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Alternative Calcium Source Effects on Commercial Egg Production and Quality*

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ABSTRACT

This study aimed at verifying the possibility of replacing calcitic limestone by marine calcium in the diet of layers. A total number of 321 Hi-sex hens, with 40 weeks of age at the beginning of the experiment, was used. A completely randomized experimental design was applied, with 5 treatments (0, 15, 30, 45, and 60 % of calcitic limestone replacement by marine calcium source) and eight replicates of eight birds each. Treatments significantly affected specific gravity ($p<0.05$), with the inclusion of 60% marine calcium (T5) presenting the worst result as compared to T1, which included only calcitic limestone as calcium source. It was concluded that marine calcium can replace up to 45% of calcitic limestone with no effects on performance or egg quality.

INTRODUCTION

Calcium is very important for layers, as eggshell consists of 90% mineral matter, out of which 98% is composed of calcium carbonate (Mendonça Junior, 1993). Losses caused by eggshell defects vary from 6 to 8% of laid eggs (Roland, 1977).In Brazil, Vicenzi (1996) estimated that losses due to cracked and/or broken eggs reach 6.0 to 12.3% annually.

According to Leeson & Summers (1997), the use of medullar bones for eggshell formation results in sudden loss of 2g of body calcium, and therefore, a calcium bone reserve must be build up before the production period.

Calcium source, as well as particle size, solubility, and inclusion level influence eggshell quality (Faria 2002).

Calcium absorption is related to several factors, such as vitamin D, protein, phosphates, free fatty acids, and zinc levels. Calcium source solubilization in the digestive tract is of utmost importance as, according to Bronner (1993), is essential, as calcium needs to solubilized in the digestive tract before being nutritionally used.

When evaluating the effects of limestone beating different solubilities on eggshell quality and performance. Thim & Craig (1990) concluded that, when replacing limestone by oyster shell, or a highly soluble limestone by a low solubility limestone and vice-versa, there were no significant differences on eggshell quality and egg production.

The relationship among calcium level, source, particle size, *in vivo* and *in vitro* solubility, and calcium retention in the gizzard was studied by Zhang & Coon (1997), who found that limestone retention in the gizzard increased when *in vitro* solubility was lower, or when high calcium levels were fed. In this study, limestone *in vivo* solubility decreased as dietary calcium level increased.

Calcium is an important nutrient in commercial layer diet, and essential for eggshell formation. Feed intake can be influenced by dietary calcium level, which seems to act on the hypothalamus, inducing the release of norepinephrine, a neurotransmitter that acts in the central nervous system, stimulating feed intake (Borges, 1999).

Eggshell works as a "package" of egg contents and protects the embryo, and must be sufficiently strong to resist egg laying, collection, grading, and transport, until it reaches the final consumer. Eggshell quality is also commercially important, as consumers are increasingly concerned with food safety, and poor eggshell quality presents potential bacterial contamination (Kussakawa *et al.,* 1998).

Calcium may be supplied by different sources, such as limestone, calcium sulfate, calcite, oyster shell meal, aragonite, eggshells, etc., the most commonly used in layer diets are calcitic limestone and oyster shell meal (Kussakawa *et al*., 1998).

Due to the high calcium requirements of commercial layers and of layer and broiler breeders, the knowledge on calcium sources that can replace or be used in association with calcitic limestone or oyster shell meal is essential in order to improve or to maintain performance and eggshell quality with the use of renewable calcium sources.

This study aimed at evaluating the possibility of the replacement of calcitic limestone by "marine calcium" in commercial layer diets.

MATERIAL AND METHODS

The experiment was carried out at the experimental poultry house of FMVZ-UNESP, Botucatu, SP, Brazil, and took three periods of 28 days. A total number of 320 Hi-sex Brown layers, with initial age of 40 weeks, was used. Birds were housed in 40 metal cages (100-cm long x 45-cm high x 45-cm deep), with eight birds per cage, resulting in a density of 562.5cm^2/bird. Five treatments (0, 15, 30, 45, and 60 % inclusion of marine calcium replacing calcitic limestone) were applied.

Marine calcium derives from the marine alga *Lithothamnium* sp., which is abundant in the northeast coast of Brazil. After collection, the algae were dried in oven at 70 ºC for 24 hours, after which they were ground. This product contains 42 % calcium, 0.04 % phosphorus, 90.5 % $CaCO_3$, 47.79 % CaO, 5.58 % MgO, 2.99 Mg, 0.75 % SiO_2, 0.16 % Al_2O_3, 0.20 % Fe_2O_3, 0.17 Na_2O, 0.03 % K_2O, 0.08 % P_3O_9, 0.02 % Mn, 0.25 % de S, 1.5 ppm Cu, 16.5 ppm Zn, 8.5 ppm Co, 30.5 ppm Ti, 11.5 ppm Ni, 9.5 ppm Cr, 9.5 ppm B, 10.5 ppm Se, 11.5 ppm V, 9.5 ppm Mo, and 16.5 ppm Cl. The product does not contain toxic factors, such as lead, cadmium, and arsenic. Product particle size, according to ABNT 0502/95, is mesh 0.4mm, passage 99%, retained 1%, and it is not soluble in water. Chemical analyses were carried out according to AOAC (1997) methodology. Product commercial availability is enough to supply the market, and the product is also used in cattle diets.

Calcitic limestone includes 38% calcium, with a particle size of 0.18 mm GMD (geometric mean diameter).

Feeds were formulated on corn and soybean meal basis, and supplemented with vitamins and minerals, in order to supply the requirements as determined by Rostagno *et al.* (2000). Feeds are shown in Table 1.

Table 1 – Percentage composition of the experimental diets

	Treatments				
Ingredients	***1***	***2***	***3***	***4***	***5***
Corn	60.213	60.084	59.955	59.825	59.696
Soybean meal	24.150	24.078	24.006	23.934	23.863
Wheat midds	5.089	5.354	5.619	5.884	6.149
Dicalcium phosphate	1.466	1.463	1.460	1.457	1.454
Limestone	8.336	7.025	5.714	4.404	3.093
DL-Methionine	0.195	0.195	0.195	0.195	0.195
Vitamin premix*	0.100	0.100	0.100	0.100	0.100
Mineral premix**	0.100	0.100	0.100	0.100	0.100
Salt	0.350	0.350	0.350	0.350	0.350
Marine calcium***	0.000	1.250	2.500	3.750	5.000
Total	100.00	100.00	100.00	100.00	100.00
Estimated composition					
ME (Kcal/kg)	2800	2800	2800	2800	2800
CP (%)	17.000	17.000	17.000	17.000	17.000
Ca (%)	3.800	3.800	3.800	3.800	3.800
NaCl (%)	0.350	0.350	0.350	0.350	0.350
Avail. P (%)	0.380	0.380	0.380	0.380	0.380
Met. (%)	0.464	0.464	0.464	0.464	0.464
Choline (%)	0.098	0.097	0.097	0.097	0.097
Lysine (%)	0.854	0.853	0.853	0.852	0.851

Vitamin premix, kg product per MT feed: Vit. A – 7000000 UI; Vit. D3 – 2000000 UI; Vit. E – 5000 mg; Riboflavin – 3000 mg; Vit. K3 – 1600 mg – Vit. B12 – 8000 µg; Niacin – 20000 mg; Pantothenic acid – 5000 mg; Antioxidant – 15000 mg; Vehicle Q.S.P. – 1000 g. ****Mineral, premix, kg product per MT feed:*** Fe – 50000 mg; Cu – 8000 mg; Mn – 70000 mg; Zn – 50000 mg; I – 1200 mg; Se – 200 mg; Vehicle Q.S.P. – 1000 g.***Percentage of inclusion of calcinated marine algae (*Lithothamnium* sp.).

Water and feed were offered "ad libitum", and feed intake and egg weight were weekly determined. Birds were submitted to 17 hours of light daily. Eggs were collected daily, in the morning.

Performance was evaluated applying a completely randomized experimental design with 5 treatments (0, 15, 30, 45, and 60%) inclusion of marine calcium, with 8 replicates (cages) of 8 birds per experimental unit, including a total number of 320 birds and 40 cages.

The following performance parameters were evaluated: mortality, egg production, individual feed intake, mean egg weight, egg mass, feed conversion ratio (per dozen eggs and per kg eggs).

In order to measure egg quality, two eggs per replicate were collected every 28 days for three consecutive days for a total period of 84 days. A completely randomized experimental design was applied, with 5 treatments (0, 15, 30, 45, and 60%) inclusion of marine calcium, with 8 replicates (cages) of 16 eggs per experimental unit, including a total number of 144 eggs per treatment, and a total number of 720 eggs. The following egg quality parameters were evaluated: eggshell percentage, yolk color, yolk percentage, albumen percentage, albumen height, egg weight, and Haugh units. Haugh unit was calculate using the formula: $UH = 100 \log (H + 7.57 – 1.7 W^{0.37})$, where H = albumen height (mm) and W = egg weight (g).

Data were submitted to analysis of variance for completely randomized experiments, and pairs of means were compared by the test of Tukey ($p<0.05$), using SAS (1999) statistical package.

RESULTS AND DISCUSSION

Performance results are shown in Table 2. There were no statistical differences ($p>0.05$) among treatments as to mortality, egg production, egg weight, egg mass, feed intake, feed conversion ratio per dozen eggs and per kg eggs, and percentage of broken eggs, which are consistent with the results found by Kussakawa *et al.* (1998), who did not find any effect of different combination of calcium sources on egg production, feed intake, or feed conversion ratio (kg/kg or kg/dozen eggs). The positive effect on calcium use, and consequent better feed conversion ratio, is associated to its mechanics, stimulating the propagation enzymes through the feed in the upper part of the digestive tract, promoting the action of digestive juices (Tortuero & Centeno 1973). However this was not observed in the present study, with the association of calcitic limestone with marine calcium.

There were no statistically significant differences ($p>0.05$) among treatment effects on yolk color and percentage, albumen percentage, eggshell thickness, and Haugh units (Table 3). However, treatments had a significant effect on specific gravity ($p<0.05$), with treatment T5 (60% marine calcium + 40% calcitic limestone) presented the worst result (1.0923 b) as compared to the control treatment T1 (1.0952 a), but these results were not different from T2 (1.0933 ab), which presented 15% marine calcium and 85% calcitic limestone in the diet; T3 (1.0949 ab), with dietary

Table 2 – Performance parameters of 40 to 84-week-old layers fed different calcium combinations.

Parameters	*Treatments***					*CV (%)*
	T1	*T2*	*T3*	*T4*	*T5*	
Lay (%/hen/day)*	90.60	91.80	93.20	92.90	91.50	5.24
Egg mass (g)*	59.70	62.20	59.80	60.00	61.20	6.04
Feed intake (g/hen/day)*	120	120	121	119	117	3.71
feed conversion ratio (kg feed /kg eggs)*	2.01	2.01	1.95	1.97	1.98	6.20
Feed conversion ratio (kg feed /dozen eggs)*	1.57	1.57	1.57	1.54	1.54	6.21
Broken eggs (%)*	1.30	1.30	1.60	1.60	1.30	30.59

*P>0.05. **T 1: 0% inclusion marine calcium replacing calcitic limestone, T 2: 15% inclusion marine calcium replacing calcitic limestone, T3: 30% inclusion marine calcium replacing calcitic limestone, T4: 45% inclusion marine calcium replacing calcitic limestone; T5: 60% inclusion marine calcium replacing calcitic limestone.

Table 3 – Egg quality parameters of 40 to 84-week-old layers fed different calcium combinations.

Parameters	*Treatments***					*CV (%)*
	T1	*T2*	*T3*	*T4*	*T5*	
Mean egg weight (g)	66.0	65.0	65.0	66.0	66.0	3.45
Yolk color*	6	6	6	6	6	4.96
Eggshell (%)**	9.70	9.70	9.80	9.50	9.50	3.49
Albumen (%)	65.20	64.60	64.40	65.40	65.20	1.80
Yolk (%)	25.10	25.70	25.70	25.10	25.30	4.26
Eggshell thickness (mm)	0.396	0.390	0.399	0.388	0.388	3.86
Specific gravity	1.0952a	1.0933ab	1.0949ab	1.0941ab	1.0923b	0.26
Haugh units	87.62	85.84	85.46	86.52	86.48	4.29

Means followed by different letters in the same row are significantly different by the test of Tukey (p<0.05); T 1: 0% inclusion marine calcium replacing calcitic limestone, T 2: 15% inclusion marine calcium replacing calcitic limestone, T3: 30% inclusion marine calcium replacing calcitic limestone, T4: 45% inclusion marine calcium replacing calcitic limestone; T5: 60% inclusion marine calcium replacing calcitic limestone.
*Yolk color was determined using Roche Colorimetric Fan, with color scores ranging from 1 to 15.
** Eggshell dried in oven for 3 days at 60ºC.

inclusion of 30% marine calcium and 70% calcitic limestone; and T4 (1.0941 ab) with 45% marine calcium and 65% calcitic limestone. These egg quality results are different from those found by Kussakawa *et al.* (1998), who observed differences in egg weight and eggshell percentage, but no effect on specific gravity when providing different calcium source combinations in layer diets. On the other hand, Makled & Charlés (1987) found improved apparent egg density due to the association of calcium sources.

The different results found in other studies are related to the chemical composition and to the physical characteristics of different calcium sources (Fassani *et al.* 2004).

Roland (1986), Rao *et al.* (1992), and Cheng & Coon (1990) carried out studies to evaluate optimal particle size of calcium sources in terms of performance and egg quality. Rabon Junior & Roland (1985) evaluated 44 calcium sources and found 63% variation in calcium solubility *in vitro*. Leeson & Summer (1997) observed that calcium sources with larger particles were retained for longer in the upper digestive tract, releasing calcium slowly and consistently during eggshell formation period.

The present experiment showed that, for all analyzed performance and egg quality parameters, except for specific gravity, treatments containing marine calcium promoted similar results as to the control treatment, which contained only calcitic limestone.

Calcitic limestone GMD was 0.18mm, whereas marine calcium GMD was 0.4mm, and therefore it was expected that limestone would promote better results, particularly as to egg weight and eggshell weight, as demonstrated by Guinote & Nys (1991). However, our results are consistent with those of Fassani *et al.* (2004), who worked with limestone GMDs between 0.15 and 0.60 mm, and did not observe differences in limestone intake, limestone intestinal retention, or *in vitro* solubility. It must be noted that the limestone particle sizes used by Fassani *et al.* (2004) are similar to those found for calcitic limestone and marine calcium used in the present study. This is important as limestone with lower solubility, which is found in limestone with larger particles, increases calcium intestinal retention, thereby improving eggshell quality, as observed by Guinote & Nys (1991). On the other hand, Fassani *et al.* (2004), working with particle sizes of 2.00-4.00 mm found different limestone intake, limestone intestinal retention, and *in vitro* solubility.

CONCLUSIONS

Based on the results obtained under the conditions of the present study, it was concluded that it is possible to include up to 45% of the product containing calcium of marine origin in layer diets in substitution of calcitic limestone with no harmful effects on performance or egg quality.

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