Productive Performance and Egg Quality of Brown Egg-Laying Hens in the Late Phase of Production as Influenced by Level and Source of Calcium in the Diet

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ABSTRACT A total of 1,152 Lohmann Brown laying hens were used to study the influence of level (3.5 and 4.0%) and source (coded FIN, COA, and OYS) of Ca in the diet on productive performance and egg quality from 58 to 73 wk of age. The FIN diet contained all the Ca carbonate as fine limestone (LIM). In the COA and OYS diets, 40% of the fine LIM was substituted with either coarse LIM or oyster shell. Each treatment was replicated 8 times (24 hens). Productive performance and egg quality traits were recorded every 4 wk, and tibia characteristics and shell quality traits were determined at 73 wk of age. An increase in Ca intake from 4.08 to 4.64 g/hen per day improved egg production (71.2 vs. 74.9%; $P < 0.001$), egg mass (49.0 vs. 51.4 g; $P < 0.05$), and feed conversion ratio (2.43 vs. 2.30 kg of feed/kg of egg; $P < 0.001$). In addition, an increase in Ca intake improved shell weight (9.98 vs. 10.20%; $P < 0.05$), shell thickness (0.342 vs. 0.351 mm; $P < 0.01$), and shell density (82.0 vs. 83.8 mg/cm^2; $P < 0.001$). Calcium source had no effect on productive performance, tibia characteristics, or egg quality except for shell density, which was greater for hens fed COA than for hens fed FIN, with hens fed OYS being intermediate (81.9 vs. 84.0 vs. 82.7 mg/cm^2, respectively; $P < 0.05$). It was concluded that Brown egg-laying hens in the late phase of production require more than 3.5% Ca in the diet (4.08 g of Ca/hen per day) and that the substitution of 40% of fine LIM with COA or OYS does not affect productive performance and has little impact on shell quality and tibia characteristics.

Key words: Brown egg-laying hen, calcium level, limestone, oyster shell, shell quality

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INTRODUCTION

Egg loss produced during egg handling from farm to consumer accounts for 5 to 7% of all eggs laid (Roland, 1988). Most losses are related to the poor shell quality of eggs produced at the end of the production cycle. Grobas et al. (1999) found that the percentage of broken eggs from Brown egg-laying hens on the farm increased from 0.43% at 22 wk to 1.81% at 74 wk of age. Al-Batshan et al. (1994) observed that the percentage of shell decreased from 9.8 to 8.9% and shell thickness decreased from 0.403 to 0.373 mm from 22 to 57 wk of age. These authors indicated that aged hens were less efficient in absorbing Ca than younger ones. Roland (1980) observed that in older hens, a drastic increase in egg size increased shell deposition, but that the increase was not enough to prevent a decline in shell quality, because shell thickness decreased. Therefore, increasing the Ca content of the diet at the end of the laying cycle might be a good strategy to attempt to reduce the incidence of broken eggs. Keshavarz et al. (1993) reported that the specific gravity of the shell of eggs laid by Single Comb White Leghorn (**SCWL**) hens from 38 to 62 wk of age increased from 1.0835 to 1.0843 when the Ca level of the diet was increased from 3.0 to 3.5%. However, many authors have not found any benefit on hen productivity (Roland and Bryant, 1994; Bar et al., 2002) or specific gravity (Keshavarz et al., 1993) when the Ca level of the diet was increased beyond 3.5%. Most of the research conducted on the influence of Ca level on egg production and egg quality has been conducted with SCWL hens, and little information is available for Brown egg-laying hens. The NRC (1994) recommends 3.25 and 3.60 g of Ca/hen per day for SCWL and Brown egg-laying hens, respectively (3.25% Ca in the diet). However, no clear explanation is given for the recommendation.

The source of Ca might affect hen productivity and egg quality. Scott et al. (1982) reported that shell quality was improved when part of the fine limestone (**LIM**) in the diet was substituted by particulate LIM or oyster shell. Recently, Lichovnikova (2007) recommended supplying two-thirds of the Ca in the diet as

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Table 1. Ingredient composition and calculated nutrient analysis of the experimental diets (% as-fed basis, unless stated otherwise)[1]

	3.5% Ca			4.0% Ca		
Item	FIN	COA	OYS	FIN	COA	OYS
Ingredient						
Yellow corn	58.60	58.60	58.60	58.60	58.60	58.60
Soybean meal, 44% CP	21.33	21.33	21.33	21.33	21.33	21.33
Sunflower meal, 32% CP	4.93	4.93	4.93	4.93	4.93	4.93
Soybean oil	3.82	3.82	3.82	3.82	3.82	3.82
Met hydroxyl-analog, 88%	0.17	0.17	0.17	0.17	0.17	0.17
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30
Sodium bicarbonate	0.06	0.06	0.06	0.06	0.06	0.06
Vitamin and mineral premix[2]	0.40	0.40	0.40	0.40	0.40	0.40
Fine limestone	8.75	5.25	5.25	9.91	5.95	5.95
Coarse limestone	—	3.50	—	—	3.96	—
Oyster shell	—	—	3.50	—	—	3.96
Dicalcium phosphate	0.25	0.25	0.25	0.48	0.48	0.48
Sepiolite[3]	1.39	1.39	1.39	—	—	—
Calculated analysis						
AME_n (kcal/kg)	2,780	2,780	2,780	2,780	2,780	2,780
CP	15.50	15.50	15.50	15.50	15.50	15.50
Total Lys	0.77	0.77	0.77	0.77	0.77	0.77
Total Met	0.42	0.42	0.42	0.42	0.42	0.42
Total Thr	0.59	0.59	0.59	0.59	0.59	0.59
Total Trp	0.18	0.18	0.18	0.18	0.18	0.18
Total ash	12.30	12.30	12.30	12.30	12.30	12.30
Ca	3.50	3.50	3.50	4.00	4.00	4.00
Total P	0.38	0.38	0.38	0.42	0.42	0.42
Available P	0.29	0.29	0.29	0.33	0.33	0.33

[1]FIN = 100% fine limestone; COA = 60% fine and 40% coarse limestone; OYS = 60% fine limestone and 40% oyster shell.

[2]Provided the following (per kg of diet): vitamin A (*trans*-retinyl acetate), 8,000 IU; vitamin D_3 (cholecalciferol), 1,750 IU; vitamin E (all-*rac*-tocopherol acetate), 5 mg; thiamine (thiamine mononitrate), 1 mg; riboflavin, 3 mg; pyridoxine (pyridoxine·HCl), 1 mg; vitamin B_{12} (cyanocobalamin), 0.01 mg; vitamin K (bisulfate menadione complex), 1 mg; nicotinic acid, 16 mg; pantothenic acid (D-calcium pantothenate), 7 mg; Mn ($MnSO_4 \cdot H_2O$), 70 mg; Zn (ZnO), 50 mg; Fe ($FeSO_4 \cdot H_2O$), 30 mg; Cu ($CuSO_4 \cdot 5H_2O$), 4 mg; I (KI), 1 mg; Co, 0.2 mg; Se (Na_2SeO_3), 0.1 mg; choline (choline chloride), 240 mg; canthaxantin (carophyll red, DSM, Madrid, Spain), 200 mg; phytase (Natuphos 5000, BASF Española, Barcelona, Spain), 300 phytase units; ethoxyquin, 110 mg.

[3]A complex magnesium silicate clay.

large particles to ensure eggshell quality in the last third of the laying period. In the gizzard, large particles are solubilized more slowly than fine particles (Scott et al., 1971; Zhang and Coon, 1997a), allowing for a better provision of Ca during the first part of the dark period, when shell calcification is in process and hens do not have access to feed. Scheideler (1998) found an improvement in the specific gravity of the shell when 50% fine LIM was substituted by coarse LIM, probably because of its lower solubility and greater residence time in the proximal part of the digestive system. In addition, Guinotte and Nys (1991) reported that the breaking strength of the tibiae and the percentage of tibia ash were increased when coarse Ca sources (LIM or oyster shell) rather than fine LIM sources were used in Brown egg-laying hens from 64 to 77 wk of age. However, practical observations in the field (G. G. Mateos, Universidad Politécnica de Madrid, Madrid, Spain; personal communication) do not support these findings because shell quality is not impaired and the percentage of broken eggs is not increased when feed is offered as crumbles, a feed form in which the Ca has to be supplied in powdered form. In addition, in many instances, no benefits on eggshell quality have been observed when extra amounts of oyster shell (approximately 2 g/hen per day) were offered to hens 1 to 2 h before the beginning of the dark period (G. G. Mateos, Universidad Politécnica de Madrid, Madrid, Spain; personal communication). The lack of response to the inclusion of oyster shell might be due to poor handling and mixing of the oyster shell with the feed, the reduced particle size of the source used, or merely a lack of effect of the Ca supplied in this form. We hypothesize that Brown egg-laying hens late in the production cycle require more than 3.5% Ca in the diet and that substituting part of the fine LIM with coarse LIM or oyster shell could reduce the incidence of eggshell problems associated with the lower level of Ca inclusion.

MATERIALS AND METHODS

Husbandry and Experimental Diets

All experimental procedures used in this experiment were approved by the Animal Ethics Committee of the University of Madrid and were in compliance with the Spanish guidelines for the care and use of animals in research (Boletín Oficial Estado, 2005). A total of 1,152 Lohmann Brown laying hens with an initial BW of

1,845 ± 10.8 g were obtained from a commercial flock (Cantos Blancos, Guadalajara, Spain) at 58 wk of age and used in this experiment. The preexperimental diet was a commercial diet based on corn and soybean meal that contained 2,750 kcal of AME_n/kg, 17% CP, 3.9% Ca, and 0.33% available P. The hens were selected at random, weighed individually, and stratified by BW into 6 groups of 192 hens each. The experimental unit consisted of a group of 24 hens (4 from each BW group) housed in groups of 4 in 610 × 450 mm battery cages (Big Dutchman, Vechta, Germany). Six experimental diets were randomly distributed within the 48 replicates. Room temperature was kept at 22 ± 3°C, and the light program consisted of 16 h of light/d throughout the experiment (58 to 73 wk of age).

The experimental diets were formulated to have similar AME_n and amino acid contents (Fundación Española Desarrollo Nutrición Animal, 2003) but to differ in the levels of available P and total Ca (0.29 and 3.5% or 0.33 and 4.0%, respectively) and in the source of Ca used (coded **FIN**, **COA**, and **OYS**). The Ca and available P content differed among the diets, but the Ca:available P ratio was the same (Table 1). All the Ca was supplied as fine LIM in the FIN diets, whereas in the COA and OYS diets, 40% of the fine LIM was substituted with either coarse LIM or oyster shell. The LIM (Tricalsa, Burgos, Spain) contained 38.3% Ca and was supplied as powder or in particles with a theoretical mean particle size (**MPS**) of 3,400 μm. The oyster shell, which was obtained from a Danish fossil bank (Oytaco Ltd., Frederikssund, Denmark), contained 37.9% Ca and was supplied in particles with an expected MPS of 3,500 μm. All diets were offered for ad libitum consumption in meal form. The determined analyses of the Ca sources and the diets are presented in Tables 2 and 3, respectively.

Analytical Evaluation of Feeds and Tibiae

Feeds were analyzed for DM by the oven-drying method (method 930.15), total ash by a muffle furnace (method 942.05), nitrogen by combustion (method 990.03) using a Leco instrument (model FP-528, Leco Corporation, St. Joseph, MI), ether extract by Soxhlet fat analysis after 3 *N* HCl acid hydrolysis (method 920.39), and crude fiber by sequential extraction with diluted acid and alkali (method 962.09) as described by AOAC International (2000). Gross energy was determined by combustion with an adiabatic bomb calorimeter (model 356, Parr Instrument Company, Moline, IL). The P content was determined colorimetrically, and the Ca content of the feeds and the Ca, Mg, Cu, Zn, Fe, and Mn contents of the Ca sources were determined by atomic absorption as described by Boletín Oficial Estado (1995). To determine the distribution and MPS of the Ca sources and diets, 3 subsamples of 100 g were sieved through a Retsch shaker (Retsch, Stuttgart, Germany) provided with 8 sieves ranging in mesh from 5,000 to 40 μm. The method outlined by the American Society of Agricultural Engineers (1995) was used. The solubility of the Ca sources was determined in vitro as indicated by Zhang and Coon (1997a). Briefly, a 2.0-g sample of the Ca source was poured into a 400-mL beaker containing 200 mL of 0.2 *N* HCl solution and was warmed at 42°C until the temperature of the solution became constant (approximately 15 min). After allowing 10 min for reaction, the undissolved material was filtered onto a preweighed Whatman ashless filter paper, dried in a 60°C oven for 20 h, and weighed. Solubility was expressed as the percentage of weight loss (**WL**) of the original sample. In addition, the solubility of the Ca sources was estimated as indicated by Zhang and Coon (1997b). Briefly, WL was predicted by using

Table 2. Particle size distribution, mean particle size (MPS ± SD, μm), in vitro solubility, and mineral content of the Ca sources[1]

Item	Fine limestone	Coarse limestone	Oyster shell
Sieve size (μm)		Retained (%)	
2,500	—	94.82	56.79
1,250	—	4.39	39.72
630	15.94	0.30	3.39
315	43.82	—	0.10
160	26.10	0.10	—
80	11.45	0.40	—
40	2.69	—	—
MPS ± SD[2]	337 ± 2.0	3,360 ± 1.3	2,557 ± 1.5
Solubility (%)			
In vitro[3]	57.47	33.48	50.65
Predicted[4]	62.42	44.56	54.70
Mineral content			
Ca (%)	38.30	38.30	37.90
Mg (%)	0.17	0.16	0.11
Cu (ppm)	5.3	4.5	4.4
Zn (ppm)	4	5	11
Fe (ppm)	299	266	3,989
Mn (ppm)	11	7	260

[1]In triplicate samples.

[2]Log normal SD.

[3]Solubility (%) = weight loss (g) × l00/initial sample weight (g).

[4]Solubility (%) = [1.809 + 0.601 × ln(pH change)] × l00/initial sample weight (g).

Table 3. Determined chemical composition (% as-fed basis unless stated otherwise) and mean particle size (MPS ± SD, μm) of the experimental diets[1]

	Calcium, 3.5%			Calcium, 4.0%		
Item	FIN	COA	OYS	FIN	COA	OYS
Gross energy (kcal/kg)	3,743	3,773	3,746	3,739	3,795	3,776
DM	89.0	88.9	89.2	89.0	88.8	88.9
CP	16.1	16.0	15.3	15.4	15.6	15.7
Ether extract	6.6	6.7	6.2	6.4	6.6	6.6
Crude fiber	4.7	4.5	4.7	4.7	4.5	5.0
Total ash	12.9	12.1	13.0	13.4	12.6	13.5
Ca	3.63	3.35	3.55	4.22	3.90	4.32
Total P	0.41	0.38	0.42	0.42	0.42	0.43
MPS ± SD[2]	1,112 ± 2.3	1,215 ± 2.3	1,298 ± 2.3	1,098 ± 2.5	1,118 ± 2.6	1,186 ± 2.5

[1]In triplicate samples. FIN = 100% fine limestone; COA = 60% fine and 40% coarse limestone; OYS = 60% fine limestone and 40% oyster shell.

[2]Log normal SD.

the pH change between a transferred blank of 0.2 *N* HCl and the supernatant after filtration according to the following formula: WL (g) = 1.809 + 0.601 × ln (pH change). The solubility of the Ca source was then calculated according to the following formula: solubility (%) = WL (g) × l00/initial sample weight (g).

At the end of the experiment, 2 hens per replicate were randomly selected, weighed, and killed by cervical dislocation. The left tibiae were excised, cleaned of connective tissue, and stored in individual plastic bags at −20°C. Before analysis, the frozen tibiae were thawed inside the plastic bags at room temperature for 24 h and tibia weight, length, and volume (water replacement) were measured in 2 tibiae per replicate. The relative weight (g/kg of BW), length (cm/kg of BW), volume (mL/kg of BW), and density (g/cm^3) were calculated from these data as indicated by Nelson et al. (1992). Afterward, the tibiae from 1 of the 2 hens of each replicate was ashed without previous fat extraction as indicated by Yan et al. (2005), and the other tibiae were dried in an oven at 103°C for 24 h, submerged in diethyl ether for 48 h, and dried again, and the dry-defatted weight was calculated (Cheng and Coon, 1990). All the tibiae were ashed at 600°C for 36 h and the ash weight and the percentages of ash, Ca, and P of the nondefatted and defatted tibiae were determined from these data following the same procedures as used for the feeds.

Productive Performance and Egg Quality

The number of total, dirty, and broken and shell-less eggs and mortalities were recorded daily by replicate. An egg was considered dirty when a spot of any kind or size was detected on the shell, as evaluated by an independent observer that was blinded to treatments. Feed intake was recorded by replicate every 28 d and BW of the hens was controlled at the beginning and at the end of the experiment to determine BW gain by replicate. All the eggs produced on the last day of each week were individually weighed and graded (European Economic Community, 1989). The 4 categories recorded for egg size were extra large (>73 g), large (73 to 63 g), medium (63 to 53 g), and small (<53 g). Egg production, egg mass, average daily feed intake (**ADFI**), feed conversion ratio (**FCR**) by kilogram and by dozen of eggs, and mortality rate were calculated from these data by period and cumulatively. Egg quality was measured in 10 eggs collected randomly from each replicate on the last day of each 28-d period, and the average value was used to analyze the data by period and cumulatively. The eggs were individually weighed, and the external and the internal quality was determined by using an egg multitester instrument (QCM-System, TSS, York, UK). Shell color was measured by using a QCR shell color reflectometer (TSS). All the eggs were then broken and their contents were removed. The shell with the membranes and the yolk was separated from the albumen and weighed, and the relative proportions were determined (Grobas et al., 2001). The surface area of the egg was calculated as indicated by Mueller and Scott (1940), and the shell density (mg/cm^2) was calculated as the weight of the dry shell divided by the surface area. Albumen height (±0.1 mm) was measured as indicated by Keener et al. (2006), using an electronic height gauge (QCH-System, TSS). Yolk color was determined by using the Roche Color Fan (QCC-System, TSS). Haugh units were calculated as indicated by Haugh (1937) on the input of egg weight and albumen height (QCM-System, TSS). The incidence of blood and meat spots on the eggs was determined by a single controller that was blinded to treatments, and the air cell cavity was measured by using a ruler with a precision of 1 mm (Luftkammer-Messer 872, Bruja, Hammelburg, Germany). On the last day of the trial, 12 eggs of each replicate were collected at random to measure egg length and egg width, and the egg shape index was calculated by dividing egg length by egg width. In addition, shell thickness was measured in these eggs by using a digital micrometer (model IT-014UT, Mitutoyo, Kawasaki, Japan).

Statistical Analysis

The experiment was conducted as a completely randomized design with 6 treatments arranged factorially,

and main effects (level and source of Ca) and their interaction were analyzed by using the GLM procedure of SAS (SAS Institute, 1990). Duncan's multiple range test was carried out to detect differences among Ca sources. All differences were considered significant at $P < 0.05$. The variances in the data were homogeneous, as indicated by the HOVTEST option of the GLM procedure. The experimental unit was a group of 24 hens for all traits studied. For tibia traits, only 2 hens per replicate were used, and for the determination of ash, Ca, and P in the nondefatted and defatted tibiae, only 1 hen per replicate was used. Nonnormally distributed data (mortality rate, shell and yolk color, percentage of yolk and albumen in the egg, incidence of blood and meat spots, percentage of broken and shell-less eggs, dirty eggs, and egg grade) were analyzed by using the CATMOD procedure (SAS Institute, 1990). Results in the tables are presented as means.

RESULTS

The MPS of the Ca sources were 337 μm for fine LIM, 3,360 μm for coarse LIM, and 2,557 μm for oyster shell (Table 2). The percentage of particles greater than 1,250 μm was 0% for fine LIM, 99.21% for coarse LIM, and 96.51% for oyster shell. The MPS of oyster shell was lower than expected (2,557 vs. 3,500 μm, respectively). The in vitro solubility of the Ca sources was greatest for fine LIM and least for coarse LIM, with oyster shell being intermediate (57.47, 33.48, and 50.65%, respectively). The Ca, Mg, and Cu contents were similar for all the Ca sources, but Zn, Fe, and Mn contents were greater for oyster shell than for LIM. The determined chemical values of the experimental diets were similar to the expected values (Table 3), and as expected, MPS was lowest for the FIN diets.

Productive Performance and Egg Quality

The interaction between level and source of Ca was not significant for all traits studied; therefore, only the main effects are presented. Increasing the Ca intake from 4.08 to 4.64 g/d (3.5 to 4.0% Ca in the diet) improved egg production (71.2 vs. 74.9%; $P < 0.001$), egg mass (49.0 vs. 51.4 g; $P < 0.05$), and FCR per kilogram (2.43 vs. 2.30; $P < 0.001$) and per dozen (2.01 vs. 1.89; $P < 0.001$) of eggs (Table 4). However, BW gain, egg weight, ADFI, and mortality were not affected. The beneficial effects of increasing the Ca level of the diet on productive performance were evident in all periods (Figure 1). Calcium level did not affect egg grade or egg quality traits (Table 4). Source of Ca did not have any effect on productive performance or egg quality traits in any of the periods studied.

Eggshell and Tibia Characteristics

The interaction between level and source of Ca was not significant for all traits studied; therefore, only the main effects are presented. An increase in Ca intake from 4.08 to 4.64 g/hen per day (3.5 to 4.0% Ca in the diet) improved weight (9.98 vs. 10.20%; $P < 0.05$), thickness (0.342 vs. 0.351 mm; $P < 0.01$), and density (82.0 vs. 83.8 mg/cm^2; $P < 0.001$) of the shell, and reduced the percentage of broken and shell-less eggs (0.64 vs. 0.54%; $P < 0.05$; Table 5). Most of the observed improvement in shell weight and shell density occurred in the last 8 wk of the trial (Figure 2). In addition, an increase in Ca intake increased the Ca content of the nondefatted tibiae (11.7 vs. 12.4; $P = 0.06$), but no other traits were affected. Source of Ca had little effect on shell quality and tibia characteristics. In fact, the only significant effect observed was for shell density, which was greater in eggs from hens fed COA than in eggs from hens fed FIN, with hens fed OYS being intermediate (84.0 vs. 81.9 vs. 82.7 mg/cm^2, respectively; $P < 0.05$).

DISCUSSION

Most published research has reported that oyster shell is less soluble than fine or coarse LIM (Zhang and Coon, 1997b; Lichovnikova, 2007). However, in the current study the solubility was greater for oyster shell than for coarse LIM, probably because of the small MPS of the oyster shell used. Zhang and Coon (1997b) reported that the in vitro solubility of Ca sources depended not only on their origin, but also on the MPS. In fact, they found that the solubility of 2 sources of Ca with a similar range of MPS (250 to 4,800 μm) varied from 78.2 to 26.7% for LIM and from 55.0 to 21.0% for oyster shell. Moreover, Zhang and Coon (1997a) reported that the in vitro solubility of a coarse LIM source ranged from 63.1% for LIM with an MPS of 500 μm to 29.8% for LIM with an MPS of 4,700 μm, confirming that the solubility was reduced as the MPS was increased.

Productive Performance and Egg Quality

An increase in Ca intake from 4.08 to 4.64 g/hen per day (3.5 to 4.0% of the diet) improved most productive traits, including egg production, egg mass, and FCR. However, Castillo et al. (2004) reported that these traits were not affected when Ca intake increased from 3.68 to 4.26 g/d (3.22 to 3.83% of the diet) in SCWL hens from 55 to 70 wk of age. In addition, Roland and Bryant (1994) indicated that as Ca intake increased from 3.58 to 4.35 g/d (3.3 to 4.1% of the diet), ADFI decreased, but neither egg production nor egg weight was affected in SCWL from 24 to 27 wk of age. Moreover, Atteh and Leeson (1985) found that Ca intakes ranging from 3.91 to 4.43 g/hen per day (3.0 to 4.2% of the diet) had little effect on the productivity of SCWL hens from 30 to 37 wk of age, and Keshavarz (1998a) found no effect on the performance of SCWL hens from 43 to 49 wk of age when Ca intake was increased from 3.62 to 4.04 g/d (3.4 to 3.7% of the diet). Similarly, Keshavarz

Table 4. Influence of level and source of Ca on productive performance, egg quality, and egg grade of laying hens from 58 to 73 wk of age

	Ca (%)		Ca source[1]				*P*-value[3]	
Item	3.50	4.00	FIN	COA	OYS	SEM[2]	1	2
Productive performance								
BW gain[4] (g)	81	75	77	73	85	20.4	NS	NS
Egg production (%)	71.2	74.9	72.8	73.7	72.7	1.62	***	NS
Egg weight (g)	68.9	68.6	68.9	68.3	69.0	0.43	NS	NS
Egg mass (g)	49.0	51.4	50.1	50.3	50.2	1.14	*	NS
Feed intake (g/d)	116.7	116.0	116.5	116.1	116.5	0.99	NS	NS
Feed conversion (kg/kg)	2.43	2.30	2.37	2.35	2.37	0.056	***	NS
Feed conversion (kg/dozen)	2.01	1.89	1.96	1.93	1.96	0.047	***	NS
Mortality[5] (%)	1.39	1.43	1.49	1.51	1.23		NS	NS
Egg quality								
Shape index	129.6	130.0	129.8	130.4	129.2	0.61	NS	NS
Haugh units	79.5	79.7	79.2	80.3	79.2	0.66	NS	NS
Air cell cavity (mm)	1.21	1.20	1.22	1.19	1.21	0.023	NS	NS
Albumen weight[5] (%)	64.33	64.11	64.19	64.20	64.26		NS	NS
Yolk weight[5] (%)	25.68	25.68	25.81	25.54	25.69		NS	NS
Yolk color[5]	11.7	11.8	11.8	11.7	11.8		NS	NS
Dirty eggs[5] (%)	0.53	0.61	0.49	0.60	0.61		NS	NS
Blood and meat spots[5] (%)	0.146	0.158	0.150	0.181	0.125		NS	NS
Egg grade[5] (%)								
>73 g	18.90	18.81	18.91	18.92	18.79		NS	NS
63 to 73 g	63.50	63.32	63.40	63.51	63.29		NS	NS
53 to 63 g	17.30	17.52	17.40	17.22	17.60		NS	NS
<53 g	0.30	0.35	0.29	0.35	0.32		NS	NS

[1]FIN = 100% fine limestone; COA = 60% fine and 40% coarse limestone; OYS = 60% fine limestone and 40% oyster shell.

[2]SEM (8 replicates of 24 hens each per treatment).

[3]1 = Ca level effect; 2 = Ca source effect. The interaction between Ca level and Ca source was not significant ($P > 0.05$).

[4]The average initial BW of the hens was 1,845 g.

[5]Analyzed by CATMOD (SAS Institute, 1990).

*$P < 0.05$; ***$P < 0.001$.

et al. (1993) observed no difference in egg production, egg weight, or FCR of SCWL hens from 38 to 62 wk of age when the Ca content of the diet was increased from 3.85 to 4.40 g/d (3.5 to 4.0% of the diet). However, all of these studies were conducted with SCWL hens that were younger than the hens in the current trial. Probably, a deficiency in dietary Ca in young hens is compensated for by the use of bone Ca reserves. Bar et al. (2002) reported that egg production, egg weight, and ADFI of Lohmann Brown hens from 66 to 78 wk of age were not affected when the Ca intake increased from 4.20 to 5.78 g/d (3.6 to 4.9% of the diet). However, in a second trial, these authors found a trend toward increased egg production and decreased egg weight when dietary Ca was increased from 3.60 to 5.02 g/d (3.5 to 4.8% of the diet) in hens from 57 to 65 wk of age.

The source of Ca did not affect the productive performance of hens, which agrees with the report of Scheideler (1998), who did not find any effect on productivity when 25 or 50% fine LIM in the diet was substituted with either oyster shell or large LIM in SCWL hens in their first or third production cycle. Moreover, Keshavarz et al. (1993) did not observe any effect on productivity when 33% fine LIM was substituted with oyster shell in the diets of SCWL hens from 38 to 62 wk of age, which contained 3.0, 3.5, or 4.0% total Ca. In contrast, Ahmad and Balander (2003) reported that the replacement of 50% LIM with oyster shell significantly improved egg production in SCWL hens from 28 to 64 wk of age. However, the increase in egg production observed in this trial was only 0.8 percentage units.

In the current research, neither the level nor source of Ca affected the shape index, Haugh units, air cell cavity, relative proportion of albumen and yolk, incidence of blood and meat spots, or percentage of dirty eggs. We have found no information in the literature comparing the effects on these traits of Ca level or source of Ca.

Eggshell and Tibia Characteristics

The Ca content of the diet affected most shell quality traits. Keshavarz (1998b) observed that an increase in daily Ca intake from 3.51 to 4.25 g/hen (3.1 to 3.8% of the diet) improved shell thickness (from 0.372 to 0.386 mm) and specific gravity (from 1.0765 to 1.0786) of eggs in SCWL hens from 42 to 48 wk of age. However, shell weight and shell percentage were not affected. In addition, Keshavarz (1998a) reported that SCWL hens from 43 to 49 wk of age responded with an increase in specific gravity (from 1.0786 to 1.0803) to increases in daily Ca intake from 3.62 to 4.04 g/hen (3.4 to 3.7% of the diet). In contrast, Rao et al. (2003) observed no benefit on eggshell weight or shell thickness when the Ca intake was increased from 3.51 to 4.82 g/hen per day (3.25 to 4.50% of the diet).

The results of the current trial indicate that Brown egg-laying hens late in the production cycle require more than 4.08 g of Ca/hen per day (3.5% of the diet), which agrees with the report of Lichovnikova (2007), who recommended 4.51 g of Ca/hen per day (4.1% of the diet) to ensure eggshell quality in the last third of the production cycle. In contrast, Leeson et al. (1993) did not observe any effect on eggshell deformation in Brown egg-laying hens when Ca intake, provided as LIM, was greater than 3.4 g/d. The reason for the discrepancy among authors with respect to the Ca requirement and shell quality of eggs is not apparent, but might be due to differences in the strain, age, egg production, and nutrient specifications of the diets used. Franco-Jimenez and Beck (2005) indicated that at the end of the production cycle, Brown egg-laying hens had a greater bone-breaking strength than did SCWL hens. Therefore, Brown and SCWL hens might respond differently to supplementary Ca levels. These authors suggested that the greater bone frame and stronger bone structure of Brown egg-laying hens allow them to store a greater amount of Ca; consequently, Brown egg-laying hens would be better equipped to satisfy Ca requirements for eggshell formation when needed.

The substitution of 40% fine LIM with coarse LIM or oyster shell had little effect on shell quality. In fact, the only significant effect observed was for shell density, which was greater for hens fed COA diets than for hens fed FIN diets. Ahmad and Balander (2003) did not find any benefit in eggshell thickness when 50% LIM was substituted with oyster shell in the diets of SCWL hens from 28 to 64 wk of age, in agreement with our results. In contrast, Keshavarz and Nakajima (1993) observed a beneficial effect on eggshell specific gravity when 50% fine LIM was substituted with an equivalent amount of oyster shell. Similarly, Scheideler (1998) found an improvement in specific gravity when 50% fine LIM was substituted with coarse LIM. Large particles of Ca are solubilized more slowly and are retained in the gizzard longer than fine particles of Ca (Zhang and Coon, 1997a). In consequence, the substitution of fine LIM by coarse LIM or oyster shell should improve shell quality and bone structure because more of the dietary Ca will be utilized for eggshell formation during the first part

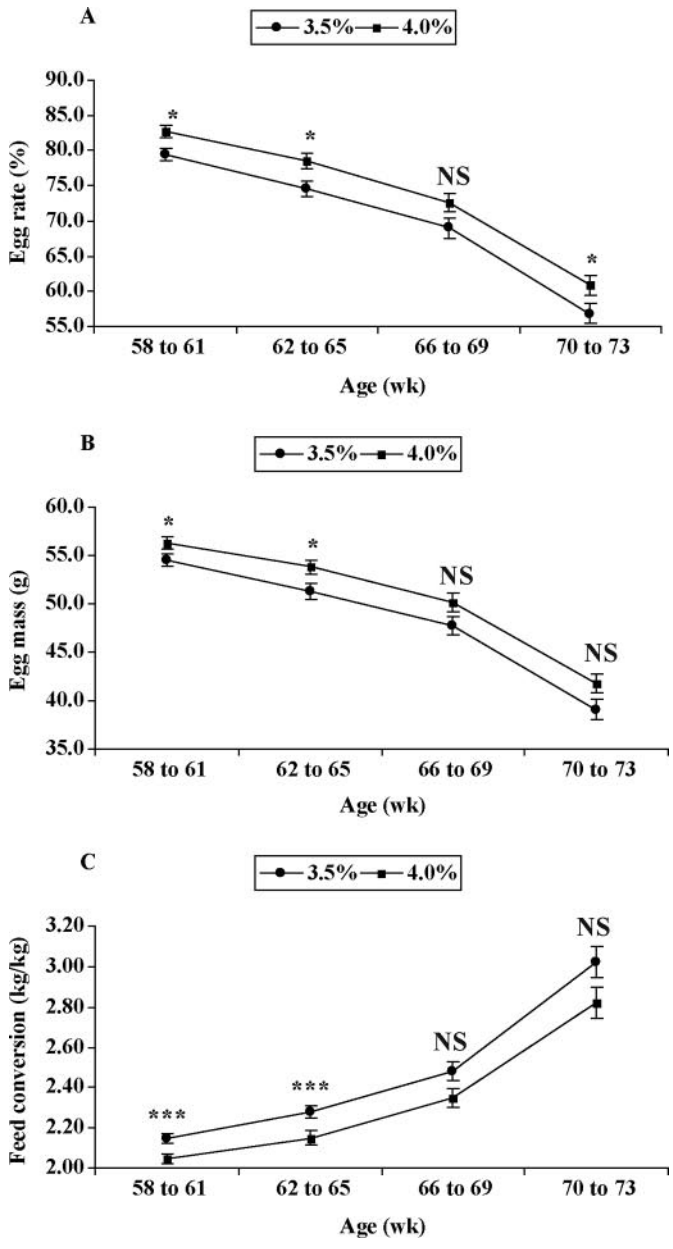

Figure 1. Influence of the level of Ca in the diet on egg production (a), egg mass (b), and feed conversion ratio per kilogram of eggs (c) of laying hens from 58 to 73 wk of age. $^{*}P < 0.05$; $^{***}P < 0.001$; NS = not significant.

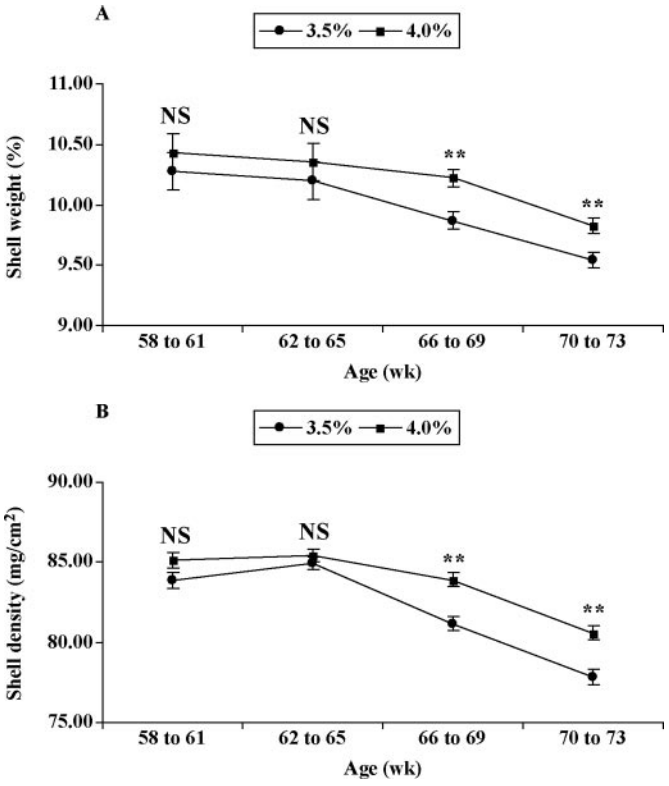

Figure 2. Effect of Ca level in the diet on shell weight (a) and shell density (b) of laying hens from 58 to 73 wk of age. $^{*}P < 0.05$; $^{**}P < 0.01$; NS = not significant.

Table 5. Influence of level and source of Ca on eggshell quality from 58 to 73 wk of age and tibia characteristics at 73 wk of age

	Ca (%)		Ca source[1]				*P*-value[2]	
Item	3.50	4.00	FIN	COA	OYS	SEM[3]	1	2
Shell quality traits								
Weight (%)	9.98	10.20	10.04	10.13	10.10	0.113	*	NS
Thickness (mm)	0.342	0.351	0.348	0.344	0.347	0.004	**	NS
Density (mg/cm^2)	82.0	83.8	81.9^b	84.0^a	82.7^{ab}	0.77	***	*
Color[4]	26	26	26	26	26		NS	NS
Broken and shell-less eggs[4] (%)	0.64	0.54	0.55	0.57	0.66		*	NS
Tibia traits								
Weight (g/kg of BW)	5.55	5.56	5.54	5.54	5.59	0.115	NS	NS
Length (cm/kg of BW)	6.24	6.28	6.22	6.29	6.26	0.157	NS	NS
Volume (mL/kg of BW)	4.63	4.71	4.57	4.71	4.74	0.155	NS	NS
Density (g/cm^3)	1.23	1.24	1.23	1.23	1.24	0.020	NS	NS
Ash (%)								
Fresh tibiae	33.3	33.9	33.2	33.3	34.4	0.83	NS	NS
Dry-defatted tibiae	55.5	55.8	55.7	54.4	56.8	1.24	NS	NS
Ca (% of ash)								
Nondefatted tibiae	11.7	12.4	11.8	12.2	12.2	0.43	NS	NS
Defatted tibiae	37.7	38.0	37.9	38.0	37.6	0.27	NS	NS
P (% of ash)								
Nondefatted tibiae	5.3	5.4	5.3	5.5	5.2	0.17	NS	NS
Defatted tibiae	16.9	17.0	16.8	17.0	17.0	0.22	NS	NS

[a,b]Means within a row and main effects not sharing a common superscript are different ($P < 0.05$).

[1]FIN = 100% fine limestone; COA = 60% fine and 40% coarse limestone; OYS = 60% fine limestone and 40% oyster shell.

[2]1 = Ca level effect; 2 = Ca source effect. The interaction between Ca level and Ca source was not significant ($P > 0.05$).

[3]SEM (8 replicates of 24 hens each per treatment).

[4]Analyzed by CATMOD (SAS Institute, 1990).

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

of the dark period. In fact, Lichovnikova (2007) recommended that at least two-thirds of the Ca should be included as large particles (coarse LIM or oyster shell) to maintain eggshell quality in the last third of the laying period. However, the results of the current experiment do not support this suggestion.

Calcium content of the diet did not affect tibia characteristics, in agreement with the data of Keshavarz et al. (1993), who observed no effect on the dry weight or ash content of the tibiae of 62-wk-old SCWL hens when the Ca intake was increased from 3.85 to 4.40 g/hen per day (3.5 to 4.0% of the diet). In contrast, Atteh and Leeson (1985) reported that tibia ash was increased by 1.5 percentage units when Ca intake increased from 3.91 to 4.43 g/hen per day (3.6 to 4.2% of the diet) although tibia Ca content was not modified. The source of Ca did not affect any tibia trait, an observation that agrees with the data of Keshavarz et al. (1993), who observed no improvement in tibia weight or tibia ash content when 33% powdered LIM was substituted with oyster shell. In contrast, Guinotte and Nys (1991) reported that when fine LIM was substituted with coarse LIM or oyster shell, tibia characteristics were improved. In addition, Fleming et al. (1998) indicated that, compared with powdered LIM, particulate LIM reduced the loss of cancellous bone at 25 wk of age and increased tibia breaking strength at 50 wk of age in pullets that started the trial at 15 wk of age. These authors observed that coarse Ca particles increased the accumulation of medullary bone in the proximal tarsus-metatarsus, with differences with respect to fine Ca that were more apparent at 70 than at 25 or 50 wk of age.

The reasons for the discrepancies among authors with respect to source of Ca and shell quality and tibia strength are not known but might be explained, at least in part, by the age of the hens when the supply of particulate Ca began and by the duration of the trial. For example, Fleming et al. (1998) supplied the particulate LIM before the beginning of the laying period (from 15 to 70 wk of age) and Lichovnikova (2007) supplied the particulate LIM for only a 2-wk period (56 to 57 wk of age), whereas in the current experiment, the supply of the particulate sources of Ca began at 58 wk of age and the trial lasted for 15 wk. The effect of particulate Ca, either coarse LIM or oyster shell, on eggshell quality might be more evident when the coarse source is introduced into the diet at younger ages. In addition, the beneficial effects might be more evident for the first days after the introduction of particulate Ca in the diet. This hypothesis is consistent with the improvement in shell density observed from 58 to 61 wk of age, but not after 61 wk, in hens fed coarse LIM.

We conclude that Brown egg-laying hens late in the production cycle require more than 3.5% Ca in the diet (4.08 g of Ca/d) to maintain productivity and shell quality. In addition, substitution of part of the fine LIM in the diet with coarse LIM with an MPS of 3,360 μm or oyster shell with an MPS of 2,557 μm does not affect productive performance and tibia characteristics although it might improve some shell quality traits. Therefore, the use of oyster shell with an MPS of 2,557

μm to improve productivity and eggshell quality in old hens is not justified.

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