existing fibers or a fusion process with others can take place to form new fibers (Moss, 1968). Satellite cells are responsible for secondary generation nuclei in the myofibrils (Bischoff, 1975). This process is responsible for approximately 98% of the final DNA content of the myofiber, so the cytoplasm volume is maintained as well as the number of micronuclei, thus increasing the protein synthesis capability (Halevy et al., 2003; Duclos et al., 2007).

Vignale et al. (2015) also have shown participation of vitamin D in protein synthesis processes. These authors propose that the mechanism takes place by signaling pathways that are dependent on mTOR protein kinase (rapamycin-sensitive). However, there is not enough information to determine if the muscle hyperplasia measured by satellite cells is controlled by the mTOR pathway as a response to 25(OH)D3 supplementation.

Apart from the proposed mechanisms, Yarger et al. (1995b) also reported an increase in the breast weight when birds were supplemented with 25(OH)D3. On the other hand, studies proposing mechanisms that explain the direct participation of minerals from different sources in meat yield are not available.

The inclusion of CAPC mineral source in the diet resulted in higher wing weight (p<0.05) when compared to diets with inorganic mineral source (Table 5), however there was no positive effect on wing yield (p>0.05). Vitamin supplementation had no significant effect (p>0.05) on absolute or relative weight of wings.

Table 5 shows that birds fed diets with optimum vitamin levels or CAPC mineral source had a lower percentage of abdominal fat (p<0.05).

Recent molecular studies have demonstrated that vitamin E has not only an antioxidant effect but is also a regulating factor of gene expression. Azzi et al. (2004) reported that the transcription of a gene for cytosolic phospholipase, a key enzyme involved in phospholipid oxidation, was regulated by vitamin E. Higher levels of vitamin E could potentially alter fatty acids profile and composition and also the body fat deposition (Li et al., 2009). Bhat et al. (2014) report that vitamin D (25(OH)D3) supplementation can alter body composition and fat deposition in broilers.

In addition, Jianhua et al. (2000) state that the increase of Se levels in the diet has a direct relationship with increased blood levels of T3 hormone and deiodinase iodothyronine enzyme. Thyroid hormones regulate the metabolic activity in animals and can stimulate the mobilization of fat reserves and reduce deposition on the carcass (Olkowski & Classen, 1995).

The pH and temperature measured on the broilers breast right after slaughter (initial) and after 24 hours (final) were not influenced (p<0.05) by the treatments (Table 7).
Lu et al. (2014) evaluated diets with oxidant factors associated with two different levels of vitamin E and obtained similar results as vitamin E supplementation resulted in lower loss by dripping.

Table 9 presents the results of evaluating two parameters of shear force: force necessary for tearing, elasticity of muscle tissue, or the distance covered by the probe to cause the tearing and evaluation of the skin resistance that followed two principles: force necessary to tear the skin and elasticity.

No significant difference ($p>0.05$) was found for shear force for tearing. However, there was a significant effect ($p<0.10$) of the mineral source on the elasticity measurement. Supplementation of diets with CAPC mineral sources resulted in a less elastic muscle tissue, that is, the muscle fibers were teared with less resistance because they were more tender. Thus, the value $p=0.0905$, can be considered as tendency ($p<0.10$), and there is a favorable effect of supplementation with CAPC mineral on skin tearing.

The skin of birds supplemented with CAPC minerals is more resistant to tearing than the skin of birds supplemented with inorganic sources.

Perić et al. (2009) stress that selenium yeast supplementation results in lower water loss and, as a consequence, a higher degree of juiciness and tenderness. When using sources that are more bioavailable than the inorganic sources. Boiago (2006) found lower shear force and, consequently, the meat was more tender. Medeiros et al. (2012) supplemented the diets with increasing levels of selenium yeast and also observed a positive linear effect on shear force, that is, adding selenium resulted in more tender meat. However, Gomes et al. (2012) and Oliveira et al. (2014) did not observe an effect of different mineral sources supplementation on the tenderness of breast meat from broilers slaughtered at 42 days of age.

It is important to consider that when the trace mineral requirements are met the mineral supplementation, whatever the source, may not result in significant

| Table 8 – Evaluation of losses by dripping, pressure, freezing and cooking that occur in the meat of broilers fed diets supplemented with different vitamin programs and mineral sources at 42 days of age. |
|-----------------------------------------------|
| Vitamin programs | Loss by Dripping, % | Loss by Pressure, % | Loss by Freezing, % | Loss by Cooking, % |
| Commercial | 2.084$^a$ | 7.686 | 6.261 | 27.06 |
| Optimum | 1.861$^b$ | 7.700 | 6.283 | 26.12 |
| Mineral source | | | | |
| Inorganic | 2.020 | 7.470 | 6.479 | 26.05 |
| CAPC* | 1.925 | 7.919 | 6.062 | 27.14 |
| CV, % | 27.53 | 41.84 | 34.39 | 15.98 |
| Variance analysis | | | | |
| Vitamins (V) | 0.0262 | 0.9762 | 0.9549 | 0.2292 |
| Minerals (M) | 0.3138 | 0.4665 | 0.2955 | 0.1628 |
| V x M | 0.3473 | 0.9331 | 0.7935 | 0.2438 |
| *CAPC: Carbo-Amino-Phospho-Chelate. CV: Coefficient of variation. |

| Table 9 – Breast shear force and skin resistance of broilers fed diets supplemented with different vitamin programs at 42 days of age. |
|-----------------------------------------------|
| Breast shear force | Skin resistance |
| | Force for tearing, kg | Elasticity, mm | Force for tearing, kg | Elasticity, mm |
| Vitamin programs | | | | |
| Commercial | 2.33 | 13.54 | 6.488 | 9.562 |
| Optimum | 2.22 | 13.18 | 6.385 | 9.469 |
| Mineral sources | | | | |
| Inorganic | 2.29 | 13.87$^c$ | 6.159 | 9.623 |
| CAPC* | 2.27 | 12.86$^c$ | 6.722 | 9.405 |
| CV, % | 31.13 | 15.71 | 27.82 | 14.71 |
| Variance analysis | | | | |
| Vitamins (V) | 0.4207 | 0.3426 | 0.7646 | 0.4156 |
| Minerals (M) | 0.8746 | 0.0095 | 0.0905 | 0.3047 |
| V x M | 0.4233 | 0.1517 | 0.4779 | 0.9804 |
| *CAPC: Carbo-Amino-Phospho-Chelate. CV: Coefficient of variation. |

Averages followed by different letters in the column are statistically different.
differences in performance, yield, or meat quality. However, excretion of the supplement that was not utilized is lower (Nollet et al., 2007).

Rossi et al. (2007) found an increase in the number of epithelial cell layers, higher amount of collagen, reduced skin inflammation and increased skin resistance in broilers fed diets supplemented with organic zinc.

Minerals with higher bioavailability can result in higher concentration of circulating minerals, thus potentiating their physiological functions (Scottá et al., 2014). Copper is a component of lysyl oxidase, an enzyme that catalyzes formation of collagen and elastin, and favors crosslinking between the collagen fibers, responsible for structural rigidity and elasticity. Zinc plays an important role in hormone secretion, keratin formation, and collagen and nucleic acids synthesis in the skin. Manganese is required for collagen and elastin glycosylation (Underwood & Suttle, 1999).

Thiobarbituric acid reactive substances (TBARS) resulting from the lipid oxidation of fresh meat samples from thighs and drumsticks, expressed as MDA (mg/kg), showed that there was no statistical difference (p>0.05) between the treatments (Table 10).

**Table 10** – Average values of TBARS (MDA mg/kg) in fresh and frozen thighs and drumsticks from broilers supplemented with different vitamin programs and mineral sources.

| Vitamin programs | Fresh meat 10 days | 20 days | 40 days | 50 days |
|------------------|-------------------|---------|---------|--------|
| Commercial       | 0.310             | 0.54    | 0.69    | 0.44b  | 0.48   |
| Optimum          | 0.283             | 0.57    | 0.59    | 0.31a  | 0.47   |

| Mineral sources | Inorganic | CAPC* | CV, %   |
|-----------------|-----------|-------|---------|
|                 | 0.275     | 0.316 | 71.42   |
|                 | 0.59      | 0.52  | 46.11   |
|                 | 0.62      | 0.66  | 42.74   |
|                 | 0.32      | 0.42  | 62.98   |
|                 | 0.49      | 0.45  | 45.42   |

Variance analysis

Vitamins (V) 0.6741 0.5542 0.1532 0.0244 0.7939
Minerals (M) 0.2098 0.2068 0.5901 0.0779 0.4349
V x M 0.2441 0.0110 0.5642 0.1418 0.6724

*CAPC: Carbo-Amino-Phospho-Chelate. CV: Coefficient of variation.

There was a significant interaction between the vitamin levels and mineral source in the frozen meat analyzed 10 days after slaughter. In the interaction analysis it can be observed that the use of optimum vitamin programs associated with CAPC mineral source resulted in a lower MDA index (Table 11).

**Table 11** – Analysis of the interaction between vitamin programs and mineral sources and their effect on TBARS (MDA mg/kg) average values in frozen thighs and drumsticks analyzed 10 days after slaughter.

| Vitamin programs | Inorganic | CAPC* | p value |
|------------------|-----------|-------|---------|
| Commercial       | 0.050     | 0.058 | 0.2823  |
| Optimum          | 0.070a    | 0.045a| 0.0208  |

*CAPC: Carbo-Amino-Phospho-Chelate. Averages followed by different letters in the line are statistically different

Analyses performed with samples frozen for 40 days after slaughter showed lower MDA indices in samples from birds fed the diet with optimum vitamin programs (p<0.05), whatever the mineral source supplemented. For the other periods that were evaluated there were no statistical differences between the treatments (p>0.05). The lower MDA indices found in the meat that was frozen for 40 days can be explained by the hypothesis raised by Rao et al. (1996). These authors found a reduction in the MDA values of frozen buffalo meat between 30- and 60-days storage and attributed this decrease to the interaction of proteins present in the food. According to Shamberger et al. (1977), MDA can combine with other food chemical components, such as proteins, forming stable compounds that lead to an underestimate of the MDA final value.

Delles et al. (2014) used different oils (oxidized) in association with selenium and found that independent of the oil quality given to the birds, the diets with more bioavailable selenium source had lower TBARS indices in broiler breast meat.

Studies show that due to a higher bioavailability, more efficient absorption and retention, mineral sources with higher biological value are deposited in the birds’ muscles at higher concentrations than inorganic sources (McDowell, 1992; Downs et al., 2000).
Supplementation of broiler diets with high levels of α-tocopherol and selenium delay the start of oxidative action during meat storage (Ryu et al., 2005). Vitamin E acts by inhibiting phospholipase A2 and stabilizing mitochondrial membranes, thus reducing exudative losses of cells that contribute to extend the shelf-life of meat products (Block et al., 1995; Al Khader et al., 1996).

Souza et al. (2006) and Li et al. (2009) used increasing levels of vitamin E as a supplement and found that the amount of MDA was smaller in the breast and drumsticks of birds supplemented with high levels of vitamin E after 5 and 7 days of storage, respectively.

The increase in vitamin E levels in the diet can contribute to maintain the original flavor of the product and extend the shelf-life of broiler meat, reducing the extension of lipid oxidation (Young et al., 2004).

Macroscopic analysis of dorsal cranial myopathy lesions did not demonstrate statistical differences (p<0.05) between the vitamin and mineral associations that were tested, as shown by the data on Table 12. For the classification of white striping no statistical differences (p>0.05) were found in the associations of vitamin programs and mineral sources.

Dorsal myopathy has caused major economic losses due to carcass condemnation. And males of heavy lines with higher average weight at slaughter had the highest condemnation rates. Lesions are characterized by swelling and a yellowish discoloration of the skin that covers the affected muscle. When the skin is sectioned, subcutaneous edema, muscular superficial hemorrhage, pallor, adherence, increased thickness and density are seen. Based on the occurrence and severity of these characteristics, dorsal myopathy is classified as normal, moderate and severe (Zimermann et al., 2012).

White striping lesions are characterized by parallel white lines that run parallel to the muscle fiber, their amount and thickness being variable. Histological examination of the affected breast muscle showed that these lines are formed by adipose tissue (Bailey et al., 2015).

Other authors report that breasts with severe white striping lesions can have more connective tissue, with different degrees of microscopic degeneration and regeneration of myofibrils (Petracci et al., 2013). Kuttappan et al. (2012) report that when white striping severity increases there is an increase in the fat percentage and also in the dry matter content of the muscle, with histological characteristics of adipogenesis in the affected muscle tissues.

When samples are collected from breasts evaluated macroscopically, the analysis of the muscular tissue composition (fat, collagen and protein) confirms these reports (Table 13). However, no significant effect of the treatments (p>0.05) was found in fat, collagen and protein percentages.

Table 13 – Evaluation of muscle fat, collagen and protein percentages (%) of breasts from broilers supplemented with different vitamin programs associated with mineral sources.

| Vitamin programs | Fat, % | Collagen, % | Protein, % |
|------------------|--------|-------------|------------|
| Commercial       | 12.83  | 8.30        | 77.60      |
| Optimum          | 11.71  | 7.72        | 80.57      |
| Mineral sources  |        |             |            |
| Inorganic        | 12.76  | 7.99        | 78.56      |
| CAPC*            | 11.74  | 7.96        | 79.75      |
| CV, %            | 40.22  | 57.58       | 10.64      |

| Variance analysis | Vitamins (V) | Minerals (M) | VxM |
|-------------------|--------------|--------------|-----|
|                   | 0.2819       | 0.6215       | 0.1118 |
|                   | 0.3238       | 0.9923       | 0.4592 |
|                   | 0.8115       | 0.4993       | 0.7182 |

*CAPC: Carbo-Amino-Phospho-Chelate. CV: Coefficient of variation.

Poultry breast muscles are very valuable. For economic reasons, it became necessary to slaughter birds with a rapid weight gain at an earlier age and the resulting intensive genetic selection has caused abnormal physiological behaviors, with damages to

Table 12 – Classification of dorsal cranial myopathy and white striping macroscopic lesions in broilers supplemented with different vitamin programs associated with mineral sources.

| Treatments | Normal | Moderate | Severe | Normal | Moderate | Severe |
|------------|--------|----------|--------|--------|----------|--------|
| Commercial vitamins + inorganic minerals | 58.93  | 39.29    | 1.79   | 2.74   | 79.45    | 17.81  |
| Optimum vitamins + inorganic minerals | 55.93  | 37.50    | 7.14   | 5.41   | 78.38    | 16.22  |
| Commercial vitamins + CAPC* minerals | 64.58  | 27.08    | 8.33   | 2.74   | 80.82    | 16.44  |
| Optimum vitamins + CAPC* minerals | 60.00  | 40.00    | 0.00   | 2.70   | 81.08    | 16.22  |
| CV, % | 49.21  | 48.05    | 19.82  | 40.17  | 50.43    | 25.11  |

*CAPC: Carbo-Amino-Phospho-Chelate. CV: Coefficient of variation.
the muscle tissue (Petracci et al., 2015). Besides the genetic component, nutritional and management factors, even health factors, may be involved in the occurrence of different myopathies: deep pectoral myopathy, dorsal myopathy, white striping, woody breast. In this sense, Bailey et al. (2015) also stressed the importance of understanding both the environmental and the management factors that contribute to more than 65% variance in white striping incidence in breast muscle and more than 90% variance in woody breast and deep pectoral myopathy incidence in broilers.

Vitamin E deficiency can cause nutritional muscular dystrophy, similar to white striping lesions, in several species (Van Vleet et al., 1976). However, attempts to reduce myopathy by providing vitamin E supplementation have not been successful (Kuttappan et al., 2012). And this suggests that white striping is not associated with nutritional muscular dystrophy as a result of vitamin E deficiency.

Bauermeister et al. (2009) did not have favorable results using optimum vitamin levels and organic minerals to preserve muscle fibers integrity in broilers affected by this myopathy. As the causes are still unknown and there are indications that this is a lesion resulting from ischemic hypoxia, antioxidants could be used as an alternative as the muscle involved is oxidative phosphorylation dependent (Carmo-Araújo et al., 2007).

As it is known that white striping is the result of a degenerative process in muscle fibers, followed by repair with adipose tissue and also support connective tissue (Petracci et al., 2013), these alterations interfere directly with meat quality. The increase in lipid levels can favor lipid peroxidation and the product’s tenderness can change due to collagen deposition (Kranen et al., 2000; Bailey et al. 2015).

Definition of meat quality depends on the consumer market. New quality concepts have recently been incorporated such as food safety and production systems observing human, animals and environment well-being. Organic minerals have a higher availability and are therefore more readily absorbed by animals, decreasing their excretion and environmental pollution. They can also bring additional benefits as improving skin integrity and meat quality.

In conclusion, diets with optimum vitamin programs associated with a more bioavailable mineral source contributed positively to broilers performance (feed conversion and carcass weight at 42 days of age), yield (heavier breasts and less abdominal fat deposition), and meat quality (water loss by dripping in breasts, lower lipid peroxidation rates in thigh and drumstick meat after 10 and 40 days freezing).

**CONFLICT OF INTEREST STATEMENT**

The authors declare no conflict of interest.

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