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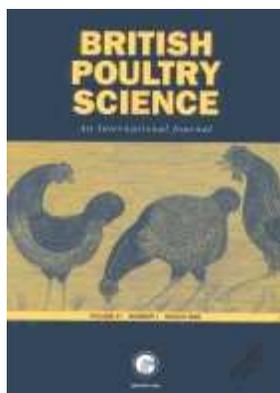
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**The effect of the calcium source, level and the particle size on calcium retention, eggshell quality and the overall calcium requirement in laying hens**

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

M. LICHNIKOVA

*Department of Animal Breeding, Mendel University of Agriculture and Forestry in  
Brno, Zemedelska 1, 613 00 Brno, Czech Republic*

**RUNNING TITLE: CALCIUM SOURCE AND SHELL QUALITY**

Telephone: ++420 54513 3229

Email: [lichov@mendelu.cz](mailto:lichov@mendelu.cz)

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1  
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3  
4 25 **Abstract** 1. Four different sources of calcium in isonitrogenous and isoenergetic  
5  
6 26 diets were fed to laying hens for two weeks when they were 56 and 57 weeks old.  
7  
8 27 The calcium source blends were as follows: 29% fine limestone (LF) + 71% large  
9  
10 28 limestone (LG), 32% fine limestone (LF) + 68% eggshell, 32% fine limestone (LF) +  
11  
12 29 68% oyster shell, 50% fine limestone + 50% large limestone (LG). The contents of  
13  
14 30 these blends of calcium in the diets were as follows: 103.3 g/kg, 93.3 g/kg, 93.3 g/kg  
15  
16 31 and 93.3 g/kg respectively.  
17  
18 32 2. The coefficients of calcium retention were significantly higher in 50:50 LF:LG  
19  
20 33 (0.578) and 32:68 LF:eggshell (0.576). The midnight feeding significantly improved  
21  
22 34 the coefficient of calcium retention in all mixtures except 50:50 LF:LG.  
23  
24 35 3. In the mixture 29:71 LF:LG and 32:68 LF:oyster shell, there was significantly  
25  
26 36 greater eggshell quality, eggshell weight, eggshell thickness and eggshell strength.  
27  
28 37 Midnight feeding had no significant effect on eggshell quality.  
29  
30 38 4. In the ration with oyster shells, 96.5% of the retained calcium was deposited in the  
31  
32 39 eggshell, but in ration 32:68 LF:eggshells and 50:50 LF:LG the utilisation was only  
33  
34 40 73.9% and 78.6% respectively.  
35  
36 41 5. To ensure good quality eggshells in the last third of production, the  
37  
38 42 recommendation for calcium is 4.1 g/kg (900 g/kg dry matter, feed intake 110 g/d).  
39  
40 43 As a source of calcium in this stage of production, a feed mixture containing two-  
41  
42 44 thirds large particles should be used (limestone grit or oyster shell).  
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45

#### 45 INTRODUCTION

46 The quality of the eggshells has a major influence on the economics of egg  
47  
48 47 production. Damaged or broken shells account for 6-8% of all the eggs laid (Bain,  
49  
50 48 1997) and the highest incidence of cracked eggs occurs mainly in the last third of the  
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52 49 laying period (after 53 weeks of age). The source and particle size of mineral  
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4 74 A total of 24 laying hens were divided into 4 groups and they were kept in individual  
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6 75 balance cages. They were all fed on the same basic diet (corn 407 g/kg%, wheat 340  
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8 76 g/kg, soybean meal 138 g/kg). The 4 diets, however, varied in the source of calcium  
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10  
11 77 as follows: 29% fine limestone (LF) + 71% large limestone (LG: 1 – 2 mm x 1 mm),  
12  
13 78 32% fine limestone and 68% eggshell (2 - 5 mm x 0.4 mm in the first digestion trial  
14  
15 79 and 1-2 mm x 0.4 mm x 0.8 mm in the second digestion trial), 32% fine limestone  
16  
17 80 and 68% oyster shell (2 - 5 mm, Oyta Oystershells no. 1), 50% fine limestone and  
18  
19 81 50% large limestone (1 - 2 mm). The contents of these sources of calcium in the diets  
20  
21 82 were as follows: 103.3 g/kg, 93.3 g/kg, 93.3 g/kg and 93.3 g/kg respectively;  
22  
23 83 consequently, the content of calcium was higher in the diet 29:68 LF:LG. The fine  
24  
25 84 limestone and the large limestone were from the same source. The compositions of  
26  
27 85 the diets are shown in Table 1. After a 5-d adjustment period, when the hens were  
28  
29 86 fed *ad libitum*, two digestion trials were done. Each digestion trial took 5 d and  
30  
31 87 between digestion trials there was a 2-d pause. In both trials the same hens received  
32  
33 88 the same diets. The results in each group were calculated as average of 12 samples (2  
34  
35 89 x 6). The hens were 56 and 57 weeks of age. Light was provided for 16 h per d from  
36  
37 90 0400 h to 2000 h. During the first digestion trial the hens were given 120 g of the  
38  
39 91 mixtures at 0830 h. In the second digestion trial, a midnight feeding was added and  
40  
41 92 the hens were fed 90 g of the mixtures during the light period and 30 g during the  
42  
43 93 dark period. At the end of each digestion trial the remainder of the diets in the  
44  
45 94 feeders were weighed and average daily food intake was calculated for each hen.  
46  
47 95 During the dark period the light was turned on for 1 h from 2400 h to 0100 h. Water  
48  
49 96 was provided *ad libitum* throughout the day. Droppings were collected every 24 h  
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51 97 and dried at 65 °C each day after collection. The retention of calcium, energy,  
52  
53 98 nitrogen, ash, phosphorus and fat were estimated using the indicator method.  
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4 99 Insoluble ash in 3 M HCl was used as an indicator. The content of nitrogen was  
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6 100 determined according to Kjeldahl. The content of fat was determined according to  
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8 101 Soxhlet. Ash was determined after combustion at 550 °C. The content of calcium and  
9  
10 102 phosphorus were determined by spectrophotometry. The energy was determined in  
11  
12 103 an automatic bomb calorimeter PARR 1281 (Parr Instrument Company, Illinois,  
13  
14 104 USA). An acid correction was not done. All the eggs were collected and weighed.  
15  
16 105 The strength of the eggshells (N) was measured manually by destructive methods.  
17  
18 106 Eggs were compressed between two parallel plates by a steadily increasing load until  
19  
20 107 failure resulted. The force was recorded throughout each test and the strength of the  
21  
22 108 eggshell was given in terms of the force at failure. The force was measured vertically  
23  
24 109 to the axis. Also the thickness (the average of both ends and at the middle) of  
25  
26 110 eggshell was evaluated and dry eggshells were weighed.

Table 1 near here

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

114  $Ca_p = 100 * Ca_a / Ca_r$

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

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

117  $Ca_a = Wt_e \times Ca_e / 100$

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

119  $Ca_e$  = calcium content in the eggshells (%)

120  $Ca_r$  = calcium retention (g/hen.d)

121  $Ca_r = Ca_i \times Ca_c$

122  $Ca_i$  = calcium intake (g/hen.d)

123  $Ca_c$  = coefficient of calcium retention

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4 124 Data were analysed throughout by one-way analysis of variance (ANOVA)  
5  
6 125 using the software package *Unistat 5.1* (UNISTAT Ltd, England). Tukey-HSD  
7  
8 126 (eggshell quality), and Least Significant Difference (the coefficients of nutrient  
9  
10 127 retention) were used as the *post hoc* test for all possible pair-wise comparisons within  
11  
12 128 groups.

13  
14  
15 129 The *in vitro* solubility of the limestone used in the study was determined by  
16  
17 130 the method described by Zhang and Coon (1997). A 2.0 g limestone sample was  
18  
19 131 poured into a 400 ml beaker containing 200 ml of 0.2 N HCl solution that was  
20  
21 132 warmed at 42 °C until the temperature of the solution became constant in a water  
22  
23 133 bath oscillation at 80 Hz. After allowing 10 min for reaction, the undissolved  
24  
25 134 limestone was filtered onto a preweighed filter paper and weighted after drying in a  
26  
27 135 60 °C oven for 20 h. The *in vitro* solubility of limestone was expressed as the  
28  
29 136 percentage weight loss.

## 30 137 RESULTS AND DISCUSSION

31  
32  
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35 138 The coefficients of the retention of the dry matter, fat, energy, calcium, nitrogen, ash  
36  
37 139 and phosphorus are shown in Table 2. The coefficients of the calcium retention were  
38  
39 140 significantly higher ( $P=0.0271$ ) in 50:50 LF:LG and LF:Eggshell in comparison with  
40  
41 141 29:71 LF:LG and LF:Oystershell. Also, Scheideler (1998) found that the highest  
42  
43 142 retention of calcium was from eggshells. The midnight feeding significantly  
44  
45 143 improved the retention of calcium ( $P<0.01$ ) in all of the groups except 50:50 LF:LG  
46  
47 144 (Table 4). The highest retention of calcium and the highest retention of all others  
48  
49 145 nutrients (energy, nitrogen, ash and phosphorus) were found in the rations with  
50  
51 146 eggshells.

Tables 2,3,4 near here

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53  
54  
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57  
58 147 The weight of the eggs and the quality of the eggshells is shown in Table 3.  
59  
60 148 The weight of the eggs was significantly lower ( $P=0.0422$ ) in the 29:71 LF:LG. In

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4 149 this group the amount of calcium was the highest. The retention of energy was  
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6 150 significantly lower ( $P<0.05$ ). However, Rao *et al.* (2003), Chandramoni *et al.*  
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8 151 (1998), and Keshavarz and Nakajima (1993) did not find any indications of calcium  
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10 152 contents greater than 32.5 g/kg (max. 55.0 Ca g/kg) of the egg weight. The midnight  
11  
12 153 feeding significantly increased the weight of the eggs ( $P<0.05$ ) except in the 50:50  
13  
14 154 LF:LG group (Table 4). The midnight feeding did not have a significant effect on the  
15  
16 155 eggshell quality. The eggshell weight ( $P<0.05$ ), thickness ( $P<0.001$ ) and strength  
17  
18 156 ( $P=0.001$ ) were significantly higher in the group with oyster shell and 29:71 LF:LG  
19  
20 157 in comparison with the diets containing eggshell and 50:50 LF:LG. The same  
21  
22 158 improvement of eggshell strength was reported by Richter *et al.* (1999) when the  
23  
24 159 hens were given limestone with a particle size of 0.5 - 2.0 mm, or a mixture of one-  
25  
26 160 third finely ground limestone and two-thirds oyster shell. The beneficial effects of  
27  
28 161 oyster shell on eggshell quality were consistent with the reports of Keshavarz and  
29  
30 162 Nakajima (1993). Although also in the group 32:68 LF:eggshell the greater the  
31  
32 163 amount of large particles the worse was the quality of the eggshell (eggshell weight,  
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34 164 eggshell weight ratio and eggshell thickness). This may have been due to the low  
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36 165 solubility of the eggshells. The *in vitro* solubility was as follows: fine limestone  
37  
38 166 85.0%, eggshells 14.0%, oyster shell 44.0% and large limestone 49.5%. Cheng and  
39  
40 167 Coon (1990) reported that the eggshell quality (and bone status) were more closely  
41  
42 168 related with limestone *in vitro* solubility than particle size. The researchers indicated  
43  
44 169 a potential difference in calcium retention for layers when two calcium sources of the  
45  
46 170 same particle size with different *in vitro* solubility were compared. On the other  
47  
48 171 hand, Rao and Roland (1990) reported that *in vivo* calcium solubilisation in laying  
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50 172 hens, for the particle size tested, was not influenced by *in vitro* limestone solubility.  
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4 173 The source and the particle size of the calcium probably have a greater effect  
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6 174 on eggshell quality than does the calcium level. Although in the 29:71 LF:LG diet  
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9 175 the content of calcium was about 12% higher than in the 32:68 LF:oyster shell diet,  
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11 176 the quality of the eggshell was not improved. There was no significant difference in  
12  
13 177 eggshell strength, thickness, weight ratio or eggshell weight between these groups.  
14  
15 178 Neither Chandramoni *et al.* (1998) who used calcium concentrations of 32.5, 36.0  
16  
17 179 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  
18  
19 180 g/kg, observed any improvement in the eggshell quality (eggshell weight, eggshell  
20  
21 181 weight per unit surface area and eggshell weight, and eggshell thickness  
22  
23 182 respectively). However, Chandramoni *et al.* (1998) used limestone powder and bone  
24  
25 183 meal. Rao *et al.* (2003) used oyster shell (powder and grit 1 - 2 mm) but the weights  
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27 184 of the eggs in their experiments were almost 10 g lighter than the eggs in this  
28  
29 185 experiment were, and consequently the requirement of calcium was lower.  
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34 186 Contrary to Clunies *et al.* (1992), there was no significant difference in the  
35  
36 187 total retention of calcium between the groups, which allowed higher and lower  
37  
38 188 eggshell quality (thickness). Also Keshavarz and Nakajima (1993) did not find any  
39  
40 189 significant difference in the total retention of calcium among concentrations of 35.0 -  
41  
42 190 55.0 g/kg in the diets. Probably the ability to utilise the calcium from the retained  
43  
44 191 calcium for eggshell formation plays an important role. The utilisation of calcium for  
45  
46 192 the formation of the eggshell was calculated and shown in Table 3. It was calculated  
47  
48 193 on the basis of calcium retention, eggshell production (Figure 1) and the calcium  
49  
50 194 content of the eggshells. The daily intake of calcium and the content of calcium in  
51  
52 195 the eggshells are shown in Table 3. In the group of hens given oyster shells, 96.5 %  
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54 196 of the retained calcium was deposited in the eggshells. In the groups given eggshells  
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56 197 and 50:50 LF:LG the utilisation was only 73.9 and 78.6 % respectively, although the  
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4 198 coefficients of calcium retention were significantly higher ( $P<0.05$ ) in these groups.  
5  
6 199 There was no difference in the daily eggshell production (g) among the groups  
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8 200 (Figure 1). This was probably due to the fact that the hens digest the calcium from  
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10 201 the large particle oyster shell (2 - 5 mm) slowly but constantly, and it is utilised  
11  
12 202 immediately for eggshell formation.

Figure 1 near here

### 203 **Conclusions**

17  
18 204 Two thirds of the calcium source should be fed in the form of large particles  
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20 205 (limestone grit or oyster shell) in the last third of the laying period to ensure good  
21  
22 206 eggshell quality. Leeson *et al.* (1993) indicated that 3.4 g calcium/d is enough for  
23  
24 207 brown-egg layers because they did not observe any effect of higher levels of calcium  
25  
26 208 on eggshell deformation in these hens, but they used only limestone as a source of  
27  
28 209 calcium in all of the rations. On the basis of the present study, eggshell quality can be  
29  
30 210 improved by using suitable sources and particle sizes of calcium. Higher retention or  
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32 211 concentration of calcium does not, in itself, mean better eggshell quality.

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42  
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44  
45 216 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	32:68 LF:eggshell	32:68 LF:oyster shell	50:50 LF:LG	<i>P</i> -values
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	
Retention of nutrients					
Dry matter	0.714 ± 0.0139 <sup>a</sup>	0.784 ± 0.0136 <sup>b</sup>	0.748 ± 0.0173	0.751 ± 0.0121	<0.05
Energy	0.758 ± 0.0135 <sup>a</sup>	0.818 ± 0.0136 <sup>b</sup>	0.786 ± 0.0168	0.786 ± 0.0123	<0.05
Nitrogen	0.367 ± 0.0353 <sup>a</sup>	0.541 ± 0.0290 <sup>b</sup>	0.465 ± 0.0441	0.455 ± 0.0290	<0.05
Ash	0.488 ± 0.0134 <sup>b</sup>	0.577 ± 0.0134 <sup>a</sup>	0.518 ± 0.0165 <sup>b</sup>	0.515 ± 0.0131 <sup>b</sup>	<0.001
Calcium - <b>Ca<sub>c</sub></b>	0.506 ± 0.0142 <sup>a</sup>	0.576 ± 0.0177 <sup>b</sup>	0.515 ± 0.0334 <sup>a</sup>	0.578 ± 0.0123 <sup>b</sup>	<0.05
Phosphorus	0.219 ± 0.0464 <sup>a</sup>	0.415 ± 0.0420 <sup>b</sup>	0.353 ± 0.0388 <sup>b</sup>	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.

<sup>a,b,c</sup>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	32:68 LF:Eggshell	32:68 LF:Oyster shell	50:50 LF:LG	<i>P</i> -values
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	
Egg production (eggs)	52	49	46	53	-
Food intake (g/d)	109.3	108.5	104.9	109.3	-
Ca intake - $Ca_i$ (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 <sup>a</sup>	63.7 ± 0.66 <sup>b</sup>	64.0 ± 0.59 <sup>b</sup>	63.9 ± 0.78 <sup>b</sup>	<0.05
Eggshell weight (g)	6.63 ± 0.058 <sup>b</sup>	6.24 ± 0.086 <sup>a</sup>	6.69 ± 0.110 <sup>b</sup>	6.40 ± 0.130	<0.01
Eggshell weight ratio (%)	10.8 ± 0.087 <sup>b</sup>	9.81 ± 0.108 <sup>ac</sup>	10.4 ± 0.126 <sup>bc</sup>	10.0 ± 0.154 <sup>c</sup>	<0.001
Eggshell thickness (mm)	0.432 ± 0.0034 <sup>b</sup>	0.397 ± 0.0045 <sup>a</sup>	0.428 ± 0.0050 <sup>b</sup>	0.406 ± 0.0067 <sup>a</sup>	<0.001
Eggshell strength (N)	38.1 ± 1.01 <sup>b</sup>	35.7 ± 0.97	39.1 ± 1.14 <sup>b</sup>	32.6 ± 1.65 <sup>a</sup>	<0.01
Ca in eggshell - $Ca_e$ (g/kg)	359.7 ± 1.75	359.1 ± 1.30	359.8 ± 1.26	359.4 ± 1.26	NS
Ca eggshell : Ca retention - $Ca_p$ (%)	84.1 ± 4.18	73.9 ± 8.04	96.5 ± 10.0	78.6 ± 3.97	NS

SE - standard error.

NS - non-significant.

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

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**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
<b>Retention of calcium</b>				
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
<i>P</i> -values	<0.01	<0.001	<0.001	NS
<b>Weight of eggs</b>				
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
<i>P</i> -values	<0.05	<0.01	<0.01	NS

SE - standard error.  
NS - non-significant.

