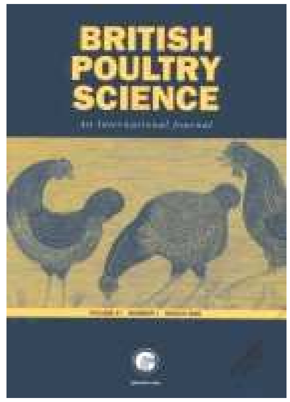
The effect of the calcium source, level and the particle size on calcium retention, eggshell quality and the overall calcium requirement in laying hens

Martina Lichovnikova



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Journal:	*British Poultry Science*
Manuscript ID:	CBPS-2006-246.R1
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	20-Oct-2006
Complete List of Authors:	Lichovnikova, Martina; Mendel University of Agriculture and Forestry
Keywords:	Digestion, Egg shell, Laying hens

CBPS_2006_246

edited M G MacLeod, November 2006

The effect of dietary calcium source, concentration and particle size on calcium retention, eggshell quality and overall calcium requirement in laying hens

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Accepted for publication 16th November 2006

Abstract 1. Four different sources of calcium in isonitrogenous and isoenergeric diets were fed to laying hens for two weeks when they were 56 and 57 weeks old. The calcium source blends were as follows: 29% fine limestone (LF) + 71% large limestone (LG), 32% fine limestone (LF) + 68% eggshell, 32% fine limestone (LF) + 68% oyster shell, 50% fine limestone + 50% large limestone (LG). The contents of these blends of calcium in the diets were as follows: 103.3 g/kg, 93.3 g/kg, 93.3 g/kg and 93. 3 g/kg respectively.

2. The coefficients of calcium retention were significantly higher in 50:50 LF:LG (0.578) and 32:68 LF:eggshell (0.576). The midnight feeding significantly improved the coefficient of calcium retention in all mixtures except 50:50 LF:LG.

3. In the mixture 29:71 LF:LG and 32:68 LF:oyster shell, there was significantly greater eggshell quality, eggshell weight, eggshell thickness and eggshell strength. Midnight feeding had no significant effect on eggshell quality.

4. In the ration with oyster shells, 96.5% of the retained calcium was deposited in the eggshell, but in ration 32:68 LF:eggshells and 50:50 LF:LG the utilisation was only 73.9% and 78.6% respectively.

5. To ensure good quality eggshells in the last third of production, the recommendation for calcium is 4.1 g/kg (900 g/kg dry matter, feed intake 110 g/d). As a source of calcium in this stage of production, a feed mixture containing two-thirds large particles should be used (limestone grit or oyster shell).

INTRODUCTION

The quality of the eggshells has a major influence on the economics of egg production. Damaged or broken shells account for 6-8% of all the eggs laid (Bain, 1997) and the highest incidence of cracked eggs occurs mainly in the last third of the laying period (after 53 weeks of age). The source and particle size of mineral nutrients, mainly calcium, play an important role in maintaining eggshell quality. It is known that hens given ground limestone as a single source of calcium had lower eggshell quality than hens fed blends of particulate and ground limestone (Guinotte and Nys, 1991; Richter *et al.*, 1999; Pavlovski *et al.*, 2003; Koreleski and Swiatkiewicz, 2004).

Rao *et al.* (1992) reported that limestone solubility in laying hens improves if retention time is prolonged in the gizzard, which means feeding a minimum particle size of 1.0 mm. A minimum particle size less than 1.0 mm did not sustain retention in the gizzard. Scheideler (1998) reported significantly greater specific gravity (P<0.05) of the eggs from the hens on the diets, which included large particle size calcium (fine and large limestone 50:50 or 75:25 limestone:oyster shell in the diets) compared with hens fed with diets containing 100% fine limestone or 100% ground eggshell. Scheideler (2004), on the basis of her results, recommends that laying hens be fed at least 25% of their calcium from a large particle calcium source. Also, Ahmad and Balander (2003) reported higher egg specific gravity with the partial replacement of limestone (50%) with oyster shell as the calcium source. However, not enough data exists at this time to justify increasing the large particle portion to more than 50% of the ration. Also the requirement of calcium may not be the same, as reported in earlier studies or NRC (1994), to meet the demand for relatively high production in the last third of the laying period. The aim of the present study was to evaluate the effects of a higher proportion of the large particle calcium (taken from different sources) and higher calcium concentration on the calcium retention and the eggshell quality in the last third of the laying period.

MATERIALS AND METHODS

A total of 24 laying hens were divided into 4 groups and they were kept in individual balance cages. They were all fed on the same basic diet (corn 407 g/kg%, wheat 340 g/kg, soybean meal 138 g/kg). The 4 diets, however, varied in the source of calcium as follows: 29% fine limestone (LF) + 71% large limestone (LG: 1 – 2 mm x 1 mm), 32% fine limestone and 68% eggshell (2 - 5 mm x 0.4 mm in the first digestion trial and 1-2 mm x 0.4 mm x 0.8 mm in the second digestion trial), 32% fine limestone and 68% oyster shell (2 - 5 mm, Oyta Oystershells no. 1), 50% fine limestone and 50% large limestone (1 - 2 mm). The contents of these sources of calcium in the diets were as follows: 103.3 g/kg, 93.3 g/kg, 93.3 g/kg and 93.3 g/kg respectively; consequently, the content of calcium was higher in the diet 29:68 LF:LG. The fine limestone and the large limestone were from the same source. The compositions of the diets are shown in Table 1. After a 5-d adjustment period, when the hens were fed *ad libitum*, two digestion trials were done. Each digestion trial took 5 d and between digestion trials there was a 2-d pause. In both trials the same hens received the same diets. The results in each group were calculated as average of 12 samples (2 x 6). The hens were 56 and 57 weeks of age. Light was provided for 16 h per d from 0400 h to 2000 h. During the first digestion trial the hens were given 120 g of the mixtures at 0830 h. In the second digestion trial, a midnight feeding was added and the hens were fed 90 g of the mixtures during the light period and 30 g during the dark period. At the end of each digestion trial the remainder of the diets in the feeders were weighed and average daily food intake was calculated for each hen. During the dark period the light was turned on for 1 h from 2400 h to 0100 h. Water was provided *ad libitum* throughout the day. Droppings were collected every 24 h and dried at 65 ^{o}C each day after collection. The retention of calcium, energy, nitrogen, ash, phosphorus and fat were estimated using the indicator method.

Insoluble ash in 3 M HCl was used as an indicator. The content of nitrogen was determined according to Kjeldahl. The content of fat was determined according to Soxhlet. Ash was determined after combustion at 550 ^{o}C. The content of calcium and phosphorus were determined by spectrophotometry. The energy was determined in an automatic bomb calorimeter PARR 1281 (Parr Instrument Company, Illinois, USA). An acid correction was not done. All the eggs were collected and weighed. The strength of the eggshells (N) was measured manually by destructive methods. Eggs were compressed between two parallel plates by a steadily increasing load until failure resulted. The force was recorded throughout each test and the strength of the eggshell was given in terms of the force at failure. The force was measured vertically to the axis. Also the thickness (the average of both ends and at the middle) of eggshell was evaluated and dry eggshells were weighed.

Table 1 near here

On the basis of the weight of the eggshells, the calcium content in the eggshells and the calcium retention, the proportions of retained calcium deposited in the eggshells were determined in each group. The following equations were used:

$Ca_p = 100 * Ca_a / Ca_r$

Ca_p = proportion of retained Ca deposited into the eggshells (%)

Ca_a = amount of calcium deposited to the eggshells (g/hen/d)

$Ca_a = Wt_e \times Ca_e / 100$

Wt_e = weight of dry eggshells (g/hen.d)

Ca_e = calcium content in the eggshells (%)

Ca_r = calcium retention (g/hen.d)

$Ca_r = Ca_i \times Ca_c$

Ca_i = calcium intake (g/hen.d)

Ca_c = coefficient of calcium retention

Data were analysed throughout by one-way analysis of variance (ANOVA) using the software package *Unistat 5.1* (UNISTAT Ltd, England). Tukey-HSD (eggshell quality), and Least Significant Difference (the coefficients of nutrient retention) were used as the *post hoc* test for all possible pair-wise comparisons within groups.

The in vitro solubility of the limestone used in the study was determined by the method described by Zhang and Coon (1997). A 2.0 g limestone sample was poured into a 400 ml beaker containing 200 ml of 0.2 N HCl solution that was warmed at 42 °C until the temperature of the solution became constant in a water bath oscillation at 80 Hz. After allowing 10 min for reaction, the undissolved limestone was filtered onto a preweighed filter paper and weighted after draying in a 60 °C oven for 20 h. The *in vitro* solubility of limestone vas expressed as the percentage weight loss.

RESULTS AND DISCUSSION

The coefficients of the retention of the dry matter, fat, energy, calcium, nitrogen, ash and phosphorus are shown in Table 2. The coefficients of the calcium retention were significantly higher (P=0.0271) in 50:50 LF:LG and LF:Eggshell in comparison with 29:71 LF:LG and LF:Oystershell. Also, Scheideler (1998) found that the highest retention of calcium was from eggshells. The midnight feeding significantly improved the retention of calcium (P<0.01) in all of the groups except 50:50 LF:LG (Table 4). The highest retention of calcium and the highest retention of all others nutrients (energy, nitrogen, ash and phosphorus) were found in the rations with eggshells.

Tables 2,3,4 near here

The weight of the eggs and the quality of the eggshells is shown in Table 3. The weight of the eggs was significantly lower (P=0.0422) in the 29:71 LF:LG. In this group the amount of calcium was the highest. The retention of energy was significantly lower (*P*=<0.05). However, Rao *et al.* (2003), Chandramoni *et al.* (1998), and Keshavarz and Nakajima (1993) did not find any indications of calcium contents greater than 32.5 g/kg (max. 55.0 Ca g/kg) of the egg weight. The midnight feeding significantly increased the weight of the eggs (*P*<0.05) except in the 50:50 LF:LG group (Table 4). The midnight feeding did not have a significant effect on the eggshell quality. The eggshell weight (*P*<0.05), thickness (*P*<0.001) and strength (*P*=0.001) were significantly higher in the group with oyster shell and 29:71 LF:LG in comparison with the diets containing eggshell and 50:50 LF:LG. The same improvement of eggshell strength was reported by Richter *et al*. (1999) when the hens were given limestone with a particle size of 0.5 - 2.0 mm, or a mixture of one-third finely ground limestone and two-thirds oyster shell. The beneficial effects of oyster shell on eggshell quality were consistent with the reports of Keshavarz and Nakajima (1993). Although also in the group 32:68 LF:eggshell the greater the amount of large particles the worse was the quality of the eggshell (eggshell weight, eggshell weight ratio and eggshell thickness). This may have been due to the low solubility of the eggshells. The *in vitro* solubility was as follows: fine limestone 85.0%, eggshells 14.0%, oyster shell 44.0% and large limestone 49.5%. Cheng and Coon (1990) reported that the eggshell quality (and bone status) were more closely related with limestone *in vitro* solubility than particle size. The researchers indicated a potential difference in calcium retention for layers when two calcium sources of the same particle size with different *in vitro* solubility were compared. On the other hand, Rao and Roland (1990) reported that *in vivo* calcium solubilisation in laying hens, for the particle size tested, was not influenced by *in vitro* limestone solubility.

The source and the particle size of the calcium probably have a greater effect on eggshell quality than does the calcium level. Although in the 29:71 LF:LG diet the content of calcium was about 12% higher than in the 32:68 LF:oyster shell diet, the quality of the eggshell was not improved. There was no significant difference in eggshell strength, thickness, weight ratio or eggshell weight between these groups. Neither Chandramoni *et al*. (1998) who used calcium concentrations of 32.5, 36.0 and 39.0 g/kg nor Rao *et al*. (2003), who used 32.5, 35.0, 37.5, 40.0, 42.5 and 45.0 g/kg, observed any improvement in the eggshell quality (eggshell weight, eggshell weight per unit surface area and eggshell weight, and eggshell thickness respectively). However, Chandramoni *et al.* (1998) used limestone powder and bone meal. Rao *et al*. (2003) used oyster shell (powder and grit 1 - 2 mm) but the weights of the eggs in their experiments were almost 10 g lighter than the eggs in this experiment were, and consequently the requirement of calcium was lower.

Contrary to Clunies *et al*. (1992), there was no significant difference in the total retention of calcium between the groups, which allowed higher and lower eggshell quality (thickness). Also Keshavarz and Nakajima (1993) did not find any significant difference in the total retention of calcium among concentrations of 35.0 - 55.0 g/kg in the diets. Probably the ability to utilise the calcium from the retained calcium for eggshell formation plays an important role. The utilisation of calcium for the formation of the eggshell was calculated and shown in Table 3. It was calculated on the basis of calcium retention, eggshell production (Figure 1) and the calcium content of the eggshells. The daily intake of calcium and the content of calcium in the eggshells are shown in Table 3. In the group of hens given oyster shells, 96.5 % of the retained calcium was deposited in the eggshells. In the groups given eggshells and 50:50 LF:LG the utilisation was only 73.9 and 78.6 % respectively, although the coefficients of calcium retention were significantly higher (P<0.05) in these groups. There was no difference in the daily eggshell production (g) among the groups (Figure 1). This was probably due to the fact that the hens digest the calcium from the large particle oyster shell (2 - 5 mm) slowly but constantly, and it is utilised immediately for eggshell formation.

Figure 1 near here

Conclusions

Two thirds of the calcium source should be fed in the form of large particles (limestone grit or oyster shell) in the last third of the laying period to ensure good eggshell quality. Leeson *et al*. (1993) indicated that 3.4 g calcium/d is enough for brown-egg layers because they did not observe any effect of higher levels of calcium on eggshell deformation in these hens, but they used only limestone as a source of calcium in all of the rations. On the basis of the present study, eggshell quality can be improved by using suitable sources and particle sizes of calcium. Higher retention or concentration of calcium does not, in itself, mean better eggshell quality.

ACKNOWLEDGEMENTS

The author thanks Judy McPherson for the language correction and Assistant Professor Stanislav Kracmar, Dr.Sc. for the measurements of energy in the samples. The results were obtained with financial support of project 42/2005 IGA of Mendel University of Agriculture and Forestry in Brno.

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Table 1. *The composition of the diets*

Composition (g/kg)	29:71 LF:LG	32:68 LF:eggshell	32:68 LF:oyster shell	50:50 LF:LG
Maize		407		
Wheat		340		
Soybean meal		138		
Monocalcium phosphate		9.5		
Methionine		1.25		
L-lysine		3.0		
Salt		3.0		
Premix		5.0		
Limestone Fine	30	30	30	46.65
Lime Grit	73.3*	-	-	46.65
Eggshells	-	63.3	-	-
Oyster shells	-	-	63.3	-
Calcium (g/kg)	44.6	39.7	39.8	40.8
Total phosphorus (g/kg)	5.7	5.2	5.4	5.2

* The content of wheat was 330 g/kg in this diet.

Table 2. *The coefficients of nutrient retention*

	29:71 LF:LG Mean ± SE	32:68 LF:eggshell Mean ± SE	32:68 LF:oyster shell Mean ± SE	50:50 LF:LG Mean ± SE	*P* -values
Retention of nutrients					
Dry matter	0.714 ± 0.0139[a]	0.784 ± 0.0136[b]	0.748 ± 0.0173	0.751 ± 0.0121	<0.05
Energy	0.758 ± 0.0135[a]	0.818 ± 0.0136[b]	0.786 ± 0.0168	0.786 ± 0.0123	<0.05
Nitrogen	0.367 ± 0.0353[a]	0.541 ± 0.0290[b]	0.465 ± 0.0441	0.455 ± 0.0290	<0.05
Ash	0.488 ± 0.0134[b]	0.577 ± 0.0134[a]	0.518 ± 0.0165[b]	0.515 ± 0.0131[b]	<0.001
Calcium - **Ca_c**	0.506 ± 0.0142[a]	0.576 ± 0.0177[b]	0.515 ± 0.0334[a]	0.578 ± 0.0123[b]	<0.05
Phosphorus	0.219 ± 0.0464[a]	0.415 ± 0.0420[b]	0.353 ± 0.0388[b]	0.293 ± 0.0484	<0.05
Fat	0.683 ± 0.0247	0.735 ± 0.0231	0.712 ± 0.0271	0.677 ± 0.0217	NS

SE - standard error.

NS - non-significant.

[a,b,c]Values in a row not sharing a common superscript are significantly different at P<0.05.

Table 3. *Eggshell quality and calcium requirements*

	29:71 LF:LG Mean ± SE	32:68 LF:Eggshell Mean ± SE	32:68 LF:Oyster shell Mean ± SE	50:50 LF:LG Mean ± SE	*P* -values
Egg production (eggs)	52	49	46	53	-
Food intake (g/d)	109.3	108.5	104.9	109.3	-
Ca intake - $\mathbf{Ca_i}$ (g/d)	4.9 ± 0.24	4.3 ± 0.20	4.2 ± 0.30	4.5 ± 0.13	NS
Weight of hens (kg)	1.63 ± 0.074	1.66 ± 0.057	1.76 ± 0.075	1.65 ± 0.091	NS
Egg weight (g)	61.7 ± 0.45[a]	63.7 ± 0.66[b]	64.0 ± 0.59[b]	63.9 ± 0.78[b]	<0.05
Eggshell weight (g)	6.63 ± 0.058[b]	6.24 ± 0.086[a]	6.69 ± 0.110[b]	6.40 ± 0.130	<0.01
Eggshell weight ratio (%)	10.8 ± 0.087[b]	9.81 ± 0.108[ac]	10.4 ± 0.126[bc]	10.0 ± 0.154[c]	<0.001
Eggshell thickness (mm)	0.432 ± 0.0034[b]	0.397 ± 0.0045[a]	0.428 ± 0.0050[b]	0.406 ± 0.0067[a]	<0.001
Eggshell strength (N)	38.1 ± 1.01[b]	35.7 ± 0.97	39.1 ± 1.14[b]	32.6 ± 1.65[a]	<0.01
Ca in eggshell - $\mathbf{Ca_e}$ (g/kg)	359.7 ± 1.75	359.1 ± 1.30	359.8 ± 1.26	359.4 ± 1.26	NS
Ca eggshell : Ca retention - $\mathbf{Ca_p}$ (%)	84.1 ± 4.18	73.9 ± 8.04	96.5 ± 10.0	78.6 ± 3.97	NS

SE - standard error.

NS - non-significant.

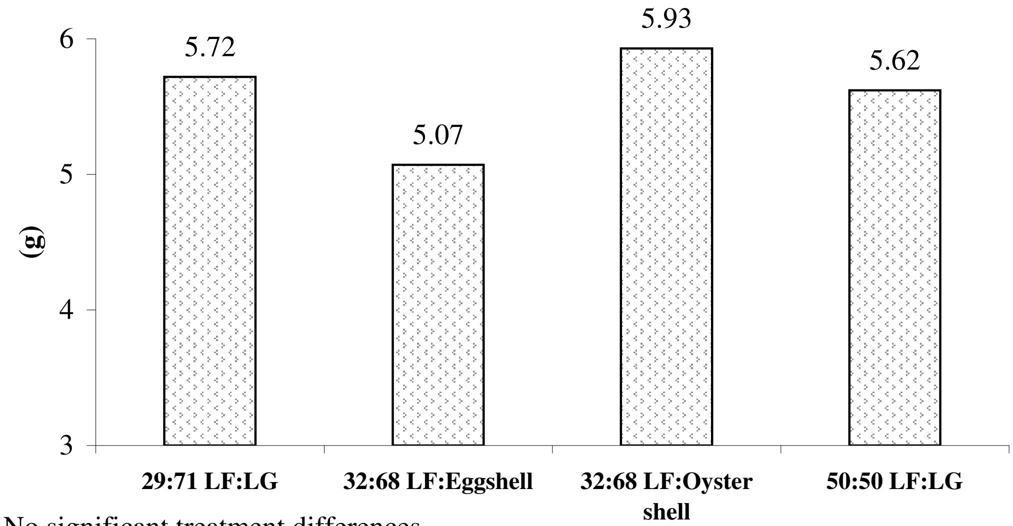
[a,b,c]Values in a row not sharing a common superscript are significantly different at *P* <0.05.

Table 4. *The effect of midnight feeding on the coefficients of calcium retention and egg weight*

	29:71 LF:LG	32:68 LF:Eggshell	32:68 LF:Oyster shell	50:50 LF:LG
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Retention of calcium				
Daily feeding	0.471 ± 0.0161	0.525 ± 0.0163	0.418 ± 0.0324	0.564 ± 0.0136
Midnight feeding	0.540 ± 0.0124	0.628 ± 0.0061	0.613 ± 0.0747	0.591 ± 0.0203
P -values	<0.01	<0.001	<0.001	NS
Weight of eggs				
Daily feeding	60.8 ± 0.62	61.7 ± 0.81	62.3 ± 0.77	62.8 ± 1.02
Midnight feeding	62.6 ± 0.60	65.4 ± 0.83	65.8 ± 0.82	64.5 ± 1.12
P -values	<0.05	<0.01	<0.01	NS

SE - standard error.
NS - non-significant.

Figure 1. *Daily eggshell production (g/hen).*



No significant treatment differences.