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Chelated Minerals and Limestone Particle Sizes on Performance and Bone Quality of Brown-Egg Layers

Author(s)

Cordeiro CN[I]
Bastos-Leite SC[II]
Vasconcelos FC[III]
Goulart CC[II]
Sousa AM[III]
Costa AC[IV]

[I] Zootecnista formada pela Universidade Estadual Vale do Acaraú – UVA.
[II] Docente do Curso de Zootecnia da Universidade Estadual Vale do Acaraú – UVA
[III] Mestrando do Programa de Pós-Graduação em Zootecnia pela Universidade Estadual Vale do Acaraú – UVA.
[IV] Graduando em Zootecnia na Universidade Estadual Vale do Acaraú - UVA.

Mail Address

Corresponding author e-mail address
Carla Nágila Cordeiro
Av João Jaime Ferreira Gomes Filho, 213, Centro, 62580-000, Acaraú-CE, Brazil. 62580-000
Tel: (55 88) 997128524
Email: carlinha_nagila@hotmail.com

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ABSTRACT

This study aimed to evaluate productive performance, egg quality, and bone quality of commercial brown-egg layers fed diets containing organic and inorganic minerals and three limestone particle sizes. A total of 288 birds at 64 weeks of age were distributed in a completely randomized design in a 2 × 3 factorial arrangement (two mineral sources and three limestone particle sizes) and eight replicates. The experiment lasted 112 days, divided into four periods. The following treatments were tested: T1 = inorganic minerals + 100% fine limestone; T2 = inorganic minerals + 50% fine limestone + 50% coarse limestone; T3 = inorganic minerals + 100% coarse limestone; T4 = inorganic + organic minerals + 100% fine limestone; T5 = inorganic + organic minerals + 50% fine limestone + 50% coarse limestone; and T6 = inorganic + organic minerals +100% coarse limestone. There was no significant interaction effect between the studied factors on any of the variables. No significant effect of the types of mineral mixes or limestone particle sizes were observed on the performance, egg quality, or bone quality variables evaluated. In conclusion, organic or inorganic minerals associated with limestone in fine, medium, or coarse particle sizes can be used in diets for brown-egg layers without affecting their productive performance, egg quality, or bone quality.

INTRODUCTION

In the last few years, the poultry industry has stood out for the level of technologies employed in the production of quality foods. Recent advances in animal nutrition are responsible for the considerable growth in this segment. In high-productivity systems like this one, any flaws in the formulation of poultry diets may lead to low efficiency, with economic losses.

Mineral supplements are usually added to balanced diets, since the ingredients utilized in larger proportions (corn and soybean) do not have sufficient amounts of these elements to meet the nutritional requirements of animals. Microminerals participate in several biochemical processes essential to animal growth and development, which makes their supplementation of great importance in the diet (Araujo *et al*., 2008), either in organic or inorganic form. Organic minerals feature a greater bioavailability in relation to usual sources (Maciel *et al*., 2010), and are thus readily absorbed and retained by birds, benefiting their productive performance, immune system and lifespan; besides reducing the excretion of trace minerals, which are potential pollutants (Brito *et al*., 2006).

In addition to demonstrating greater stability, organic minerals benefit from a biochemical protection against the different reactions that can take place in the diet itself, which would cause an expressive decrease in nutrient absorption rates (Close, 1998). According to Pessoa *et al*. (2012), organic minerals present specific actions at cellular level and a greater bioavailability in relation to inorganic minerals. Thus, the use of these minerals in the composition of poultry diets may result in better performance for these animals.

The eggshell provides protection against microbial invasion and controls the water and gas exchange through the pores present in it´s structure (Rodriguez-Navarro *et al*., 2002). Thus, shells with lower quality than desired may lead to contamination of the important content (yolk and albumen), compromising the dietary health of consumers.

In this way, there is a great concern with the integrity of eggshells and with their loss of quality throughout the productive life of layers. This decline in quality is due to factors like lower calcium retention (Pappas *et al*., 2006) and increased egg weight without a proportional increase in shell weight (Miles, 2008), since the daily deposition of calcium in the eggshell corresponds to 10% of the total stored in the bird´s body (Ebling *et al*., 2012).

The shell formation process normally occurs during the night period, when birds do not have a labile calcium source in the digestive tract (Bueno, 2013), requiring it´s partial removal from the bones. Therefore, it is argued that the limestone in larger particles remains retained in the gizzard until the release of calcium for the formation of the eggshell (Murata *et al*., 2009), thereby allowing it´s slow release, making it available for the formation of the shell during the time birds are not feeding.

Calcium has a prominent position in layer diets, not only for being the main component of the eggshell, but also because of its considerable value in the bone composition of these birds. Recent research on the bone mineral density of poultry is of great relevance for the laying-poultry segment, aiming at their productive longevity. The bone mineral density can be evaluated by many techniques, e.g., the bone mineral composition, bone resistance, and the Seedor index (Almeida Paz *et al*.,2009).

In this regard, some alternatives have been proposed to improve eggshell quality, such as the use of organic minerals and the use of limestone with different particle sizes in diets. The present study was thus developed to evaluate the effect of organic minerals and three limestone particle sizes on the productive performance, egg quality, and bone quality of commercial brown-egg layers.

MATERIAL AND METHODS

The experiment was conducted in the Poultry Unit of the Experimental Farm at Universidade Estadual Vale do Acaraú - UVA, located in Sobral - CE, Brazil, consisting of four 28-day cycles. A total of 288 Hy-Line Brown layers, at 64 weeks of age, with an average body weight of 1.88 ± 0.036 kg, were used in the trial.

The experimental design was completely randomized, in a 2 × 3 factorial arrangement (two types of mineral supplement and three limestone particle sizes), totaling six treatments, with eight replicates and six birds per experimental unit. Experimental diets were iso-nutrient and iso-caloric (Table 1), formulated according to the nutritional requirements suggested by the manual of the line (Hy-Line do Brasil, 2013). The following treatments were tested: T1 = inorganic minerals + 100% fine limestone; T2 = inorganic minerals + 50% fine limestone + 50% coarse limestone; T3 = inorganic minerals + 100% coarse limestone; T4 = inorganic + organic minerals + 100% fine limestone; T5 = inorganic + organic minerals + 50% fine limestone + 50% coarse limestone; and T6 = inorganic + organic minerals +100% coarse limestone. As for the particle size, according to the geometric diameter (Dg) of limestone particles, samples were classified as fine (Dg 0.35 mm), medium (Dg 0.827 mm), and coarse (Dg 1.762 mm) (Zanotto & Bellaver, 1996).

Birds were fed daily and had production recorded to generate performance data, based on which the following variables were evaluated: feed intake (g/bird/day), egg production (egg/bird/day), 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 28-day period, four eggs were collected per replicate for the analysis of egg quality, in which the percentages of albumen, shell, yolk, and cracked eggs; shell thickness (mm); and specific gravity (g/cm3) were determined, following the methodology described by Bezerra *et al*. (2015).

At the end of the experiment, one bird was taken at random per replicate and sacrificed by cervical dislocation for removal of drumsticks and thighs, which were frozen at −20 ºC until deboning.

For the deboning procedure, the pieces were removed from the freezer and left to thaw on countertops until they reached room temperature. Afterwards, drumsticks and thighs were immersed in boiling water for 10 min and then deboned, following the methodology described by Bruno *et al* (2007).

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

Bone resistance and deformity were analyzed at the Laboratory of Soil Mechanics at the Department of Transport Engineering of Universidade Federal do Ceará, using a mechanical press. Left tibiae were placed in the horizontal position, and a compression force was applied to the center of each bone. The maximum force applied to the bone until it´s rupture was on it´s breaking strength (kgf/cm²), measured using an electronic extensometer. Deformity (mm) was measured using an analogical extensometer until the moment of bone rupture.

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

The average of the variables of the four evaluated periods were considered. The results obtained, with the use of the two mineral sources tested, were subjected to analysis of variance (5%) by the F test; whereas the mean values for the different particle sizes were compared by Tukey's test at 5% (SAS, 2000) and subsequently analyzed by a factorial model including the effects of treatments, mineral sources, limestone particle sizes, and the interaction between the factors.

RESULTS AND DISCUSSION

No significant interaction was observed between the studied factors for any of the performance variables assessed. Likewise, there were no significant effects of the types of mineral supplement and the three limestone particle sizes on the following variables: feed intake, egg production, egg weight, egg mass,

Table 1 – Centesimal and calculated nutritional composition of experimental diets.

Ingredient	Quantity (%)					
	T1[1]	T2[1]	T3[1]	T4[1]	T5[1]	T6[1]
Ground corn	57.472	57.472	57.472	57.402	57.402	57.402
Soybean meal (45%)	27.398	27.398	27.398	27.398	27.398	27.398
Soybean oil	1.750	1.750	1.750	1.750	1.750	1.750
Dicalcium phosphate	1.416	1.416	1.416	1.416	1.416	1.416
Common salt	0.363	0.363	0.363	0.363	0.363	0.363
DL-methionine	0.149	0.149	0.149	0.149	0.149	0.149
Fine limestone (Dg =0.35mm)	11.052	5.526		11.052	5.526	
Coarse limestone (Dg =1.762mm)		5.526	11.052		5.526	11.052
Layer Premix 0.4% 500TEC*	0.400	0.400	0.400	0.400	0.400	0.400
Layer Premix 0.07% QLT**				0.070	0.070	0.070
Calculated Nutritional Composition						
Metabolizable energy (Cal/kg)	2750	2750	2750	2750	2750	2750
Crude protein (g/kg)	17.50	17.50	17.50	17.50	17.50	17.50
Calcium (g/kg)	4.70	4.70	4.70	4.70	4.70	4.70
Available phosphorus (g/kg)	0.36	0.36	0.36	0.36	0.36	0.36
Sodium (g/kg)	0.18	0.18	0.18	0.18	0.18	0.18
Chlorine (%)	0.25	0.25	0.25	0.25	0.25	0.25
Dig. methionine + cystine (g/kg)	0.690	0.690	0.690	0.690	0.690	0.690
Dig. methionine (g/kg)	0.449	0.449	0.449	0.449	0.449	0.449
Lysine (g/kg)	0.810	0.810	0.810	0.810	0.810	0.810

[1]T1 = inorganic minerals + 100% fine limestone; T2 = inorganic minerals + 50% fine limestone + 50% coarse limestone; T3 = inorganic minerals + 100% coarse limestone; T4 = inorganic + organic minerals + 100% fine limestone; T5 = inorganic + organic minerals + 50% fine limestone + 50% coarse limestone; and T6 = inorganic + organic minerals + 100% coarse limestone.

*PREMIX - 0.4% 500 (inorganic) - guaranteed levels (composition per kg of product): iron (min) 10.00 mg/kg; copper (min): 2,500.00 mg/kg; zinc (min): 20.00 g/kg; manganese (min) 20.00 mg/kg; iodine (min) 208.00 mg/kg; selenium (min): 75.15 mg/kg; vitamin A (min): 2,000,000.00 IU/kg; vitamin D3 (min): 625,000.00 IU/kg; vitamin E (min): 3,000.00 IU/Kg; vitamin K3 (min): 395.92 IU/kg; folic acid (min): 74.25 mg/kg; choline (min): 100.00 g/kg; niacin (min): 5,025.74 mg/kg; pantothenic acid (min) 1,805.16 mg/kg; vitamin B1 (min): 250.09 mg/kg; vitamin B2 (min): 1,000.00 mg/kg; vitamin B6 (min): 250.1mg; vitamin B12 (min): 2,400.00mcg/kg; methionine (min): 125,00 g/kg; colistin (min): 1,750.00 mg/kg.

**PREMIX - 0.07% QLT (chelated) - guaranteed levels (composition per kg of product):copper (min) 7,143.00 mg/kg; zinc (min) 57.00 g/kg; manganese (min) 57.00 g/kg; vitamin A (min) 1,428,600.00 IU/kg; vitamin D3 (min) 1,428,550.00 IU/kg; vitamin E (min) 5,714,000.00 IU/kg.

conversion per egg mass, or conversion per dozen eggs (Table 2). In this way both mineral supplements employed here, show to be adequate to maximize these productive parameters with no additional effects of the use of chelated minerals in the diets.

The results of the present study corroborate those reported by Fernandes *et al*. (2008), Maciel *et al*. (2010), Passos *et al*. (2011), and Dikmen *et al*. (2015), who, working with chelated minerals in the feeding of layers, similarly did not find positive effects of their addition on poultry performance. Studies conducted by Ito *et al*. (2006) using two limestone particle sizes, reported no differences in feed intake, feed conversion, egg production, egg weight, or mass of eggs produced.

By contrast, Figueiredo Junior *et al*. (2013) worked with supplementation of 33, 66, and 100% of substitution of inorganic minerals, by organic minerals in diets for brown-egg layers at 68 weeks of age and reported that better results for production, egg weight, and conversion per mass and per dozen were found when organic minerals replaced the inorganic by 66%.

Regarding the limestone particle sizes, similar results were obtained by Murata *et al*. (2009), who used 0, 25, 50, 75, and 100% coarse limestone to substitute the fine version, in the feeding of commercial layers. The variables: egg production, feed conversion, and egg weight were not influenced by the particle sizes tested. Similarly, working with limestone in fine, coarse, and gravel sizes in diets for white-egg layers, Jardim Filho *et al*. (2005) reported that they did not influence performance variables.

Conflicting results were reported by Garcia *et al*. (2012), who worked with five levels of inclusion of coarse limestone, replacing it´s fine version (0, 25, 50, 75, and 100%) in diets for brown-egg layers at 53 weeks of age; and observed that intake, egg production, and egg mass decreased as the inclusion of coarse limestone in the diet was increased.

Better results were expected for intake in the treatments that involved the limestone in coarse particles, because older birds prefer larger particles, and, when they ingest them, they would do so also with the smaller particles of the diet, improving their intake as a whole, which was not observed. The limestone particle sizes were not sufficient to influence intake, probably due to the good intake capacity shown by the birds, demonstrating that after the initial adaptation, brown-egg layers normalize their intake throughout the production cycle.

No significant interaction was observed between the studied factors on any of the egg-quality variables evaluated. Likewise, there was no significant effect of the two mineral sources and limestone particle sizes on the variables percentage of albumen, yolk and shell; shell thickness, specific gravity or percentage of cracked eggs (Table 3).

Although the organic mineral sources have a greater availability (Pessoa *et al*., 2012), promoting better performance when ingested; in the present study, no improvement was recorded with regards to the internal or external quality of the eggs. In the literature, results for the use of chelated minerals are highly controversial as to their influence on the egg´s

Table 2 – Mean values referring to performance parameters of brown-egg layers fed diets containing inorganic or organic minerals and three limestone particle sizes.

Factors	Variables					
	FI (g/hen/day)	EP (%)	Egg weight (g)	EM (g/hen/day)	FC/EM (kg/kg)	FC/DZ (kg/kg)
Minerals						
Inorgânic	111,11	78.33	64.86	50.80	2.158	1.673
Inorg+Organic	109,81	78.09	64.40	50.40	2.163	1.699
Limestone						
Fine	108,44	77.62	64.60	50.20	2.155	1.691
Fine+Coarse	111,19	77.44	64.16	49.79	2.193	1.705
Coarse	111,76	79.58	65.15	51.82	2.132	1.661
CV² (%)	5,72	4.44	2.36	4.81	5.33	5.23
Mean	110,46	78.21	64.64	50.60	2.160	1.686
ANOVA²	p-value					
Minerals (M)	0,4817	0,8126	0,3011	0,5725	0,8964	0,3314
Limestone (L)	0.2934	0,1690	0,1995	0,0553	0,4088	0,4102
MxL	0.2597	0,7440	0,6829	0,6060	0,7745	0,8090

[1]CV- coefficient of variation; FI - feed intake; EP - egg production; EM - egg mass; FC/EM-feed conversion per egg mass; FC/DZ- feed conversion per dozen eggs.

[A, B]Means followed by different uppercase letters in the row differ statistically by Tukey's test at 5%.

[a, b]Means followed by different lowercase letters in the row differ statistically by Tukey's test at 5%.

Table 3 – Mean values referring to quality of eggs from commercial brown-egg layers fed diets with inorganic or organic minerals and three limestone particle sizes.

Factores	Variables					
	Albumen (%)	Yolk (%)	Shell (%)	ST[2] (mm)	SG[3] (g/cm³)	Cracked eggs (%)
Mineral						
Inorganic	62.98	25.38	9.82	0.384	1.096	1.23
Organic	62.87	25.08	9.87	0.384	1.096	1.22
Limestone						
Fine	63.07	25.26	9.90	0.386	1.096	1.24
Fine+Coarse	62.58	25.46	9.86	0.384	1.097	1.19
Coarse	63.13	24.97	9.77	0.381	1.095	1.26
CV[1] (%)	2.34	3.92	3.41	3.32	0.183	19.26
Mean	62.92	25.23	9.84	0.384	1.096	1.23
ANOVA²	p-value					
Minerals (M)	0,7651	0,2974	0,6473	0,9961	0,5823	0,9724
Limestone (L)	0,5021	0.3856	0,5378	0,6223	0,5328	0,6924
MxL	0,4099	0.6584	0,7649	0,8352	0,6028	0,5706

[1]CV- coefficient of variation;[2] ST - shell thickness; [3] SG-specific gravity.

[A, B] Means followed by different uppercase letters in the row differ statistically by Tukey's test at 5%.

[a, b] Means followed by different lowercase letters in the row differ statistically by Tukey's test at 5%.

quality. This fact can be explained in part, by the large variety of chelated molecules existing and their differences in bioavailability and stability as well as their metabolism in the animal´s body (Maciel *et al*., 2010).

Lack of effects of chelated minerals on the egg´s quality parameters was also reported by Swiatkiewicz & Koreleski (2008), Saldanha *et al*. (2009), and Geraldo *et al*. (2012). On the contrary, Maciel *et al*. (2010), working with layers at the end of their laying period, using diets containing 50% organic mineral supplementation, associated (Zn+Cu+Mn) or isolated, concluded that associated supplementation provided better results than zinc, copper, or manganese separately, as it resulted in lower loss of eggs, greater specific gravity and heavier eggs.

Working with brown-egg layers fed diets with and without organic minerals, Figueiredo Júnior *et al*. (2013) reported that the percentage of albumen, specific gravity and shell thickness had better results when inorganic minerals were replaced with organic minerals by 66%.

The quality variables were not influenced by any of the limestone particle sizes tested. Better results were expected for percentage of shell, shell thickness and specific gravity in the treatments with coarse limestone, since this particle size was assumed to provide more calcium for the formation of the shell, due to it´s longer permanence in the tract, thereby improving its quality, which was not observed in this experiment. Probably, for presenting a greater feed intake capacity (Jardim Filho *et al*., 2005), the brown-egg layers consequently consumed a sufficient amount of nutrients for the formation of the eggshell, irrespective of the limestone particle size utilized in the diet.

Additionally, literature reports describe that in situations in which diets have appropriate calcium levels, coarse limestone is not necessarily essential to the supply of this mineral (Witt *et al*., 2009).

Working with limestone in five particle sizes in the feeding of white-egg layers, Murata *et al*. (2009) concluded that the use of granulated limestone did not benefit the egg quality, which this study corroborates. Similar results were reported by Garcia *et al*. (2012), who increasingly replaced finely ground limestone (0.145 mm) by its coarse form (3.18 mm) in the diet of layers at the end of production and concluded that particle size did not affect the internal or external egg quality.

The results of the present study differ from those presented by Lemos *et al*. (2011), who evaluated the external quality of eggs from brown-egg layers and observed that the coarse limestone particle size (2 mm) improved the percentage of the eggshell, thereby improving the egg quality. Jardim Filho *et al*. (2005) also observed improved eggshell quality after feeding the birds with coarse limestone (2.00 mm).

No significant interaction was observed between the studied factors for any of the bone-quality variables. Similarly, there was no significant effect of either mineral source or limestone particle size on the following variables: tibia weight, length, Seedor index, resistance, deformity and mineral matter (Table 4).

Table 4 – Mean values for weight, length, Seedor index (SI), resistance, deformity and mineral matter of tibiae from brown-egg layers fed diets containing inorganic or organic minerals and three limestone particle sizes.

Factores	Variables					
	Weight (g)	Length (mm)	SI (mg/mm)	Resistance (kgf/cm²)	Deformity (mm)	Mineral matter (g/kg)
Mineral						
Inorganic	10.59	118.74	89.09	5.04	2.29	43.56
Organic	10.21	120.80	84.32	5.07	2.17	45.27
Limestone						
Fine	10.63	120.82	87.98	5.76	2.41	44.34
Fine+Coarse	10.19	119.03	85.38	4.26	2.15	43.79
Coarse	10.37	119.46	86.75	4.97	2.11	45.12
CV (%)	11.64	3.17	9.59	25.69	24.42	9.72
Mean	10.40	119.77	86.70	5.06	2.23	44.41
ANOVA²	p-value					
Minerals (M)	0.3986	0.1506	0.1295	0.9555	0.5813	0.2906
Limestone (L)	0.7195	0.5556	0.7845	0.0733	0.4331	0.7905
MxL	0.7575	0.4563	0.6635	0.5046	0.5657	0.6905

[1]CV- coefficient of variation.
[A, B] Means followed by different uppercase letters in the row differ statistically by Tukey's test at 5%.
[a, b] Means followed by different lowercase letters in the row differ statistically by Tukey's test at 5%.

The bone is a type of connective tissue formed by an organic matrix, in which microminerals (copper, manganese, and zinc), as enzymatic cofactors, have a fundamental role in its synthesis and a mineral portion, constituted of calcium phosphate (Nunes *et al*. 2013). Therefore, a balanced diet provides the nutrients necessary for formation of bones with quality, resulting in more resistant bones, and decreasing animal loss caused by their fragility, which comes with age. Hence, the results found in the present experiment, demonstrated that the two mineral supplements provided the nutrients necessary for the good bone quality obtained. It can thus be inferred that, in this case, the organic minerals utilized do not show advantages in relation to the inorganic source as to bone quality.

Similar results to those found in this trial 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 from 30 to 70 weeks of age. Conflicting results were published by Carvalho (2013), who described an improvement in bone resistance of layers fed diets containing organic mineral supplementation.

Brito *et al*. (2006) worked with chelated minerals in diets for replacement pullets of 7 to 12 weeks of age and reported that treatments did not influence length, weight or ash content of the tibia of these birds. These results are similar to those found in the present study. Likewise, Swiatkiewicz & Koreleski (2008) also reported no effect of organic mineral supplementation on the length, deformity and ash content of the tibia from brown-egg layers.

Working with increasing levels of coarse limestone for white-egg layers, Oliveira *et al*. (2013) observed that the limestone particle size did not influence the Seedor index or the mineral matter; the present study corroborates these findings.

Contrasting results were reported by Cufadar *et al*. (2011), who utilized three levels of calcium and three limestone particle sizes (2 mm, 3 to 5 mm, and over 5 mm) in the diet of brown-egg layers. These authors suggested the use of limestone of medium particle size as the most suitable to maintain poultry bone quality.

CONCLUSION

Diets containing inorganic minerals or these associated with organic minerals, along with different limestone particle sizes – fine, medium, or coarse –, can be used in the feeding of commercial layers without affecting their production performance, egg quality, or bone quality.

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