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Organic minerals with different chemical characteristics in diets for Hy-Line White laying hens: performance, biometry of digestive organs, and bone quality

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ABSTRACT - Two trials were carried out to evaluate the effect of groups of organic minerals with different chemical characteristics on the performance, egg quality, biometry of digestive organs, and bone quality of laying hens in the first and second laying cycles. In the first cycle, 180 layers at 72 weeks were used in a completely randomized design (CRD) with four treatments and five replicates with nine birds each. In the second cycle, 216 layers at 94 weeks of age were used in a CRD with four treatments and six replicates with nine birds each. Birds were fed the following diets: treatment 1 - basal diet (inorganic minerals); treatment 2 - basal diet + amino acid chelated minerals (Cu, Fe, Mn, and Zn) + selenium yeast; treatment 3 - basal diet + mineral-amino acid complex (Mn, Zn, and Cu); and treatment 4 - basal diet + metal chelate (Mn, Zn, and Cu) + methionine hydroxy analogue. The following performance variables were evaluated: feed intake (g/bird/day), egg production (%), egg weight (g), egg mass (g/bird/day), conversion per mass (kg/kg), and conversion per dozen eggs (kg/dz). In the egg, the percentages of albumen, yolk, and shell; eggshell thickness (mm); and specific gravity (g/cm^3) were determined. The relative weights (%) of the proventriculus, gizzard, liver, pancreas, and intestines were also measured. Lastly, in the tibiae, the weight (g), length (mm), resistance (kgf/cm^2), deformity (mm), Seedor index (mg/mm), and mineral matter content (g/kg) were measured. The variables were not influenced by the groups of organic minerals used. Groups of organic minerals with different chemical characteristics can be used in layer diets without affecting their performance, egg quality, digestive organs, or bone quality.

Keywords: bone resistance, chelated amino acid, laying, Seedor index

Introduction

The commercial-poultry production system in Brazil hit the mark of 788,260,000 dozen eggs in the first trimester of 2017, which represents an increase of 31,060,000 dozens when compared with the same period in the previous year (IBGE, 2017). These figures prove the visible growth in the laying-poultry sector in the country. Such growth, in turn, is a result of the combined action of factors influencing production, one of which is the nutritional management, given that nutrition is one of the foundations of animal production (Alves et al., 2015).

For an animal to present a good nutritional status, it must receive adequate quantities of all nutrients essential to its organism, among which are minerals. For many years, minerals originating from inorganic sources have been used to meet the mineral requirements of birds; however, their bioavailability is low, which results in losses during the absorption process that compromise the animal performance (Carvalho et al., 2016).

A strategy that has been adopted to elevate the bioavailability of these minerals and thus reduce losses during the absorption process is the use of minerals from organic sources replacing or supplementing conventional sources, with a view to increasing production efficiency (Pacheco et al., 2010).

Organic minerals are defined as metal ions chemically linked to an organic molecule. These are classified by the Association of American Feed Control Officials (AAFCO, 2000) into the following groups: metal-amino acid chelates, metal-amino acid complex, specific metal-amino acid complex, metal proteinate, and polysaccharide metal complex.

There are many types of commercially available products containing the groups of organic minerals described above, and each carries a different proposal, with a higher or lower bioavailability of the involved minerals (Saleh et al., 2018). In this way, questions arise as to the molecule to be chosen as a ligand of the mineral used in the product. The efficiency of each of group of these may, however, vary according to how it is produced.

In view of this scenario, the present study tested the hypothesis that groups of organic minerals with different chemical characteristics influence the performance, egg quality, biometry of digestive organs, and bone quality of white-egg laying hens in their first and second laying cycles.

Material and Methods

The project was approved by the local ethics committee (case no. 001.05.016.UVA.504.03). The two trials were carried out in Sobral, CE, Brazil (03°41'10" S latitude, 40°20'59" W longitude, and 69 m asl).

Birds were housed in galvanized-wire cages measuring 30 × 45 × 45 cm, equipped with a frontal metal trough feeder and a nipple drinker per cage. All birds received the same daily management during the evaluated period, with water available *ad libitum*. A lighting program of 16 h per day was adopted, consisting of 12 h of natural plus 4 h of artificial illumination.

Before the start of the experiments, birds were weighed and selected to generate experimental plots with uniform body weights and egg production, following recommendations proposed by Sakomura and Rostagno (2007).

In the first trial, 180 Hy-Line White laying hens at 72 weeks of age, in their first laying cycle, weighing 1.673±0.109 kg, were used in a completely randomized design with four treatments and five replicates with nine birds each. The experimental period was 84 days, divided into three 28-day sub-periods.

The experimental diets were isonutritive and isocaloric (Table 1), formulated according to the nutritional requirements suggested by the Hy-Line White manual (Hy-Line do Brasil, 2016), and the composition of ingredients used in the formulation followed the recommendation of Rostagno et al. (2011). The following experimental diets were tested: Treatment 1 - basal diet (corn, soybean meal, a calcium and phosphorus source, a vitamin premix, vegetable oil, and common salt containing only inorganic minerals); Treatment 2 - basal diet + amino acid chelated minerals (Cu, 5 ppm; Fe, 35 ppm; Mn, 40 ppm; and Zn, 55 ppm) + selenium yeast (16 ppm); Treatment 3 - basal diet + mineral-amino acid complex (Mn, 40 ppm; Zn, 40 ppm; and Cu, 7 ppm); and Treatment 4 - basal diet + metal chelate (Mn, 40 ppm; Zn, 40 ppm; and Cu, 8 ppm)-methionine hydroxy analogue [MHA]).

Egg production was recorded daily until the end of each 28-day period, when these data, together with those pertaining to feed intake, were used to calculate the performance variables. The following variables were assessed: feed intake (g/bird/day), egg production (%), egg weight (g), egg mass (g/bird/day), conversion per egg mass (kg/kg), and conversion per dozen eggs (kg/dz).

At the end of each period, the following egg quality parameters were also evaluated: percentages of albumen, yolk, and shell; eggshell thickness (mm); and specific gravity (g/cm^3). For these measurements, four eggs were selected per replicate, two of which for the determination of specific gravity and the other two for the remaining quality analyses.

The second trial involved 216 Hy-Line White layers at 94 weeks of age, in the second laying cycle, weighing 1.689±0.100 kg, for 105 days that were divided into five 21-day periods. A completely randomized design was adopted with four treatments and six replicates with nine birds each. Experimental diets were similar to those of the first trial, with the same nutritional levels except for calcium, which was 4% in the second trial.

After every 21-day period, the following egg quality parameters were assessed: percentages of albumen, yolk, and shell; eggshell thickness (mm); and specific gravity (g/cm^3). For these measurements, four eggs were selected per replicate, two of which for the determination of specific gravity and the other two for the remaining quality analyses.

At the end of 105 days, 20 birds per treatment were chosen at random and identified. These were euthanized by the cervical-dislocation method (following Normative Resolution no. 13/2013 – CONCEA, 2013) and taken to the laboratory, where they were weighed individually and had their organs removed and emptied in a necropsy for biometric evaluations of the gizzard, liver, pancreas, and intestines, using a 0.01-g precision scale (adapted from Braz et al., 2011). All weight data were expressed as a percentage of body weight.

Table 1 - Calculated nutritional composition of the experimental diet (as-fed basis; g/kg)

Ingredient (g/kg)	Inorganic	Amino acid chelate	Amino acid complex	Metal chelate-MHA
Corn grain	607.00	607.00	607.00	607.00
Soybean meal	219.50	219.50	219.50	219.50
Limestone	84.00	84.00	84.00	84.00
Meat meal	54.80	54.80	54.80	54.80
Vitamin-mineral supplement[1]	4.00	4.00	4.00	4.00
Common salt	2.72	2.72	2.72	2.72
Soybean oil	25.70	25.70	25.70	25.70
DL-methionine	1.22	1.22	1.22	1.22
Inert	1.00	0	0	0.39
Amino acid chelates + selenium yeast	0	1.00	0	0
Amino acid complex (Mn+Zn+Cu)	0	0	1.00	0
Zn metal chelate-MHA	0	0	0	0.25
Mn metal chelate-MHA	0	0	0	0.31
Cu metal chelate-MHA	0	0	0	0.05
Metabolizable energy (kcal/kg)	2900	2900	2900	2900
Crude protein (g/kg)	175.00	175.00	175.00	175.00
Calcium (g/kg)	41.00	41.00	41.00	41.00
Available phosphorus (g/kg)	4.30	4.30	4.30	4.30
Sodium (g/kg)	1.80	1.80	1.80	1.80
Digestible met + cys (g/kg)	6.60	6.60	6.60	6.60
Digestible methionine (g/kg)	4.16	4.16	4.16	4.16
Digestible lysine (g/kg)	7.64	7.64	7.64	7.64
Digestible threonine (g/kg)	5.60	5.60	5.60	5.60
Digestible tryptophan (g/kg)	1.69	1.69	1.69	1.69

MHA - methionine hydroxy analogue.

[1] Provides per kg of product: iron (min), 10.00 g; copper (min), 2,500.00 mg; zinc (min), 20.00 g; manganese (min), 20.00 g; iodine (min), 208.00 mg; selenium (min), 75.15 mg; vitamin A (min), 2,000,000.00 IU; vitamin D3 (min), 625,000.00 IU; vitamin E (min), 3,000.00; vitamin K3 (min), 395.92 mg; folic acid (min), 74.25 mg; choline (min), 100.00 g; niacin (min), 5,025.74 mg; pantothenic acid (min), 1,805.16 mg; vitamin B1 (min), 250,09 mg; vitamin B2 (min), 1,000.00 mg; vitamin B6 (min), 250.1 mcg; vitamin B12 (min), 2,400.00; methionine (min), 125.00 g; colistin (min) 1,750.00 mg.

Right and left tibiae were also removed to analyze the following bone characteristics: weight (g), length (mm), resistance (kgf/cm^2), deformity (mm), Seedor index (mg/mm), and mineral matter (g/kg). Bone length was measured using a digital caliper, and its weight was obtained on an electronic scale with 0.01-g precision. Bone density was evaluated based on the Seedor index, which was calculated by dividing the weight (mg) by the length (mm) of the evaluated bone (Seedor et al., 1991).

Analyses of bone resistance and deformity were performed using a mechanical press, where the left tibiae were placed in the horizontal position, and then a compression force was applied to the center of each one of them. The maximum force applied onto the bone until it broke was considered the breaking strength (kgf/cm^2), which was measured using a digital strain gauge. Deformity (mm) was measured using an analogical stain gauge until the moment of bone rupture.

After deboning, the right tibiae were weighed and dried in a forced-air oven at 105 °C for 72 h (Abudabos, 2012). Next, they were weighed and crushed with a mortar and pestle. Crushed samples were then identified for the determination of the mineral matter, following the methodology described by Silva and Queiroz (2002).

In the two experiments, the statistical procedures were applied using the SAS statistical software (Statistical Analysis System, version 8). The average data of the period were subjected to an analysis of variance, and means were compared by the SNK test at the 5% probability level.

Variables were analyzed according to the following mathematical model:

$$Yij = \mu + \beta i + \varepsilon ij,$$

in which Yij = observation j of experimental unit subjected to treatments i, μ = general constant, βi = effects of rations with organic minerals with different chemical characteristics, and εij = random error associated to each observation.

Results

In the first trial, regardless of the chemical characteristics of the groups of organic minerals used, no significant differences were observed (P>0.05) for any of the performance or egg quality variables (Tables 2 and 3). The same result was found for the second trial (Table 4).

In the biometric analysis of the digestive organs, no significant differences were observed (P>0.05) for the relative weights of gizzard, liver, pancreas, proventriculus, and intestines (Table 5).

Irrespective of the chemical characteristics of the groups of organic minerals used, no significant differences were observed (P>0.05) for weight, length, Seedor index, resistance, deformity, or mineral matter content of the tibiae (Table 6).

Table 2 - Mean values for performance traits of Hy-Line White layers fed diets containing groups of minerals with different chemical characteristics, in the period of 72 to 84 weeks of age

Supplement[1]	Intake (g/bird/day)	Production (%)	Egg weight (g)	Egg mass (g/bird/day)	CM (kg/kg)	CDZ (kg/dz)
1	89.73	62.72	65.47	41.06	2.204	1.732
2	93.58	62.92	65.22	41.08	2.291	1.792
3	92.93	63.93	65.25	41.71	2.245	1.758
4	91.54	64.91	66.13	42.55	2.157	1.696
Mean	91.95	63.62	65.53	41.59	2.123	1.744
CV (%)	5.44	7.61	1.89	7.90	7.78	8.25
P-value	0.6347	0.8838	0.9696	0.8753	0.6585	0.7517

CM - conversion per egg mass; CDZ - conversion per dozen eggs; CV - coefficient of variation.

[1] Supplement 1 - inorganic minerals; Supplement 2 - inorganic minerals + amino acid chelated minerals + selenium yeast; Supplement 3 - inorganic minerals + mineral-amino acid complex (Mn+Zn+Cu); Supplement 4 - inorganic minerals + metal chelate (Mn+Zn+Cu)-methionine hydroxy analogue.

Discussion

With respect to performance (Table 2) — because the birds used in this experiment were at an advanced age and thus outside of their production peak, when the requirements and nutritional challenges are much higher — the inorganic premix used was sufficient to optimize their production and, consequently, the effect of organic supplementations was not evidenced. The obtained results confirm others found

Table 3 - Mean values for measurements of eggs from Hy-Line White layers fed diets containing groups of minerals with different chemical characteristics, in the period of 72 to 84 weeks of age

Supplement[1]	Albumen (%)	Yolk (%)	Shell (%)	ST (mm)	SG (g/cm^3)
1	60.02	28.00	8.49	0.342	1.084
2	59.77	28.02	8.82	0.358	1.085
3	59.57	28.11	8.49	0.355	1.082
4	60.28	27.60	8.59	0.357	1.084
Mean	59.91	27.93	8.60	0.353	1.084
CV (%)	1.38	2.62	3.21	3.49	0.20
P-value	0.5765	0.7061	0.2379	0.1933	0.2618

ST - eggshell thickness; SG - specific gravity; CV - coefficient of variation.

[1] Supplement 1 - inorganic minerals; Supplement 2 - inorganic minerals + amino acid chelated minerals + selenium yeast; Supplement 3 - inorganic minerals + mineral-amino acid complex (Mn+Zn+Cu); Supplement 4 - inorganic minerals + metal chelate (Mn+Zn+Cu)-methionine hydroxy analogue.

Table 4 - Percentages of albumen, yolk, and shell, eggshell thickness (ST), and specific gravity (SG) of Hy-Line White layers fed diets containing groups of minerals with different chemical characteristics, in the period of 94 to 109 weeks of age

Supplement[1]	Albumen (%)	Yolk (%)	Shell (%)	ST (mm)	SG (g/cm^3)
1	61.23	27.92	8.36	0.344	1.082
2	60.42	27.79	8.51	0.348	1.082
3	59.93	27.81	8.58	0.349	1.083
4	60.78	27.82	8.40	0.350	1.081
Mean	60.59	27.84	8.46	0.348	1.082
CV (%)	1.94	3.04	3.00	3.53	0.19
P-value	0.3003	0.9958	0.4608	0.8606	0.2007

ST - eggshell thickness; SG - specific gravity; CV - coefficient of variation.

[1] Supplement 1 - inorganic minerals; Supplement 2 - inorganic minerals + amino acid chelated minerals + selenium yeast; Supplement 3 - inorganic minerals + mineral-amino acid complex (Mn+Zn+Cu); Supplement 4 - inorganic minerals + metal chelate (Mn+Zn+Cu)-methionine hydroxy analogue.

Table 5 - Relative weight of digestive organs of Hy-Line White layers fed diets containing groups of minerals with different chemical characteristics, in the period of 94 to 109 weeks of age

Supplement[1]	Gizzard (%)	Liver (%)	Pancreas (%)	Proventriculus (%)	Intestines (%)
1	1.13	2.58	0.20	0.34	3.10
2	1.20	2.51	0.19	0.32	3.38
3	1.09	2.40	0.17	0.30	3.19
4	1.16	2.41	0.18	0.32	3.01
Mean	1.15	2.48	0.19	0.32	3.18
CV (%)	14.06	13.98	21.81	15.97	9.77
P-value	0.7048	0.8158	0.6813	0.6138	0.2541

CV - coefficient of variation.

[1] Supplement 1 - inorganic minerals; Supplement 2 - inorganic minerals + amino acid chelated minerals + selenium yeast; Supplement 3 - inorganic minerals + mineral-amino acid complex (Mn+Zn+Cu); Supplement 4 - inorganic minerals + metal chelate (Mn+Zn+Cu)-methionine hydroxy analogue.

in the literature, which state that the larger bioavailability attributed to chelated minerals was not verified by the performance variables of the layers.

Manangi et al. (2015) worked with organic (Zn, Cu, and Mn metal chelate + MHA) and inorganic minerals in the production of commercial white-egg layers in the period of 24 to 80 weeks of age and observed that the feed intake, egg production, and egg mass variables were not influenced by supplementation in organic form. However, Sun et al. (2012) used Cu-MHA, Mn-MHA, and Zn-MHA supplementation in diets for brown-egg layers at 39 weeks of age and found different results for egg weight, which increased with organic supplementation.

Although the organic mineral sources had a higher bioavailability (Pessôa et al., 2012), promoting better results when consumed, in both trials (Tables 3 and 4), no improvements in terms of egg internal/external quality were seen with the supplements. A lack of effect of the chelated minerals on egg quality parameters was also reported by Swiatkiewicz and Koreleski (2008), Saldanha et al. (2009), and Geraldo et al. (2012).

However, Sun et al. (2012) worked with chelated minerals in the feeding of brown-egg layers (at 39 weeks of age) using a control diet with minerals in sulfated form, treatment 2 with supplementation of 10 mg/kg Cu-MHA, treatment 3 with supplementation of 20 mg/kg Zn-MHA, and treatment 4 with supplementation of 20 mg/kg Mn-MHA, and reported that shell thickness increased with Zn-MHA and Mn-MHA. The positive effect of Zn-Cu-Mn-MHA on eggshell thickness was also reported by Manangi et al. (2015).

According to Rutz et al. (2004), after absorption, organic minerals are readily transported to the body tissues, where they remain stored for longer periods than inorganic minerals. Selenium-methionine, for instance, is stored in tissues like the liver, pancreas, and kidney (Funari Junior, 2008) and may cause an increase in the relative weight of those organs when in excess, which was not observed in the current study (Table 5). Thus, we can infer that all mineral supplements provided the adequate levels of nutrients necessary for the good biometric quality of the organs observed.

Gheisari et al. (2011) worked with broilers fed diets containing organic and inorganic (sulfated and oxide forms) minerals in the following compositions: control - basal diet (Zn, Mn, and Cu in oxide form; 100, 100, and 10 mg/kg respectively); diet 2 (140, 140, and 17 mg/kg Zn, Mn, and Cu oxides associated with 40, 40, and 7 mg/kg thereof in organic form); diet 3 (40, 60, and 8 mg/kg of Zn, Mn, and Cu in sulfated and oxide form); diet 4 (40, 40, and 7 mg/kg Zn, Mn, and Cu, in amino acid complex form); diet 5 (40, 40, and 7 mg/kg Zn, Mn, and Cu in sulfated form); and diet 6 (60, 60, and 10.5 mg/kg in amino acid complex form). According to the authors, the different mineral compositions of the diets did not influence the relative weight of the organs of birds (liver, spleen, and bursa).

Table 6 - Bone-related (tibia) variables of Hy-Line White layers fed diets containing groups of organic minerals with different chemical characteristics, in the period of 94 to 109 weeks of age

Supplement[1]	Weight (g)	Length (mm)	Seedor index (mg/mm)	Resistance (kgf/cm^2)	Deformity (mm)	Mineral matter (g/kg)
1	7.07	115.32	61.32	10.74	2.33	48.61
2	7.28	112.72	64.54	10.75	1.99	49.14
3	7.70	114.56	67.07	10.25	2.03	50.53
4	7.31	113.87	64.14	10.11	2.16	50.43
Mean	7.34	114.05	64.28	10.48	2.12	49.65
CV (%)	10.07	2.12	8.67	0.1618	15.04	4.21
P-value	0.6116	0.3601	0.4651	0.8954	0.3483	0.3922

CV - coefficient of variation.

[1] Supplement 1 - inorganic minerals; Supplement 2 - inorganic minerals + amino acid chelated minerals + selenium yeast; Supplement 3 - inorganic minerals + mineral-amino acid complex (Mn+Zn+Cu); Supplement 4 - inorganic minerals + metal chelate (Mn+Zn+Cu)-methionine hydroxy analogue.

Different results were reported by Netravathi et al. (2016), who worked with zinc-methionine and iron-methionine replacing inorganic minerals at the inclusion levels of 50, 100, and 150% in diets for naked-neck hens and observed a higher relative weight of the gizzard with organic supplementation.

The bone is a conjunctive tissue formed by an organic matrix, where micro-minerals (copper, manganese, and zinc), as enzymatic co-factors, play a fundamental role in their synthesis and a mineral portion constituted of calcium phosphate (Nunes et al., 2013). Therefore, a balanced diet provides nutrients necessary for high-quality bone formation, resulting in more resistant bones. On this basis, it can be inferred that all mineral supplements adequately supplied the nutrients necessary for the good bone quality found (Table 6).

Results similar to those of this study for bone resistance were reported by Nunes et al. (2013), who worked with supplementation of increasing levels of Cu, Mn, Zn, and Fe proteinates in diets for brown-egg layers in the period of 30 to 70 weeks of age.

Gheisari et al. (2011) worked with inorganic and organic minerals (Zn, Mn, and Cu) alone or associated in the feeding of broilers and also did not find an influence of organic mineralization on the ash content of the tibiae of these birds.

Conclusions

Amino acid chelated minerals plus selenium yeast; mineral-amino acid complex; and metal chelate plus methionine hydroxy analogue can be used in diets of layers without affecting their performance, egg quality, digestive organs, or bone quality.

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