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Chelated minerals and two limestone particle sizes on production of layers in the second laying cycle

Minerais quelatados e duas granulometrias de calcário sobre a produção de poedeiras em segundo ciclo produtivo

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SUMMARY

The aim of this experiment was to evaluate the performance, egg quality, and bone quality of commercial white-egg layer hens in the second production cycle fed diets containing organic and inorganic minerals and two limestone particle sizes. A total of 215 birds with an average weight of 1.527 ± 0.092 kg were distributed in a completely randomized design in a 2 × 2 factorial arrangement (two mineral sources and two limestone particle sizes) with six replicates. The following four treatments were tested: T1 = inorganic minerals + 100% fine limestone; T2 = inorganic minerals + 50% fine limestone + 50% coarse limestone; T3 = organic minerals + 100% fine limestone; and T4 = organic minerals+ 50% fine limestone + 50% coarse limestone. No significant interaction occurred between the studied factors for any variable. Egg production, egg mass, bone resistance and bone deformity were higher with organic supplementation. Eggshell weight, percentage of eggshell, and specific gravity were higher in the treatments with the fine+coarse limestone association. In conclusion, organic mineral supplementation improves the performance based on egg production and egg mass. The association between fine and larger-sized limestone improves the external quality of eggs. The use of organic minerals improves the bone quality of birds in the second production cycle.

Keywords: bone quality, calcium sources, egg laying, mineral supplementation, organic mineral

RESUMO

Neste experimento objetivou-se de avaliar o desempenho, qualidade dos ovos e qualidade óssea das poedeiras comerciais leves em 2º ciclo de produção, submetidas às dietas contendo minerais orgânicos e inorgânicos e duas granulometrias do calcário. Foram utilizadas 216 aves, com peso médio de 1,527 ± 0,092 kg, distribuídas em um delineamento inteiramente casualizado em um esquema fatorial 2 X 2 (duas fontes de minerais e duas granulometrias do calcário), com seis repetições. Foram empregados quatro tratamentos a seguir: T1 = minerais inorgânicos + 100% de calcário fino; T2 = minerais inorgânicos + 50% de calcário fino + 50% de calcário grosso; T3 = minerais orgânicos + 100% de calcário fino e T4 = minerais orgânicos + 50% de calcário fino + 50 % de calcário grosso. Não houve interação significativa entre os fatores estudados para nenhuma variável. A produção de ovos, massa de ovo, resistência e deformidade óssea foram melhores com a suplementação orgânica. O peso da casca, percentagem da casca e a gravidade específica foram melhores nos tratamentos com a associação do calcário fino + grosso. Concluiu-se que suplementação mineral orgânica melhora o desempenho com base na produção de ovos e massa dos ovos. A utilização da associação do calcário fino com o de maior granulometria melhora a qualidade externa dos ovos. A utilização de minerais orgânicos melhora a qualidade óssea das aves, em 2º ciclo produtivo.

Palavras–chave: fontes de cálcio, mineral orgânico, postura, qualidade óssea, suplementação mineral

INTRODUCTION

The egg production scenario in the poultry industry has shown some substantial evolution in the last few years, as a consequence of the enhancement of techniques of management, sanitation, nutrition, automation of facilities, and genetic breeding, resulting in increasingly precocious and productive lines. However, despite this evolution, there is a concern about the maintenance of the eggshell quality and the bone structure of layers during the productive cycle, aiming to reduce economic losses due to the egg calcification. In this regard, supplementation of minerals is a necessary practice because of their participation in important biochemical processes that are essential to the growth and development of the bird, especially the processes of bone tissue growth and the egg formation (BRITO et al., 2006).
The use of trace minerals in organic form relatively to the conventional forms has been emphasized, based on the assumption that they can be easily absorbed by birds, improving their egg laying life span. Regarding the participation of minerals in the physiological processes, they act in the activation of several enzymes in the egg-formation process (ARAUJO et al., 2008).The trace minerals zinc, magnesium, and copper act on bone growth and development (NUNES et al., 2013).
Chelated minerals are more easily retained and absorbed by birds (FIGUEIREDO JÚNIOR et al., 2013), improving their performance and reducing environmental contamination by the lower excretion of minerals (NOLLET et al., 2007) that are potentially pollutant. However, research results are still controversial (BRITO et al., 2006).
Nutritional deficiencies in layers can lead to a reduction of productivity and low egg shell quality occurring a frequently mobilization of bone calcium (ALMEIDA PAZ et al., 2009). Thus, a good uptake of dietary calcium is recommended for birds in the laying period, because it is targeted towards the egg calcification. In addition to the dietary calcium level, questions have emerged about the particle size of the calcium source utilized. In this context, it is arguing that limestone particles should be large enough to be retained in the gizzard of the birds for a longer period, allowing the release and absorption of calcium (AMERAH et al., 2008).
Therefore, this study was conducted aiming to evaluate the effect of organic minerals and two particle size of limestone on the productive performance, egg quality, and bone quality of commercial layers in the second production cycle.

MATERIAL AND METHODS

The experiment was conducted in the poultry section of the Experimental Farm at UVA, located in Sobral - CE, Brazil. The experimentation animal protocol of number nº 00212 was approved by the Institutional Committee of Ethics on Animal Use – CEUA/UVA. A total of 216 Hy-Line White layer hens with an average weight of 1.512 ± 0.051 kg, at 88 weeks of age, were used in the experiment. The experimental period was 112 days, divided into four 28-day periods. A completely randomized experimental design was adopted, in a 2 × 2 factorial arrangement, with six replicates of nine birds.

The birds were subjected to forced molting by the feed-deprivation method, according to the procedures indicated in the manual of the line (HY LINE DO BRASIL, 2011).

The experimental diets were formulated according to the requirements suggested by Rostagno et al. (2011) as shown in Table 1.

The following treatments were employed: T1 = inorganic minerals (Fe, Cu, Zn, Mn, I, Se) + and 100% fine limestone; T2 = inorganic minerals + 50% fine limestone + 50% coarse limestone; T3 = organic minerals (Cu, Mn, and Zn;and the other minerals in inorganic form) + 100% fine limestone; and T4= organic minerals + 50% fine limestone + 50% coarse limestone. As to the particle size, the samples were classified, based on the geometric diameter (GDM), as fine (GDM 0.35 mm) and coarse (GDM 2,790 mm).

Table 1. Centesimal and calculated nutritional composition of the experimental diet

Ingredient	Quantity (%)			
	T1	T2	T3	T4
Corn grain	59.750	59.750	59.750	59.750
Soybean meal (45%)	27.182	27.182	27.182	27.182
Soybean oil	1.502	1.502	1.502	1.502
Dicalcium phosphate	1.105	1.105	1.105	1.105
Fine limestone	9.539	4.769	9.539	4.769
Coarse limestone	-	4.769	-	4.769
Common salt	0.523	0.523	0.523	0.523
Methionine	0.0613	0.0613	0.0613	0.0613
Egg-laying premix 0.4% 500TEC**	0.4	0.4	-	-
Egg-laying premix 0.4% QLT*	-	-	0.4	0.4
Calculated nutritional composition				
Metabolizable energy (kcal/kg)	2.800	2.800	2.800	2.800
Crude protein (%)	17	17	17	17
Calcium (%)	4.02	4.02	4.02	4.02
Available phosphorus (%)	0.3	0.3	0.3	0.3
Sodium (%)	0.225	0.225	0.225	0.225
Total methionine + cystine (%)	0.98	0.98	0.98	0.98
Total methionine (%)	0.739	0.739	0.739	0.739
Total lysine (%)	0.812	0.812	0.812	0.812

* 7278 - Egg-laying premix II 0.4% QLT (Chelated),[1]Mineral-vitamin supplement per kg of organic premix: copper amino acid chelate: 2,500.00 mg/kg; manganese amino acid chelate: 30,000.00 mg; calcium iodate: 250.00 mg; zinc aminoacid chelate: 25,000.00 mg; ferrous sulfate: 12,500.00 mg; cobalt sulfate: 25.00 mg; sodium selenite: 75.00 mg; vitamin A: 2,000,000.00 IU; vitaminD3: 750,000.00 IU; vitamin E: 3,000.00 mg; vitamin K3: 500.00 mg; vitamin B1: 250.00 mg; vitamin B2: 1,250.00 mg; niacin: 5,000.00 mg; pantothenic acid: 1,750.00 mg; vitaminB6: 500.00 mg; folic acid: 100.00 mg; biotin: 5.00 mg; vitamin B12: 3,000.00 mcg; cholinechloride: 100,000.00 mg; methionine: 125,000.00 mg; colistin sulfate: 1,750.00 ppm.

**7422 - PX POSTURA 0.4% 500 TEC (Inorganic),[2]Mineral-vitamin supplement per kg of organic premix: ferrous sulfate: 10.00 g; copper sulfate: 2,500.00 mg; zinc sulfate: 25.00 g; manganêse monoxide: 20.00 g; calcium iodate: 208.00 mg; sodium selenite: 75.00 mg; vitamin A: 1,750,000.00 IU; vitamin D3: 625,000.00 IU; vitamin E: 2,000.00 mg; vitamin K3: 395.00 mg; folic acid: 74.00 mg; choline: 75.00 g; niacin: 5,025.00 mg; pantothenic acid: 1,805.00 mg; vitamin B1: 250.00 mg; vitamin B2: 1,000.00 mg; vitamin B6: 250.00 mg; vitaminB12: 2,400.00 mcg; methionine: 125.00 g; colistin: 1.750.00 ppm.

The premixes utilized in the study were two distinct commercial products. The premix with organic minerals contained only the following three chelated minerals: copper amino acid chelate, manganese amino acid chelate, and zinc amino acid chelate; all the other minerals were in inorganic form.

Intake and egg-production data were recorded throughout the experiment, to obtain the data of performance variables. These parameters were 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 every 28-day period, four eggs were collected per replicate for the analyses of egg quality, in which the percentages of albumen, yolk, and shell, in addition to shell thickness (mm), egg specific gravity (g/cm^3), broken and cracked eggs (%) were evaluated.

At the end of the experiment, one bird was chosen at random per replicate, identified, and sacrificed by cervical dislocation. After the sacrifice, the drumsticks and thighs were removed and stored in a freezer at –20ºC, where they remained until the moment of deboning.

For the deboning, the pieces were removed from the freezer and left to thaw on the platforms until they reached room temperature. Subsequently, the drumsticks and thighs were immersed in boiling water for ten minutes, and then deboned following the method described by Bruno (2002).

To evaluate bone quality, only the tibiae were used. The bone length was measured using a caliper, and the weight was obtained using an electronic scale with 0.01 g precision. Bone density was evaluated by the Seedor index, calculated by dividing the weight (mg) by the length (mm) of the evaluated bone (SEEDOR et al., 1991).

Analyses of bone resistance and bone deformity were conducted in the Laboratory of Soil Mechanics at the Department of Transportation Engineering at the Federal University of Ceará, using a mechanical press. The bones (left tibiae) were placed horizontally, with their extremities on a wooden platform, and then a compression force was applied on the center of each bone. The maximum strength applied on the bone to its breakage was considered the tensile strength (kgf/cm^2), which was measured by a digital strain gauge. The deformity (mm) was measured using an analogical strain gauge up to the breakage of the bone.

The ash content was determined at the Laboratory of Animal Nutrition (LANUT) of UVA. After deboning, the tibiae (right side) were weighed and dried in a forced-air oven at 105 ºC for 72 h. Next, they were ground with a mortar and a pestle. Ground samples were conditioned in labeled plastic bags for later determination of the mineral matter (MM) content, according to the method described by Silva & Queiroz (2002).

The statistical analyses of the data were performed using the software Statistical Analysis System (SAS, 2000), adopting 5% as the significance level. The data were subjected to analysis of variance (ProcAnova, SAS), and subsequently to a factorial model, in which the effects of treatments, mineral sources, and limestone particle size, and the interaction between the factors were included. Means were compared by Tukey's test.

RESULTS AND DISCUSSION

No significant interaction was observed (P>0.05) between the studied factors for any of the performance variables (Table 2). Likewise, no significant effect

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(P>0.05) of the types of mineral supplements or particle size of limestone was observed on the variables feed intake, egg weight, and conversion per egg mass and per egg dozen (Table 2).

The egg-production and egg-mass parameters were influenced by the mineral supplements, with the best results provided by organic supplementation. These results are assumed to be related to the bioavailability of the chelated minerals, because they are more easily absorbed and retained by the birds, which may bring benefits to performance like the above mentioned (KIEFER, 2005). The results are similar to those observed by Figueiredo Júnior et al. (2013), who studied the effect of organic mineral levels on the performance of layers and found increased production and egg mass compared with inorganic sources.

Table 2. Feed intake (FI), egg production (EP), average egg weight (AEW), egg mass (EM), and feed conversion per egg mass (FCEM) and per dozen eggs (FCDZ) of commercial layers in the 2nd production cycle fed diets with inorganic or organic minerals and two limestone particle sizes

Treatment	FI (g/bird/day)	EP (%)	AEW (g)	EM (g/bird/day)	FCEM (kg/kg)	FCDZ (kg/kg)
Mineral source						
Inorganic	90.75	73.52[B]	70.88	52.15[B]	1.734	1.471
Organic	89.16	77.88[A]	70.87	55.22[A]	1.650	1.402
Limestone						
Fine	89.41	75.71[a]	71.15	53.91[a]	1.667	1.452
Fine + Coarse	90.50	75.68[a]	70.59	53.46[a]	1.717	1.421
Mean	89.95	75.70	70.87	53.68	1.692	1.437
SEM	0.58	0.62	0.27	0.44	0.016	0.013
AnalysisVariance						
Mineral source (MS)	0,2601	0,0024	0,9927	0,0095	0,0992	0,0667
Limestone (L)	0,4344	0,9814	0,4240	0,6816	0,3132	0,4027
MS x L	0,8584	0,5373	0,6366	0,4533	0,7439	0,8375

Working with organic minerals on the performance of brown-egg layers in the second production cycle, Saldanha et al. (2009) also did not observe differences in percentage of eggs, or feed conversion per kg and dozen eggs, corroborating the results found in the present study. However, Maciel et al. (2010) observed a greater egg weight, higher specific weight and lower percentage of egg loss of commercial laying hens at the end of laying and concluded that benefits are obtained with inclusion of 50% zinc, manganese and copper in organic form.

Better results were expected for intake with the treatments that involved the association with the limestone particles, because older birds prefer larger particles (SCOTTÁ et al., 2014), and when they ingest these they also consume the smaller feed particles, improving their intake as a whole, which did not occur in the present study. Similar results were reported by Saunders-Blades et al. (2009), who stated that the particle size of the calcium source does not significantly influence feed intake, and consequently performance, since birds fed diets with different limestone particle sizes consume enough nutrients for good performance.

No significant interaction was observed between the studied factors for any of the egg-quality variables. Thus, likewise, no effect of the two mineral sources and particle sizes was detected on the variables percentage of albumen, percentage of yolk, shell thickness, broken eggs, and cracked eggs. However, the variables percentage of eggshell, and egg specific gravity were influenced by the limestone particle sizes (Table 3).

Table 3. Percentage of albumen (PA), percentage of yolk (PY), percentage of eggshell (PE), shell thickness (ST), specific gravity (SG), broken eggs (BE), and cracked eggs (CE) of commercial layers in the 2nd production cycle fed diets with inorganic or organic minerals and two limestone particle sizes

Treatment	PA (%)	PY (%)	PE (%)	ST (mm)	SG (g/cm^3)	BE (%)	CE (%)
Inorganic mineral	61.66	26.20	8.76^A	0.371	1.085^A	1.78	1.30
Organic mineral	61.55	26.05	8.59^A	0.367	1.084^A	1.98	1.35
Fine limestone	61.96	25.96	8.47^b	0.364	1.082^b	1.98	1.32
Fine + Coarse limestone	61.24	26.29	8.88^a	0.373	1.086^a	1.79	1.32
Mean	61.60	26.13	8.67	0.369	1.084	1.88	1.32
SEM	0.17	0.16	0.05	0.02	0.004	0.05	0.07
AnalysisVariance							
Mineral source (MS)	0,7973	0,7359	0,0715	0,4798	0,4722	0,1882	0,5081
Limestone (L)	0,0985	0,4631	0,0108	0,0742	0,0001	0,1975	0,9417
MS x L	0,4208	0,9296	0,2230	0,5141	0,7501	0,2457	0,7294

Considering the reduction of calcium retention as the birds age, the obtained results were presumably due to the solubility and larger particle size of this mineral retained in the mechanical stomach (gizzard), which allowed the calcium to be utilized at night, during the period of formation of the eggshell, when the layers cease to consume the feed.

This finding corroborates Ito et al. (2006), who concluded that the use of limestone with mixed or coarse particle sizes improves eggshell quality.

Opposite results were found by Jardim Filho et al. (2005), who did not observe the effect of limestone particle size on the eggshell quality of layers during the period of peak production. Likewise, Murata et al. (2009) concluded that the associates of limestone powder with limestone gravel did not have an effect on the studied parameters; these authors worked with calcium levels and limestone particle sizes on the quality of eggs from layers in the first cycle.

Although organic mineral sources have higher availability, providing better performance when consumed, in the present study no improvement was detected regarding the internal or external egg quality. In the literature, the results for the use of chelated minerals are highly controversial as to the improvement of shell quality and internal egg quality. This fact can be explained by the quantity of several chelated molecules present and

their differences in bioavailability and stability as well as their metabolism (SECHINATO et al., 2006).
No significant interaction between the studied factors was found on the bone-related variables analyzed in this study. The bone resistance and deformity parameters were influenced significantly by the mineral supplements, with the best results obtained by organic supplementation (Table 4).

Table 4. Seedor Index (SI), bone resistance (BR), bone deformity (BD), and tibia ash (ASH) of commercial layers in the 2nd production cycle fed diets with inorganic or organic minerals and two limestone particle sizes

Treatment	SI (mg/mm)	BR (kg/cm^2)	BD(mm)	ASH (%)
Inorganic mineral	66.39	5.32^B	2.45^B	48.28
Organic mineral	67.34	7.15^A	3.37^A	49.88
Fine limestone	67.36	6.22^a	2.89^a	50.59
Fine + Coarse limestone	66.26	6.36^a	2.99^a	48.84
Mean	66.88	6.28	2.93	49.59
SEM	0.98	0.34	0.09	0.82
AnalysisVariance				
Mineral source (MS)	0,7088	0,0110	0,0001	0,7331
Limestone (L)	0,6714	0,8294	0,5644	0,2328
MS x L	0,1546	0,5324	0,1074	0,9257

Concerning the better results obtained for bone resistance and deformity in the treatments with organic supplementation, it is assumed that these results are due to the bioavailability of the chelated minerals, because they are more easily absorbed and retained by the birds (BRITO et al., 2006), and so they may bring benefits to bone quality such as the abovementioned examples.
Results similar to those found in the present study for bone resistance were reported by Nunes et al. (2013), working with increasing levels of Cu, Mn, Zn, and Fe proteinates for brown-egg layers in the period of 30 to 70 weeks of age. Brito et al. (2006), working with organic and inorganic minerals in diets for replacement pullets aged 7 to 12 weeks, reported that the treatments did not influence the ash content of the birds' tibia, which are similar results to those obtained in this experiment.
Oliveira et al. (2013), working with increasing levels of coarse limestone in diets for white-egg layers, reported that the limestone particle sizes did not influence the Seedor Index. These results are similar to those obtained in the current study.
In conclusion, the supplementation with organic minerals improves the performance and bone quality based on egg production, egg mass, bone resistance, and bone deformity. The association between fine and coarse limestone improves the external quality of eggs from layer hens in the second production cycle.

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