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Replacement of inorganic trace minerals by chelated minerals in pullet diets (12 to 20 weeks of age)

Bruno M. Santos, Fernanda V. Castejon, Eduardo M. Oliveira, Fabyola B. Carvalho, Heloisa H. C. Mello, Marcos B. Café and José H. Stringhini

Federal University of Goiás, Dept. Animal Science, 74.690-900, Goiânia, Goiás, Brazil.

Abstract

Aim of study: An experiment was carried out aimed to evaluate the effects of different levels and sources of trace mineral to laying pullets with two initial body weights (BWs).

Area of study: The experiment was carried out in Federal University of Goiás, Goiânia, Goiás, Brazil

Material and methods: Two hundred and eighty eight Bovans White pullets aged 12 weeks old were allotted in a completely randomized design and a 2×3 factorial arrangement, wherein the main effects included initial BW (light-weight and heavy-weight) and three dietary trace mineral sources and levels (100% inorganic, 100% chelated and low-dose corresponding to 50% chelated), totalizing six treatments with eight replicates of six birds. The performance, the metabolizability coefficient of nutrients, and the onset of lay were evaluated at rearing phase (12 to 20 weeks). At 17th and 20th weeks of age, the relative weight of reproductive and digestive organs, abdominal fat, and tibia quality were assessed. A residual effect was evaluated at production phase on productive performance and egg quality.

Main results: The mineral source did not affect the performance of pullets. Birds fed 50% chelated mineral produced the lowest eggshell. The heavy-weight birds showed higher egg weight and eggshell quality. The lighter birds showed lower abdominal fat weight and lower tibia robustness index.

Research highlights: The replacement of 100% of inorganic mineral for chelated mineral do not result in decrease of bird performance at rearing and at production phase, but a minimum amount should be provided to ensure growth and nutrient metabolizability.

Additional key words: copper; egg quality; manganese; organic mineral; rearing phase; zinc

Abbreviations used: BW (body weight); BWG (body weight gain); CMDM (coefficient of metabolizabilization of dry matter); CMN (coefficient of metabolizabilization of nitrogen); EP (egg production); FCR (feed conversion ratio); FI (feed intake); NB (nitrogen balance); SG (specific gravity); TRI (Tibia robustness index)

Authors' contributions: Conceived and designed the experiments: BMS and JHS. Performed the experiments: BMS, FVC, EMO and FBC. Analyzed the data: BMS, MBC and JHS. Contributed reagents/materials/analysis tools: JHS and MBC. Wrote the paper: BMS, JHS and HHCM.

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Correspondence should be addressed to Heloisa H. C. Mello: heloisamello@ufg.br

Introduction

The rearing phase of commercial laying hen is known to prepare physiologically the bird to onset of lay. At this phase, the development of reproductive organs and of the liver, the increase of both calcium in medullary bones and of abdominal fat deposition occur simultaneously. Therefore, the ideal development of pullets is related to the productive efficiency of laying hen. According to Melnychuk *et al.* (2004) the lack of uniformity in body weight (BW) at the time of photostimulation leads to greater variation in the rate of reproductive development of breeder hens.

The trace mineral are nutrients required to support pullet development and to improve the productive period of laying hens (Lilburn *et al.*, 2019; Pereira *et al.*

2020). Copper (Cu) is an essential trace mineral for poultry and acts mainly to promote enzyme function, including ceruloplasmin, cytochrome oxidase, lysyl oxidase, superoxide dismutase, and tyrosinase (Klasing, 1998). Ceruloplasmin is necessary for iron (Fe) transport and plays a role in the acute phase of immune response as well as the use of high dietary level of Cu promoted the elevation of circulating ceruloplasmin concentration in broilers (Song *et al.*, 2009). Cu deprivation impairs eggshell quality, since Cu most likely plays an important role in thickening of the eggshell membrane, and, therefore, it is expected to affect eggshell breaking resistance (Berwanger *et al.*, 2018).

Manganese (Mn) and Zinc (Zn) act as cofactors of metalloenzymes responsible for carbonate and mucopolysaccharides synthesis and play an important role in eggshell formation (Swiatkiewicz & Koreleski, 2008). Xiao *et al.* (2015) reported that dietary supplementation with either organic Mn or inorganic Mn significantly enhanced the thickness, breaking strength, and elastic modulus of the eggshells. To laying hens, Zn is especially important, because plays an important role as a co-factor of carbonic anhydrase.

Trace minerals can be supplemented in diets as inorganic or chelated sources. The trace minerals complexing to small size organic molecules enhance their absorption and improve the metabolic utilization (Abdallah *et al.*, 2009). Mineral proteinates show higher retention rates and relative bioavailability values than inorganic salts and in fact, the antagonism between minerals such as Zn and Cu could be avoided by using organic forms (Ao & Pierce, 2013). Chelated minerals have been satisfactorily included in the diet in substitution of inorganic minerals for broilers (Tavares *et al.*, 2013; El-Katcha *et al.*, 2017; Mwangi *et al.*, 2017, 2019; Lopes *et al.*, 2017), laying hens (Xiao *et al.*, 2015; Sarlak *et al.*, 2020) and broiler breeders (Wang *et al.*, 2019). However, there is limited research work of chelated minerals in laying pullet diets.

The replacement of inorganic minerals by chelated minerals in poultry diets has resulted in some benefits in poultry diets. Rutz *et al.* (2007) related that the use of chelated minerals resulted in lower levels of inclusion in diets, and lower waste and excretion to environment, due the higher bioavailability. The utilization of chelated trace minerals improved eggshell thickness and immune response in laying hens (Manangi *et al.*, 2015), egg production (EP) and egg mass (Sousa *et al.*, 2017), eggshell breaking strength and resistance to fracture (Mabe *et al.*, 2003), and Fe content of eggs (Sarlak *et al.*, 2020). Zhang *et al.* (2017) verified that organic Zn enhanced carbonic anhydrase activity in laying hens. In addition, the dietary supplementation of organic trace minerals in low doses reduced fecal mineral excretion without negatively impacting hen performance and egg quality (Qiu *et al.*, 2020).

The objective of this experiment was to evaluate the effects of different levels and sources of trace mineral (inorganic, chelated and low-dose chelated) to Bovans White pullets at 12 weeks of age with two initial BWs (light and heavy) on rearing phase performance, productive performance, egg quality, organs development and nutrient metabolizability coefficients.

Material and methods

An experiment was carried out in Goiânia, Goiás, Brazil. This research project was approved by the Ethics Committee on Animal Use (CEUA; case n.013/13).

Two hundred and eighty eight Bovans White pullets aged 12 weeks old, were allotted in a completely randomized design and a 2×3 factorial arrangement, wherein the main effects included two initial BW (light-weight and heavy-weight) and three trace mineral source or level (100% inorganic, 100% chelated and low-dose corresponding to 50% chelated), totalizing six treatments with eight replicates of six birds each.

Experimental diets were isonutritive and formulated according to Rostagno *et al.* (2011) (Table 1). The diets were formulated to provide 2,900 kcal of metabolizable energy, and 100% of trace mineral inorganic, 100% of trace mineral chelated and low-dose of 50% of mineral chelated. The experimental diet relative to 50% mineral chelated was made providing reduction of 50% of recommendation of mineral chelated supplement. The Mn, Zn, Fe an Cu content (mg kg-1) in treatments 100% of trace mineral inorganic, 100% of trace mineral chelated and low-dose of 50% of mineral chelated were respectively: 60-60-30-9, 25-20-15-3, and 12.5-10-7.5-1.5. To constitute the treatments with chelated mineral, the mineral supplement "Bioplex TR SE- Poultry®" was used containing Mn, Zn, Fe, Cu, iodine (I), and selenium (Se)- amino acid chelated.

Birds were acquired from a commercial farm aged 11 weeks old and separated in two groups: light-weight (mean of 750 g ± 75g) and heavy-weight (mean of 850 g ± 85g). The standard weight recommended by Bovans White guidelines at 11 weeks of age is 775 grams. Before the experimental period, the birds were subjected to a week of adaptation to house and diets. The laying hens were housed in conventional laying shed, covered with clay tiles, and screened on sides. Galvanized metal wire cages were equipped with aluminum trough type feeders, nipple type drinkers and egg collectors. The conventional cages size was 50 x 45 cm and 32 cm of height, with two divisions of 25 cm. A 16-h lighting program was adopted using an automatic light timer, following the recommendations for the lineage manual (Bovans White Guidelines: www.bovans.com/en/product/bovans-white). At the beginning of the laying phase, 15 min of light

Table 1. Ingredients and chemical composition in the experimental diets (g kg-1) fed to pullets from 12 to 20 weeks of age and the laying hens diets fed to birds from 21 to 32 weeks of age

Treatment	100% Inorganic	100% Chelated	50% Chelated	Laying hen diets
Corn grain	747.47	747.47	747.47	621.39
Soybean meal 450.0 g kg^{-1}	152.29	152.29	152.29	251.26
Wheat bran	73.73	73.73	73.73	0.00
Calcarium	12.01	12.01	12.01	97.25
Dicalcium phosphate	11.37	11.37	11.37	11.21
Salt	1.57	1.57	1.57	2.52
Vitamin supplement [1]	1.00	1.00	1.00	1.00
Mineral supplement [2]	0.50	0.00	0.00	0.50
Mineral chelated supplement [3]	0.00	0.50	0.25	0.00
Oil vegetable	0.00	0.00	0.00	9.27
DL-Methionine	0.00	0.00	0.00	2.72
L-Lysine HCL	0.06	0.06	0.06	0.49
L-Treonine	0.00	0.00	0.00	0.46
Calculated nutritional composition				
Metabolizable energy (kcal/kg)	2,900	2,900	2,900	2,800
Crude protein	140.0	140.0	140.0	165.0
Digestible Methionine + Cystine	4.24	4.24	4.24	7.31
Digestible Methionine	2.17	2.17	2.17	5.03
Digestible Lysine	5.69	5.69	5.69	8.03
Digestible Treonine	4.70	4.70	4.70	6.10
Calcium	8.00	8.00	8.00	40.20
Avaiable phosphorus	3.10	3.10	3.10	3.00
Sodium	1.50	1.50	1.50	2.25

[1] Vitamin supplement: Vit. A 8,000 UI, Vit. E 15,000 mg, Vit. D3 2,300 UI, Vit. K3 1,000 mg, Vit. B1 200 mg, Vit. B2 3,000 mg, Vit. B6 1,700 mg, Vit. B12 10,000 mcg, Niacin 20,000 mg, Folic acid 500 mg, Biotin 15.00 mg. [2] Mineral supplement: Mn 120,000 mg, Zn 120,000 mg, Fe 60,000 mg, Cu 18,000 mg, I 2,000 mg, Ca 9,600 mg. [3] Bioplex TR SE poultry® (per kg): Mn 50 g, Zn 40 g Zn, Fe 30 g, Cu 6g, I 400 mg, Se 180 mg.

were added per week until achieve 16 h in the peak of production. The birds were fed with the experimental diets from 12 to 20 weeks old, and then they received the same diet from 21 to 32 weeks of age. Birds had free access to water and diets during the experimental period. The experimental period lasted 20 weeks.

The variables studied on the rearing phase (12 to 20 weeks) were feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR) and age at first egg: FI (g/bird/day) was calculated by the difference between the amount of feed supplied and leftovers of rearing period; FCR was determined as a direct relationship between FI and BWG; BWG (kg) was estimated by weighing each bird on the first day and the last day of the rearing period (12 to 20 weeks). Additionally, a biweekly BW (g) from 11 to 19 weeks of age were measured to attend the growth development of pullets.

The total excreta were collected at 14th and 20th weeks of age of birds to calculate the coefficient of metabolizabilization of dry matter (CMDM) and of nitrogen (CMN) and the N balance according to the method proposed by Sakomura & Rostagno (2016). The total excreta collection was performed twice a day. After collection, the excreta were conditioned in identified plastic bags and then frozen. The FI was recorded in order to calculate the CMDM and CMN. At the end of the experiment, the excreta were defrosted and homogenized, and a sample of 300 g was taken to the analysis. The dry matter content of excreta and feed samples was determined by drying in an oven (105ºC), and the crude protein by the micro-Kjeldahl method (nitrogen distiller Tecnal® TE-0364). The analysis of dry matter and N was determined according to the procedures described by Silva & Queiroz (2006). The nitrogen balance (NB) was calculated by the difference between N ingested and N excreted. The CMDM and CMN were calculated as following:

$$\mathrm{CMN} = (\mathrm{N\ ingested} - \mathrm{N\ excreted/DM\ ingested} \times 100)$$

$$\mathrm{CMDM} = (\mathrm{DM\ ingested} - \mathrm{DM\ excreted/DM\ ingested} \times 100)$$

At 17 and 20 weeks of age, four birds per treatment, totalizing eight birds per treatment, were euthanized by cervical dislocation to collect the following organs: ovary, oviduct, digestive organs, liver, pancreas, tibia, crest, dewlap, and abdominal fat. The data were presented as relative weight (g per 100 g BW). The lengths (cm) of oviduct and intestine were measured.

Tibias were cleaned of adhering tissue, dried, extracted with ethyl ether and weighed (g). A caliper was used to record the diaphysis width (mm) and length (mm). The tibia robustness index (TRI) was recorder according to Monteagudo *et al.* (1997), as follows:

$$\mathrm{TRI} = \frac{tibia\ length}{3\sqrt{tibia\ weight}}$$

At laying production phase (21 to 32 weeks) the residual effect of diets was evaluated on productive performance and egg quality. The BWG (g/bird), FI (g/bird/day) and FCR (kg/dozen) and egg production (%) were evaluated. FI (g/bird/day) was calculated by the difference between the amount of feed supplied and leftovers of production period. BWG (g/hen/day) was estimated by weighing each bird on the first day and the last day of the experiment. FCR was determined as a direct relationship between FI and dozens of eggs produced (kg/dozen). Eggs were collected once a day, and EP (%) was calculated by dividing the total amount of eggs per experimental unit by the number of laying hens.

At the last days of the 26th week of age four eggs/replication, totalizing 192 eggs, were selected to evaluate egg weight, eggshell weight, and eggshell thickness. Individual egg weights were recorded. Each egg was subsequently broken, the contents were discarded, and the eggshell were washed and air-dried for 24 hours. Shell thickness was measured with a digital calliper in two points at the middle-transversal area of the shell, from which an average measure expressed in mm was obtained for statistical analysis.

The specific gravity (SG) was determined following Hamilton (1982): eggs were immersed in saline solutions with densities varying from 1.065 to 1.110, with intervals of 0.005, and when floating, they were removed and identified as to the treatment.

Statistical analysis

Data were subjected to analysis of variance (ANOVA). Treatment differences among means with $p<0.05$ were accepted as representing statistically significant differences. Means were compared by the F test and Tukey test. A 2×3 factorial arrangement, wherein the main effects (two initial BW and three trace mineral source or level), with six treatments and eight repetitions were used. Statistical analyses were performed by the R 3.1.1 (2013) software. The proposed mathematical model was as follows:

$$Yijk = \mu + ai + bj + (ab)ij + \mathcal{E}ijk$$

in which Y_{ijk} = value observed in the initial BW_i (i = 1, 2), trace mineral source j (j = 1, 2, 3) and repetition k (k = 1, 2, 3, …, 8); μ = overall mean of the experiment; a_i = fixed effect of the initial BW_i; b_j = fixed effect of the trace mineral source j; $(ab)_{ij}$ = fixed effect of the interaction between initial BW_i and trace mineral source j; and $\mathcal{E}_{ijk}$ = random error in the initial BW_i, trace mineral source j, and repetition k.

Results

There was no significant interaction between the two factors on performance of pullets ($p>0.05$) (Table 2). The mineral source did not affect FI, BWG and FCR of pullets. The initial BW affected FI, FCR and the age at first egg. The heaviest birds presented higher FI (p=0.044), lower FCR (p= 0.034) and produced first egg early than the lighter birds ($p<0.001$) (Table 2).

The biweekly BW was evaluated to attend the growth development of pullets in the rearing phase (Table 3). It was verified that the mineral source did not influence the BW of pullets, however, the initial BW interferes on biweekly BW ($p>0.05$). Differences in the initial BW at the beginning of experiment among the groups continued to the end of experimental period, evidencing that birds that started the rearing phase below of ideal BW cannot regain the BW and achieve the target BW at production phase ($p<0.001$).

Table 2. Effects of experimental diets on feed intake (FI, g/hen/day), body weight gain (BWG, g/hen/day), feed conversion ratio (FCR, g/g), and age at first egg (AFE, days) of pullets from 12 to 20 weeks of age

Factor[1]	FI	BWG	FCR	AFE
IBW				
Light-weight	64.50B	7.27	8.91A	141.45A
Heavy-weight	66.93A	7.63	8.80B	135.83B
MS				
100% Inorganic	66.37	7.70	8.77	137.87
100% Chelated	65.44	7.45	8.83	139.62
50% Chelated	65.32	7.31	8.97	138.44
ANOVA (*p* value)				
IBW	0.044	0.131	0.034	<0.001
MS	0.595	0.598	0.840	0.987
IBW × MS	0.592	0.974	0.896	0.897
CV (%)	3.18	10.63	10.21	3.44

[1] IBW: initial body weight. MS: micromineral source. CV: coefficient of variation. Means followed by different letters in a column differ from each other by the F Test ($p<0.05$).

Table 3. Biweekly body weight (g) of pullets from 11 to 19 weeks of age

Factor [1]	Initial	13 weeks	15 weeks	17 weeks	19 weeks
IBW					
Light-weight	732.60B	908.27B	1000.90B	1072.35B	1190.89B
Heavy-weight	855.81A	1001.07A	1121.58A	1215.52A	1336.80A
MS					
100% Inorganic	792.03	962.14	1084.67	1153.66	1269.50
100% Chelated	796.84	953.08	1088.09	1158.13	1265.61
50% Chelated	793.73	951.33	1083.82	1140.79	1261.87
ANOVA (*p* value)					
IBW	<0.001	<0.001	<0.001	<0.001	<0.001
MS	0.963	0.723	0.933	0.884	0.787
IBW × MS	0.797	0.771	0.825	0.689	0.884
CV (%)	6.32	4.95	5.89	5.94	5.59

[1] IBW: initial body weight. MS: micromineral source. CV: coefficient of variation. Means followed by different letters in a column differ from each other by the F Test ($p<0.05$).

There was no significant interaction between the two factors on NB and CMDM and N of laying hens ate 14 and 20 weeks of age ($p>0.05$) (Table 4). The mineral font did not influence the nutrient metabolizability coefficients at 14 weeks of age ($p>0.05$). However, at 20 weeks of age, diets containing 50% of chelated mineral resulted in lower CMDM. It was verified that the initial BW of hens influenced the CMDM ($p<0.05$) at 14 weeks of age. The lighter birds presented higher CMDM than the highest birds.

There was no significant interaction between the two factors on relative organs weight of pullets at 17th and 20th weeks of age ($p>0.05$) (Tables 5 and 6). At 17th weeks of age the birds showed the same relative organs weight and intestine length despite the treatments ($p>0.05$). Therefore, neither the reproductive organs nor the digestive organs improved the development according to trace minerals source or initial BW of the birds. At 20th weeks of age, the initial BW but not the mineral source influenced the relative weight of spleen and abdominal fat of birds ($p<0.05$). The lighter birds showed higher spleen relative weight and lower abdominal fat weight (Table 6).

Initial BW and mineral source showed no interaction for tibia quality ($p>0.05$) (Table 7). The tibia quality was not influenced by using inorganic or chelated

Table 4. Nitrogen balance (NB) and coefficient of metabolizability of dry matter (CMDM, %) and nitrogen (CMN, %) of laying hens at 14 and 20 weeks of age

Factor [1]	14 weeks of age			20 weeks of age		
	NB (g)	CMDM	CMN	NB (g)	CMDM	CMN
IBW						
Light-weight	7.71	76.50A	47.70	11.26	75.43	46.13
Heavy-weight	7.04	73.36B	40.07	11.98	75.19	47.14
MS						
100% Inorganic	6.72	74.36	40.21	10.73	77.29A	47.71
100% Chelated	7.07	73.93	42.55	12.24	75.72AB	47.13
50% Chelated	8.33	76.49	48.90	11.90	72.93B	45.07
ANOVA (*p* value)						
IBW	0.361	0.028	0.095	0.391	0.850	0.747
MS	0.187	0.263	0.264	0.313	0.032	0.766
IBW × MS	0.220	0.230	0.112	0.435	0.807	0.553
CV (%)	23.80	4.30	24.22	17.35	4.06	16.18

[1] IBW: initial body weight. MS: micromineral source. CV: coefficient of variation. Means followed by different letters in a column differ from each other by the F Test ($p<0.05$) and Tukey test ($p<0.05$).

Table 5. Relative weight (%) of organs and intestine length (cm) of pullets at 17 weeks of age

Factor[1]	Liver	Intestine	Pancreas	Spleen	Bursa	Dewlap	Crest	Abdominal fat	Oviduct	Ovary	Intestine length
IBW											
Light-weight	1.92	3.77	0.19	0.19	0.24	0.04	0.07	1.75	0.19	0.08	123.50
Heavy-weight	2.04	3.64	0.21	0.20	0.27	0.03	0.05	1.85	0.23	0.07	124.00
MS											
100% Inorganic	2.05	3.65	0.20	0.18	0.20	0.03	0.05	1.59	0.23	0.08	120.37
100% Chelated	1.94	3.62	0.20	0.22	0.26	0.05	0.08	1.93	0.17	0.08	124.12
50% Chelated	1.95	3.83	0.19	0.19	0.29	0.03	0.05	1.88	0.24	0.07	126.75
ANOVA (*p* value)											
IBW	0.194	0.479	0.223	0.869	0.518	0.312	0.272	0.803	0.722	0.944	0.921
MS	0.607	0.593	0.955	0.252	0.211	0.087	0.074	0.738	0.827	0.366	0.583
IBW × MS	0.556	0.660	0.657	0.990	0.957	0.870	0.440	0.385	0.223	0.627	0.889
CV (%)	11.27	11.78	20.82	25.62	39.09	43.43	47.58	50.44	28.00	30.52	9.81

[1] IBW: initial body weight. MS: micromineral source. CV: coefficient of variation. ANOVA (α=5%).

minerals. The TRI was lower in lighter pullets at 17 weeks of age ($p<0.05$), while the tibia weight, tibia length, and tibia width were lower in lighter hens at 20 weeks of age ($p<0.05$).

The residual effect of the experimental diets was studied on production phase of laying hens. Initial BW and mineral source showed no interaction for performance at production phase ($p>0.05$) (Table 8). BWG, FI, FCR and EP were similar among the birds (20 to 32 weeks age) fed inorganic or chelated mineral.

There was no significant interaction between the two factors on egg quality of laying hens ($p>0.05$) (Table 9). Despite the egg weight, the eggshell weight was affected by mineral source used in diets ($p<0.05$; Table 9); birds fed 50% chelated mineral produced the lowest eggshell compared to birds fed diets supplying 100% of inorganic micromineral. The eggshell thickness and the SG were not affected by diets ($p>0.05$).

Discussion

The use of a low-dose of 50% of chelated mineral was sufficient to ensure the birds performance on rearing phase. According to Sun *et al.* (2020), replacing dietary inorganic trace mineral with low-dose complexed glycinate minerals increases the apparent bioavailability of Fe, Mn, and Zn in broiler breeders. The best bioavailability of

Table 6. Relative weight (%) of organs and intestine length (cm) of pullets at 20 weeks of age

Factor[1]	Liver	Intestine	Pancreas	Spleen	Crest	Dewlap	Abdominal fat	Oviduct	Ovary	Intestine length	Oviduct length
IBW											
Light-weight	2.38	4.30	0.18	0.14A	0.42	0.20	0.52B	3.61	2.89	116.71	56.33
Heavy-weight	2.35	4.02	0.19	0.10B	0.55	0.20	0.86A	3.45	2.97	118.50	62.08
MS											
100% Inorganic	2.30	4.04	0.16	0.12	0.47	0.22	0.78	3.68	2.91	117.62	59.62
100% Chelated	2.30	4.09	0.20	0.12	0.50	0.20	0.61	3.59	3.11	122.06	59.83
50% Chelated	2.51	4.34	0.19	0.12	0.47	0.21	0.68	3.33	2.78	113.12	58.37
ANOVA (*p* value)											
IBW	0.878	0.248	0.539	0.035	0.057	0.994	0.021	0.580	0.795	0.704	0.145
MS	0.635	0.528	0.318	0.989	0.880	0.656	0.612	0.612	0.695	0.314	0.952
IBW × MS	0.807	0.904	0.570	0.448	0.496	0.220	0.559	0.115	0.263	0.638	0.335
CV (%)	20.77	13.74	30.86	34.01	31.92	22.60	45.65	20.61	25.90	17.34	15.62

[1] IBW: initial body weight. MS: micromineral source. CV: coefficient of variation. Means followed by different letters in a column differ from each other by the F Test ($p<0.05$).

Table 7. Tibia weight (g), tibia length (mm), tibia width (mm), and tibia robustness index (TRI) of birds at 17 and 20 weeks of age

Factor[1]	17 weeks of age				20 weeks of age			
	Weight	Length	Width	TRI	Weight	Length	Width	TRI
IBW								
Light-weight	8.11	117.97	7.02	58.75B	6.72B	112.50B	6.97B	59.70
Heavy-weight	7.84	119.30	6.96	60.16A	7.56A	118.23A	7.44A	60.32
MS								
100% Inorganic	8.12	118.63	6.87	59.02	7.10	113.59	7.16	59.19
100% Chelated	7.74	117.93	7.10	59.74	7.19	116.51	7.14	60.48
50% Chelated	8.06	119.36	7.01	59.57	7.13	116.01	7.32	60.37
ANOVA (*p* value)								
IBW	0.221	0.311	0.692	0.042	0.008	0.002	0.012	0.506
MS	0.310	0.661	0.526	0.715	0.971	0.323	0.618	0.446
IBW × MS	0.413	0.700	0.449	0.394	0.462	0.890	0.393	0.632
CV (%)	6.45	2.62	5.58	2.60	9.78	3.48	5.65	3.67

[1] IBW: initial body weight. MS: micromineral source. CV: coefficient of variation. Means followed by different letters in a column differ from each other by the F Test (p<0.05).

trace minerals could explain the performance of pullets fed low dose of trace mineral chelated.

The lighter birds showed lower FI, probably due to the lower maintenance requirements. It was verified a delayed EP in light-weight hens, suggesting that the BW below to the target BW recommended by manual affects negatively the onset of lay. Van der Klein *et al.* (2018) related that the increased BW accelerated sexual maturity. According to these authors, sexual maturity is delayed, EP is reduced in hens reared on increased photoperiod and the effect of rearing photo schedule on sexual maturity and EP is dependent on BW.

At 20 weeks of age, diets containing 50% of chelated mineral resulted in lower CMDM. Zn supplementation can improve the dietary dry matter and crude protein use in broilers. According to Wang *et al.* (2016), considering the biochemical functions of several Zn-dependent enzymes and numerous other enzymes, it is possible that high dietary Zn concentrations stimulate enzyme activities

Table 8. Residual effects of experimental diets on body weight gain (BWG, g/hen·day), feed intake (FI, g/hen·day), egg production (EP, %), feed conversion ratio (FCR, kg/dozen) of laying hens from 21 to 32 weeks of age

Factor [1]	BWG	FI	EP	FCR
IBW				
Light-weight	98.84	100.80	68.12B	2.189A
Heavy-weight	79.10	101.64	79.62A	1.769B
MS				
100% Inorganic	71.72	100.60	73.42	1.958
100% Chelated	108.08	101.99	78.29	1.832
50% Chelated	87.11	101.08	69.89	2.149
ANOVA (p value)				
IBW	0.231	0.359	0.001	0.009
MS	0.198	0.449	0.125	0.245
IBW × MS	0.888	0.750	0.461	0.399
CV (%)	63.30	3.10	15.46	26.76

[1] IBW: initial body weight. MS: micromineral source. CV: coefficient of variation. Means followed by different letters in a column differ from each other by the F Test (p<0.05).

Table 9. Residual effects of experimental diets on egg weight (EW, g), eggshell weight (ESW, g), eggshell thickness (EST, mm) and specific gravity (SG) of laying hens at 26 weeks of age

Factor [1]	EW	ESW	EST	SG
IBW				
Light-weight	54.91B	4.90 B	0.38B	1095B
Heavy-weight	56.21A	5.45 A	0.39A	1097A
MS				
100% Inorganic	55.46	5.38 A	0.39	1096
100% Chelated	55.60	5.27AB	0.39	1097
50% Chelated	55.62	4.87 B	0.38	1095
ANOVA (p -value)				
IBW	0.001	0.001	0.030	0.019
MS	0.943	0.010	0.315	0.463
IBW × MS	0.224	0.314	0.597	0.295
CV (%)	4.30	17.19	0.52	8.24

[1] IBW: initial body weight. MS: micromineral source. CV: coefficient of variation. Means followed by different letters in a column differ from each other by the F Test (p<0.05) and Tukey test (p<0.05).

involved in nutrient utilization. The results indicated that it is possible to reduce the dietary level of trace mineral but there is a limit to ensure growth and nutrient metabolizability.

It was observed at 20 weeks of age, that the lighter birds showed higher spleen relative weight and lower abdominal fat weight. However, the mineral source and levels did not affect the organs development. El-Katcha *et al.* (2017) verified that organic Zn or nano Zn supplementation in diets of broilers lymphoid organs weight and improve intestinal villi length, width, and crypt depth of broiler chicken. The decreased of abdominal fat demonstrates the difficult of birds in regain BWG during the rearing phase achieving the sexual maturity with less energetic reserves, which could impact on egg productivity and egg quality.

The TRI was lower in lighter pullets at 17 weeks of age, while the tibia weight, tibia length, and tibia width were lower in lighter hens at 20 weeks of age. These results agree with the lower BW of hens observed at 19 weeks of age. Birds presenting lower initial BW at rearing phase showed lower FI, worse FCR and lower development of tibia. This result suggests that the bird under the ideal weight at onset lay has bones more fragile, and lower Ca reserves to eggshell production. In fact, in the present study we observed lower eggshell weight produced by lighter hens compared to heavier hens.

The residual effect of experimental diets was studied in the production phase of laying hens. The source of mineral had no interference on FI, FCR, BWG and EP, neither when the diets are provided on production phase. Indeed, some experiments indicated that the replacement of dietary inorganic for chelated minerals did not improve FI, BWG and FCR, but enhanced eggshell and bone quality (Manangi *et al.*, 2015; Qiu *et al.*, 2020). These results occur because the amount of inorganic mineral supplemented in diets is enough to ensure bird performance.

The highest initial BW of birds resulted in higher EP and better FCR. These results are according to Ekmay *et al.* (2012), who observed that hens reared to 20% under standard BW achieved sexual maturity later and produced fewer eggs than hens reared on the standard BW curve. Çadirci (2011) verified that FI increases wight increasing BW of layers at onset of lay. In the present study, the FCR was influenced by BW of pullets, but no the FI. The FCR was better to heavy-weight birds because they presented higher EP (79.62 *vs* 68.12%).

There were no residual effect of experimental diets offered at rearing phase on egg weight. Leeson & Caston (2008) also did not verify differences in egg weight produced by laying hens fed inorganic or organic trace mineral.

The eggshell thickness and the SG were not affected by diets. The production eggshell is related to carbonic anhydrase activity, an enzyme dependent of Zn. Zhang *et al.* (2017) cited that dietary Zn supplementation, up to 140 mg/kg feed, could increase eggshell thickness by enhancing carbonic anhydrase activity in the plasma and eggshell gland of aged layers. In addition, Min *et al.* (2018) verified that methionine hydroxyl analog chelated Zn improved Zn and Ca concentrations of eggshells and liver carbonic anhydrase activity. So, we conclude that the low dose of chelated mineral was insufficient to prove the carbonic anhydrase activity.

According to Mabe *et al.* (2003), the concentrations of Mn and Cu were generally much higher in the eggshell compared to the yolk, but levels in the eggshells did not increase with the corresponding increase in the mineral content of the diet. In addition, these authors related that the Zn levels were much lower in the eggshells than observed in the yolk and apparently decreased when hens were fed higher levels of Zn in the diet. When the chelated mineral is fed to birds in production phase, the quality of eggshell is improved (Zhang *et al.*, 2017) and the loss of egg is reduced (Qiu *et al.*, 2020). Therefore the use of organic complexes of Zn and Mn can alleviate the negative effect of hen age on eggshell breaking strength (Swiatkiewicz & Koreleski, 2008). The influence of trace mineral on eggshell structure and quality should me better explained at further studies.

The heavy-weight birds showed higher egg weight, eggshell weight, eggshell thickness, and SG than the lighter birds. According to Lacin *et al.* (2008), lighter birds have lower feed intake and produce lower egg weight.

In summary, probably there are an oversupply of trace mineral from inorganic mineral supplement, since the replacement of inorganic mineral for chelated mineral did not result in decrease of bird performance at both rearing and production phases. The replacement 100% of inorganic mineral for chelated mineral did not result in decrease of bird performance at rearing and at production phase, but there is a minimum limit that should be provided to ensure growth and metabolizability of nutrients.

References

Abdallah AG, El-Husseiny OM, Abdel-Latif KO, 2009. Influence of some dietary organic mineral supplementations. Int J Poult Sci 8: 291-298. https://doi.org/10.3923/ijps.2009.291.298

Ao T, Pierce J, 2013. The replacement of inorganic mineral salts with mineral proteinates in poultry diets. World Poult Sci J 69(1): 5-16. https://doi.org/10.1017/S0043933913000019

Berwanger E, Vieira SL, Angel CR, Kindlein L, Mayer AN, Ebbing MA, Lopes M, 2018. Copper requirements of broiler breeder hens. Poult Sci 97(8): 2785-2797. https://doi.org/10.3382/ps/pex437

Çadirci Ş, 2011. Effect of body weight on feed intake of pullets at the onset of lay. Harran Tarım ve Gıda Bilimleri Dergisi 15(3): 55-59.

Ekmay RD, Salas C, England J, Cerrate S, Coon CN, 2012. The effects of pullet body weight, dietary nonpyhtate phosphorus intake, and breeder feeding regimen on production performance, chick quality, and bone remodeling in broiler breeders. Poult Sci 91(4): 948-964. https://doi.org/10.3382/ps.2011-01931

El-Katcha M, Soltan MA, El-Badry M, 2017. Effect of dietary replacement of inorganic zinc by organic or nanoparticles sources on growth performance, immune response and intestinal histopathology of broiler chicken. Alex J Vet Sci 55(2): 129. https://doi.org/10.5455/ajvs.266925

Hamilton RMG, 1982. Methods and factors that affect the measurement of egg shell quality. Poult Sci 61: 2022-2039. https://doi.org/10.3382/ps.0612022

Klasing KC, 1998. Comparative avian nutrition. CAB Int, Wallingford, UK.

Lacin E, Yildiz A, Esenbuga N, Macit M, 2008. Effects of differences in the initial body weight of groups on laying performance and egg quality parameters of Lohmann laying hens. Czech J Anim Sci 53(11): 466-471. https://doi.org/10.17221/341-CJAS

Leeson S, Caston L, 2008. Using minimal supplements of trace minerals as a method of reducing trace mineral content of poultry manure. Anim Feed Sci Tech 142(3-4): 339-347. https://doi.org/10.1016/j.anifeedsci.2007.08.004

Lilburn MS, Pierson EEM, Robison CI, Karcher D, 2019. Supplemental magnesium in diets for growing pullets and hens: growth, skeletal development, and egg production. J Appl Poultry Res 28(4): 1202-1209. https://doi.org/10.3382/japr/pfz086

Lopes M, Paroul N, Barbosa J, Valduga E, Cansian RL, Toniazzo G, Oliveira D, 2017. Effect of partial and total replacement of inorganic by organic microminerals sources on the quality of broiler carcasses. Braz Arch Biol Technol 60. https://doi.org/10.1590/1678-4324-2017160082

Mabe I, Rapp C, Bain MM, Nys Y, 2003. Supplementation of a corn-soybean meal diet with manganese, copper, and zinc from organic or inorganic sources improves eggshell quality in aged laying hens. Poult Sci 82(12): 1903-1913. https://doi.org/10.1093/ps/82.12.1903

Manangi MK, Vazques-Anon M, Richards JD, Carter S, Knight CD, 2015. The impact of feeding supplemental chelated trace minerals on shell quality, tibia breaking strength, and immune response in laying hens. J Appl Poultry Res 24(3): 316-326. https://doi.org/10.3382/japr/pfv029

Melnychuk VL, Kirby JD, Kirby YK Emmerson DA, Anthony NB, 2004. Effect of strain, feed allocation program, and age at photostimulation on reproductive development and carcass characteristics of broiler breeder hens. Poul Sci 83(11): 1861-1867. https://doi.org/10.1093/ps/83.11.1861

Min YN, Liu FX, Qi X, Ji S, Ma SX, Liu X, Gao AY, 2018. Effects of methionine hydroxyl analog chelated zinc on laying performance, eggshell quality, eggshell mineral deposition, and activities of Zn-containing enzymes in aged laying hens. Poult Sci 97(10): 3587-3593. https://doi.org/10.3382/ps/pey203

Monteagudo MD, Hernandez ER, Seco C, Gonzalez-Riola J, Revilla M, Villa LF, Rico H. 1997. Comparison of the bone robusticity index and bone weight/bone length index with the results of bone densitometry and bone histomorphometry in experimental studies. Acta Anat 160(3): 195-199. https://doi.org/10.1159/000148011

Mwangi S, Timmons J, Ao T, Paul M, Macalintal L, Pescatore A, Dawson KA, 2017. Effect of zinc imprinting and replacing inorganic zinc with organic zinc on early performance of broiler chicks. Poult Sci 96(4): 861-868. https://doi.org/10.3382/ps/pew312

Mwangi S, Timmons J, Ao T, Paul M, Macalintal L, Pescatore A, Dawson KA, 2019. Effect of manganese preconditioning and replacing inorganic manganese with organic manganese on performance of male broiler chicks. Poult Sci 98(5): 2105-2113. https://doi.org/10.3382/ps/pey564

Pereira CG, Rabello CBV, Barros MR, Manso HEC, Santos MJBD, Faria AG, Fireman AK, 2020. Zinc, manganese and copper amino acid complexed in laying hens' diets affect performance, blood parameters and reproductive organs development. Plos One 15(11): e0239229. https://doi.org/10.1371/journal.pone.0239229

Qiu J, Lu X, Ma L, Hou C, He J, Liu B, Xu J, 2020. Low-dose of organic trace minerals reduced fecal mineral excretion without compromising performance of laying hens. As Aust J Anim 33(4): 588. https://doi.org/10.5713/ajas.19.0270

Rostagno HS, Albino LFT, Donzele JL, Gomes PC, Oliveira RF, Lopes DC, *et al.*, 2011. Tabelas brasileiras para aves e suínos. Composição de alimentos e exigências nutricionais, 3rd ed. UFV, Viçosa, MG, Brazil.

Rutz F, Anciuti MA, Xavier EG, Roll VFB, Rossi P, 2007. Avanços na fisiologia e desempenho reprodutivo de aves domésticas. Rev Bras Reprod Anim 31(3): 307-317.

Sakomura NK, Rostagno HS, 2016. Métodos de pesquisa em nutrição de monogástricos. FUNEP, São Paulo, Brazil.

Sarlak S, Tabeidian SA, Toghyani M, Shahraki ADF, Goli M, Habibian M, 2020. Effects of replacing inorganic with organic iron on performance, egg quality, serum and egg yolk lipids, antioxidant status, and iron accumulation in eggs of laying hens. Biol Trace Elem Res: 1-14. https://doi.org/10.1007/s12011-020-02284-8

Silva DJ, Queiroz AC, 2006. Análise de alimentos. Métodos químicos e biológicos, 3rd ed. UFV, Viçosa, MG, Brazil.

Song Z, Zhu L, Zhao T, Jiao H, Lin H, 2009. Effect of copper on plasma ceruloplasmin and antioxidant ability in broiler chickens challenged by lipopolysaccharide. As Aust J Anim 22(10): 1400-1406. https://doi.org/10.5713/ajas.2009.90259

Sousa AMD, Bastos-Leite SC, Goulart CDC, Barroso MLDS, Silva JDB, 2017. Chelated minerals and two limestone particle sizes on production of layers in the second laying cycle. Rev Bras Saude Prod Anim 18(1): 103-112. https://doi.org/10.1590/s1519-99402017000100010

Swiatkiewicz S, Koreleski J, 2008. The effect of zinc and manganese source in the diet for laying hens on eggshell and bones quality. Vet Med-Czech 53(10): 555-563. https://doi.org/10.17221/1966-VETMED

Tavares T, Mourão JL, Kay Z, Spring P, Vieira J, Gomes A, Vieira-Pinto M, 2013. The effect of replacing inorganic trace minerals with organic Bioplex® and Sel-Plex® on the performance and meat quality of broilers. J Appl Anim Res 2: e10. https://doi.org/10.1017/jan.2014.2

van der Klein SAS., Bédécarrats GY, Zuidhof MJ, 2018. The effect of rearing photoperiod on broiler breeder reproductive performance depended on body weight. Poult Sci 97(9): 3286-3294. https://doi.org/10.3382/ps/pey199

Wang Z, Yu H, Wu X, Zhang T, Cui H, Wan C, Gao X, 2016. Effects of dietary zinc pectin oligosaccharides chelate supplementation on growth performance, nutrient digestibility and tissue zinc concentrations of broilers. Biol Trace Elem Res 173(2): 475-482. https://doi.org/10.1007/s12011-016-0654-y

Wang G, Liu LJ, Tao WJ, Xia, ZP, Pei X, Liu BJ, Ao TY, 2019. Effects of replacing inorganic trace minerals with organic trace minerals on the production performance, blood profiles, and antioxidant status of broiler breeders. Poult Sci 98(7): 2888-2895. https://doi.org/10.3382/ps/pez035

Sun W, Wang G, Pei X, Liu L, Xiao Z, Tao W, Pelletier W, 2020. Effects of replacing inorganic with respective complexed glycinate minerals on apparent mineral bioavailability and deposition rate in tissues of broiler breeders. Biol Trace Elem Res 192(2): 654-660. https://doi.org/10.1007/s12011-020-02102-1

Xiao JF, Wu SG, Zhang HJ, Yue HY, Wang J, Ji F, Qi GH, 2015. Bioefficacy comparison of organic manganese with inorganic manganese for eggshell quality in Hy-Line Brown laying hens. Poult Sci 94(8): 1871-1878. https://doi.org/10.3382/ps/pev138

Zhang YN, Zhang HJ, Wang J, Yue HY, Qi XL, Wu SG, Qi GH, 2017. Effect of dietary supplementation of organic or inorganic zinc on carbonic anhydrase activity in eggshell formation and quality of aged laying hens. Poult Sci 96(7): 2176-2183. https://doi.org/10.3382/ps/pew490