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Effects on performance and eggshell quality of particle size of calcium sources in laying hens' diets with different Ca concentrations

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Abstract. The objective of this study was to evaluate the effect of particle size of a dietary Ca source on egg production and eggshell quality when added to hens' diets that have different levels of calcium. The experiment was carried out on 216 ISA Brown hens (25 to 70 weeks of age), allocated to 9 groups of 12 replicates (cages), with two birds in each cage. A 3 × 3 factorial arrangement was used, with three dietary levels of calcium (3.20, 3.70 and 4.20 %) and three levels of dietary substitutions (0, 25 and 50 %) of fine particles of limestone (FPL, diameter 0.2–0.6 mm) with large particles of limestone (LPL, diameter 1.0–1.4 mm) as a Ca source.

The level of Ca in the diet had no effect on egg production, mean egg weight, feed intake, feed conversion ratio or eggshell quality parameters ($P>0.05$). Substitution of FPL with LPL did not affect laying performance indices or eggshell quality at 30, 43 and 53 weeks of age ($P>0.05$); however, it increased ($P<0.05$) eggshell percentage, thickness, density and breaking strength in older hens (69 weeks of age). In conclusion, the results of this study demonstrated that a level of 3.20 % Ca in a layer's diet is sufficient through the entire laying cycle to maintain good egg production and eggshell quality and that partial (25 or 50 %) substitution of fine- with large-particle limestone can, irrespective of the level of Ca in the diet, improve eggshell quality in aged laying hens.

1 Introduction

Eggshell quality is one of the most significant issues in the egg industry. Protection of the embryo from the detrimental effects of various environmental elements, regulation of gas and water exchange, and supplying calcium for embryonic development are the most important functions of the eggshell (Narushin and Romanov, 2002). Insufficient eggshell quality, i.e. low breaking strength or the presence of shell defects, negatively affects the economic profitability of egg production, as well as decreasing the hatchability of eggs. Moreover, eggshell quality is also a very important concern for consumers, as high resistance to breaking and lack of shell defects are necessary in order to protect against the penetration of pathogenic bacteria into eggs.

The huge impact of eggshell quality on the profitability of the egg industry was proved by several early studies showing that damaged eggs, i.e. eggs with shell defects, account for 6–10 % of all eggs produced (Washburn, 1982; Roland, 1988; Bain, 1997), and most egg deficiencies relate to eggshell problems (Pavloski et al., 2012). One of the main concerns is the decrease in eggshell quality with hens' age (Al-Batshan et al., 1994; Arpasova et al., 2010), since the incidence of cracked eggs can exceed 20 % in hens late in the production cycle (Nys, 2001). This trend can be attributed to increases in total egg weight without sufficient increases in shell deposition (Roland, 1980), and to disorders in the vitamin D_3

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metabolism in older hens (Abe et al., 1982). For these reasons, there is still a high level of interest in studies of factors affecting eggshell quality, and efficient ways of improving eggshell indices are of critical importance for the poultry industry.

Because of the chemical composition of eggshells, i.e. the fact that 95 % of the shell is made up of calcium carbonate, an optimal supply of Ca to hens is the most important nutritional factor determining eggshell quality. Providing the layer with an optimal amount of Ca is crucial in order to ensure proper calcification of the eggshell; however, the results of several earlier experiments demonstrated that the published values for hens' Ca requirements (NRC, 1994) were adequate for optimum shell formation and further increases in Ca dietary level above 3.6–3.9 % usually had no positive influence on eggshell quality indices (Leeson et al., 1993; Bar et al., 2002; Keshavarz, 2003; Valkonen et al., 2010; Pastore et al., 2012). The results of some studies have indicated, however, that replacing fine limestone with coarse limestone, which is characterised by prolonged retention times in the gizzard and is dissolved more slowly, thus supplying the hen more evenly with Ca (ensuring the maintenance of an adequate Ca blood level overnight, when the process of shell calcification is intensive), may positively influence eggshell quality (Guinotte and Nys, 1991; Pavlovski et al., 2003; Koreleski and Świątkiewicz, 2004; Lichovnikova, 2007). Therefore, the aim of this study with laying hens was to evaluate the effect of different dietary Ca levels and particle sizes of the dietary Ca source, i.e., the level of substitution of fine-particle with large-particle limestone, on eggshell quality and egg production indices throughout the entire laying period.

2 Material and methods

2.1 Birds and experimental diets

The Local Cracow Ethics Committee for Experiments with Animals approved all experimental procedures relating to the use of live animals. A total of 216 17-week-old ISA Brown hens, obtained from a commercial source, were placed in a poultry house in cages (two birds per cage) on a wire-mesh floor under controlled climate conditions. The cage dimensions were 30 cm × 120 cm × 50 cm, equating to 3600 cm^2 total floor space. During the pre-experimental period (17 to 24 weeks of age), a commercial laying hens' diet (170 $g\,kg^{-1}$ crude protein, 11.6 MJ/kg apparent metabolisable energy $(AMEn)_N$, 37.0 $g\,kg^{-1}$ calcium and 3.8 $g\,kg^{-1}$ available phosphorus) was offered *ad libitum*.

At week 25, the hens were randomly assigned to one of nine treatments, each comprising 12 replicates (cages) with two hens in each cage, and fed experimental diets until week 70. The composition of the experimental cereal–soybean diets is given in Table 1. During the experiment, the hens had free access to mash feed and water and were exposed to a 14 L:10 D lighting schedule. A 3 × 3 factorial arrangement was used, with three dietary levels of Ca (3.20, 3.70 and 4.20 %) and with three levels of substitutions (0, 25 and 50 %) of fine particles of limestone (FPL, diameter 0.2–0.6 mm) with large particles of limestone (LPL, diameter 1.0–1.4 mm) as a Ca source. The nutritional composition of the experimental diets was calculated based on the chemical composition of raw feedstuffs, and the metabolisable energy value was calculated on the basis of equations from European tables (Janssen, 1989). The chemical composition of the feed materials was analysed using conventional methods (AOAC, 2000). Amino acids were determined in acid hydrolysates, after the initial peroxidation of sulfur amino acids, in a colour reaction with a ninhydrin reagent using a Beckman System Gold 126AA automatic analyser. Calcium content was determined using flame atomic absorption spectrophotometry and phosphorus content was determined using the calorimetric method (AOAC, 2000).

2.2 Measurements

During the experiment, feed intake, and the number and weight of laid eggs were recorded and laying performance, daily egg mass, daily feed intake, and feed conversion per 1 kg of eggs and per individual egg were calculated. At weeks 30, 43, 56 and 69, one egg from each hen was collected to determine eggshell quality, using the EQM (Egg Quality Microprocessor) system (Technical Services and Supplies, York, England) as described by Krawczyk et al. (2013). Another egg was collected for the measurement of shell breaking strength using an Instron 5542 apparatus equipped with a 500 N load cell. The eggs were compressed at a constant crosshead speed of 10 $mm\,min^{-1}$ and the breaking strength was determined at the time of eggshell fracture.

2.3 Statistical analysis

The data were subjected to statistical analysis using a completely randomised design, in accordance with the general linear model (GLM) procedure of Statistica 5.0 (StatSoft, Inc., Tulsa, OK, USA). All data were analysed using two-way ANOVA. When significant differences in treatment means were detected by ANOVA (F test), Duncan's multiple range test was applied to the individual means. Statistical significance was considered to be $P \leq 0.05$.

3 Results and discussion

3.1 Laying performance

Mean egg production, averaged across all dietary treatments throughout the first phase of the laying cycle (25 to 40 weeks of age), was 95.5 %, egg weight was 59.9 g, daily egg mass was 57.3 $g\,hen^{-1}$, daily feed consumption was 113 $g\,hen^{-1}$ and feed conversion was 1.97 kg of feed/1 kg of eggs. Throughout the second phase (41 to 70 weeks) these

Table 1. Composition and nutrient content of experimental diets in grams per kilogram of air-dry matter.

Item	Reduced dietary level of Ca	Standard dietary level of Ca	Increased dietary level of Ca
Corn	417.1	423.1	456.1
Wheat	240.0	210.0	150.0
Soybean meal	230.0	236.0	244.0
Rapeseed oil	13.0	19.0	26.0
Limestone	78.0	90.0	102.0
Monocalcium phosphate	12.5	12.5	12.5
NaCl	3.0	3.0	3.0
DL-Methionine	1.4	1.4	1.4
Vitamin–mineral premix[1]	5.0	5.0	5.0
Nutrient content:			
Metabolisable energy, MJ kg^{-1} [2]	11.60	11.60	11.60
Crude protein	170.0	170.0	170.0
Lys	8.35	8.35	8.35
Met	4.10	4.10	4.10
Ca	32.0	37.0	42.0
Total P	3.15	3.15	3.15
Available P	3.90	3.90	3.90

[1] The premix provided the following per 1 kg of diet: vitamin A – 10 000 IU; vitamin D_3 – 3000 IU; vitamin E – 50 IU; vitamin K_3 – 2 mg; vitamin B_1 – 1 mg; vitamin B_2 – 4 mg; vitamin B_6 – 1.5 mg; vitamin B_{12} – 0.01 mg; Ca-pantotenate – 8 mg; niacine – 25 mg; folic acid – 0.5 mg; choline chloride – 250 mg; manganese – 100 mg; zinc – 50 mg; iron – 50 mg; copper – 8 mg; iodine – 0.8 mg; selenium – 0.2 mg; cobalt – 0.2 mg.
[2] Calculated according to European Table (Janssen, 1989) as a sum of metabolisable energy (ME) content of components.

characteristics were 92.0 %, 62.4 g, 57.7 g, 117 g hen^{-1} and 2.06 kg kg^{-1}, respectively, and throughout the entire experimental period (25 to 70 weeks) they were 93.3 %, 61.4 g, 57.2 g, 116 g and 2.03 kg kg^{-1}, respectively. Neither the dietary level nor the source of Ca (FPL vs. LPL) affected laying performance indices ($P>0.05$). The results of our study may suggest that 3.20 % Ca in the diet is sufficient to supply hens' requirements for egg production and to maintain good laying performance. Similarly, Cufadar et al. (2011) found no differences in egg production, egg weight, egg mass, feed intake and feed conversion ratio when moulted layers were fed diets with 3.0, 3.6 or 4.2 % of Ca. Pelicia et al. (2009) reported that increasing dietary levels of Ca (3.00–4.50 %) did not influence laying performance in hens at the end of the production cycle (58 to 70 weeks of age). In contrast, Saafa et al. (2008) showed that, late in the production cycle (58 to 73 weeks of age), hens require more than 3.5 % of Ca in the diet for optimal laying performance, since an increase in Ca dietary levels to 4.0 % improved egg production, egg mass and the feed conversion ratio. More recently, de Araujo et al. (2011) found that the egg performance of laying hens fed a diet with 4.14 % dietary Ca was significantly higher in comparison with the performance of layers fed diets containing 3.92 or 4.02 % of Ca.

The findings of this experiment confirm the results of several earlier studies with laying hens, in which the absence of any positive effects of increasing the particle sizes of dietary limestone on egg production and feed conversion ratio was noted (Cheng and Coon, 1990; Guinotte and Nys, 1991; Koreleski and Świątkiewicz, 2004; Saafa et al., 2008; De Witt et al., 2009). Olgun et al. (2013) even reported that the replacement of fine particles with very large particles of limestone (> 5 mm) negatively affected laying performance, egg mass and feed intake in hens from 25 to 37 weeks of age.

3.2 Eggshell quality

The mean eggshell percentage, averaged across all dietary treatments throughout the experimental period at 30 weeks of age, was 11.0 %; at 43 weeks of age, 10.8 %; at 59 weeks of age, 10.9 %; and at 69 weeks of age, 10.9 % (Tables 2–5). Mean eggshell thickness averaged 415, 392, 386 and 388 µm; eggshell density averaged 87.7, 85.5, 86.6 and 87.5 mg cm^{-2}; and eggshell breaking strength averaged 48.9, 42.5, 41.9 and 40.4 N, respectively, at 30, 43, 56 and 69 weeks of age. Eggshell quality indices were unaffected by dietary Ca levels ($P>0.05$) at 30, 43, 56 and 69 weeks of age. Thus, the results of our study confirm several earlier findings indicating that the published NRC (1994) values for the Ca requirements of hens are adequate for obtaining good eggshell quality. Similarly, no effects of dietary Ca level (3.0–4.2 %) on eggshell quality, i.e. shell breaking strength, relative weight and thickness, were observed in an experiment with moulted hens (Cufadar et al., 2011). Jiang et al. (2013) even reported that laying hens fed diets with high Ca concentrations (4.4 %) had decreased shell thickness

Table 2. Effects of dietary treatments on eggshell quality at 30 weeks of age.

Item	Dietary Ca level	% of grit in total amount of calcium carbonate used				Standard error of the mean	Effect of		
		0	25	50	Mean		Ca level	Grit supplementation	Interaction
Eggshell percent, %	Reduced	10.72	11.21	11.00	**10.98**	0.062	0.321	0.185	0.156
	Standard	10.83	11.22	10.81	**10.96**				
	Increased	11.41	11.12	10.95	**11.16**				
	Mean	**10.98**	**11.189**	**10.92**					
Eggshell thickness, µm	Reduced	419	413	401	**411**	3.887	0.335	0.891	0.333
	Standard	397	412	427	**412**				
	Increased	424	427	420	**423**				
	Mean	**413**	**418**	**416**					
Eggshell density, mg cm^{-2}	Reduced	86.5	87.1	85.0	**86.2**	0.678	0.245	0.538	0.651
	Standard	85.9	88.6	89.5	**88.0**				
	Increased	87.7	88.3	91.0	**89.0**				
	Mean	**86.7**	**88.0**	**88.5**					
Eggshell breaking strength, N	Reduced	48.1	49.1	48.1	**48.5**	0.648	0.869	0.768	0.727
	Standard	47.0	50.3	49.0	**48.8**				
	Increased	50.8	49.1	48.1	**49.3**				
	Mean	**48.6**	**49.5**	**48.4**					

Table 3. Effects of dietary treatments on eggshell quality at 43 weeks of age.

Item	Dietary Ca level	% of grit in total amount of calcium carbonate used				Standard error of the mean	Effect of		
		0	25	50	Mean		Ca level	Grit supplementation	Interaction
Eggshell percent, %	Reduced	10.70	10.95	10.89	**10.85**	0.077	0.567	0.886	0.513
	Standard	10.46	10.82	10.66	**10.64**				
	Increased	11.01	10.64	10.62	**10.79**				
	Mean	**10.72**	**10.80**	**10.72**					
Eggshell thickness, µm	Reduced	383	404	382	**390**	4.016	0.593	0.178	0.971
	Standard	385	399	384	**389**				
	Increased	401	405	389	**398**				
	Mean	**390**	**402**	**385**					
Eggshell density, mg cm^{-2}	Reduced	84.6	87.2	85.8	**85.9**	0.685	0.865	0.871	0.181
	Standard	83.8	84.9	86.3	**85.0**				
	Increased	89.3	83.0	84.6	**85.7**				
	Mean	**85.9**	**85.0**	**85.6**					
Eggshell breaking strength, N	Reduced	42.4	43.6	42.2	**42.8**	0.554	0.902	0.578	0.854
	Standard	41.1	44.1	41.2	**42.1**				
	Increased	42.5	42.2	42.8	**42.5**				
	Mean	**42.0**	**43.3**	**42.1**					

in comparison with a control group (3.7 % Ca). In contrast, Saafa et al. (2008) found, in a study with hens late in the production cycle, that eggshell quality, i.e. shell weight, thickness and density, was significantly improved when dietary Ca levels were increased from 3.5 to 4.0 %. Moreover, the results of the study with very aged laying hens (58 to 93 weeks of age) indicated that Ca requirements for optimal eggshell quality in hens at that age are slightly higher than the NRC recommendations and amounts, i.e. 3.60–4.00 % (Bar et al., 2002).

Table 4. Effects of dietary treatments on eggshell quality at 56 weeks of age.

Item	Dietary Ca level	% of grit in total amount of calcium carbonate used				Standard error of the mean	Effect of		
		0	25	50	Mean		Ca level	Grit supplementation	Interaction
Eggshell percent, %	Reduced	10.42	11.04	11.09	**10.85**	0.062	0.990	0.035	0.599
	Standard	10.71	10.99	10.89	**10.86**				
	Increased	10.78	10.98	10.87	**10.87**				
	Mean	**10.63[a]**	**11.00[b]**	**10.95[b]**					
Eggshell thickness, µm	Reduced	372	388	386	**382**	1.495	0.067	0.039	0.579
	Standard	384	387	388	**386**				
	Increased	387	392	392	**390**				
	Mean	**381[a]**	**389[b]**	**389[b]**					
Eggshell density, $mg\,cm^{-2}$	Reduced	83.8	87.3	87.1	**86.1**	0.435	0.541	0.197	0.138
	Standard	84.5	87.7	87.8	**86.6**				
	Increased	88.4	86.8	86.5	**87.2**				
	Mean	**85.6**	**87.3**	**87.1**					
Eggshell breaking strength, N	Reduced	39.0	41.8	41.3	**40.7**	0.498	0.203	0.067	0.651
	Standard	40.1	43.7	44.8	**42.9**				
	Increased	41.7	41.8	42.6	**42.0**				
	Mean	**40.3**	**42.5**	**42.9**					

[a,b] The values in the rows with different letters differ significantly ($P \leq 0.05$).

Table 5. Effects of dietary treatments on eggshell quality at 69 weeks of age.

Item	Dietary Ca level	% of grit in total amount of calcium carbonate used				Standard error of the mean	Effect of		
		0	25	50	Mean		Ca level	Grit supplementation	Interaction
Eggshell percent, %	Reduced	10.61	10.96	11.05	**10.87**	0.037	0.263	0.010	0.590
	Standard	10.92	11.04	11.09	**11.02**				
	Increased	10.84	10.99	10.98	**10.94**				
	Mean	**10.79[a]**	**11.00[b]**	**11.05[b]**					
Eggshell thickness, µm	Reduced	369	387	396	**384**	1.805	0.277	0.014	0.113
	Standard	383	389	391	**388**				
	Increased	390	392	391	**391**				
	Mean	**381[a]**	**389[b]**	**393[b]**					
Eggshell density, $mg\,cm^{-2}$	Reduced	83.6	86.9	88.2	**86.2**	0.522	0.160	0.022	0.917
	Standard	85.7	89.1	88.7	**87.8**				
	Increased	87.4	89.1	89.4	**88.6**				
	Mean	**85.5[a]**	**88.4[b]**	**88.8[b]**					
Eggshell breaking strength, N	Reduced	39.4	41.3	39.9	**39.2**	0.485	0.135	0.009	0.456
	Standard	38.5	42.7	43.2	**41.5**				
	Increased	40.2	41.0	40.4	**40.5**				
	Mean	**38.3[a]**	**41.7[b]**	**41.2[b]**					

[a,b] The values in the rows with different letters differ significantly ($P \leq 0.05$).

The source of Ca in the diet had no effect on eggshell quality at 30 and 43 weeks of age; however, substitutions of fine FPL with LPL increased eggshell percentage and thickness at week 56, as well as eggshell percent, thickness, density and breaking strength at week 69 ($P < 0.05$). These results suggest that the use of large-particle limestone may improve eggshell quality in aged laying hens irrespective of dietary Ca level. The mechanism of this effect is probably connected with the possibility that the slower solubility of large-particle limestone (grit) and its prolonged retention time in the gizzard also makes Ca available at night, when there is no feed intake by hens but when the shell calcification process is occurring intensively (Roland and Harms, 1973). This would prevent the mobilisation of bone Ca and P reserves, a process which could affect eggshell quality (Farmer et al., 1986). The results of a study by Zhang and Coon (1997) showed that large-particle limestone (> 0.8 mm) with lower in vitro solubility was retained in the gizzard for a longer time, which significantly increased in vivo solubility and could lead to improved Ca retention in layers. Results corresponding to our findings were reported by Cufadar et al. (2011), who evaluated the effects of limestone particle sizes in moulted laying hens (76 weeks of age). They found that very large (> 5 mm) limestone particles beneficially affected eggshell breaking strength; however, they observed a significant interaction between Ca dietary level and limestone particle size, such that the positive effect of large-particle limestone was pronounced only when the diet was low in Ca. Saafa et al. (2008) observed a beneficial influence of diet supplementation with coarse limestone on eggshell density; however, neither shell weight and thickness nor the percentage of broken and shell-less eggs was affected by limestone particle size. The results of an experiment by Skrivan et al. (2010) showed that the substitution of fine dietary limestone with coarse limestone (0.8–2.0 mm) can increase shell weight, shell thickness and shell Ca content, both in younger (24 to 36 weeks of age) and older layers (56 to 68 weeks), without any effect on shell breaking strength. In a recent study (Wang et al., 2014) the beneficial effect of large-particle limestone on eggshell quality, i.e. eggshell breaking strength, was observed in laying ducks. By contrast, Olgun et al. (2013) found no effect of different sizes of limestone particles on eggshell quality throughout the first phase of the laying cycle (25 to 37 weeks of age); furthermore, eggshells of hens fed diets with very large particles of limestone contained decreased amounts of Ca.

4 Conclusions

The results of this study suggest that a dietary level of 3.20 % Ca in the diet is sufficient to maintain optimal egg production and eggshell quality in laying hens throughout the entire experimental period (25 to 70 weeks of age). Partial (25 or 50 %) substitution of fine-particle (diameter 0.20–0.60 mm) with larger-particle limestone (1.00–1.40 mm) can improve eggshell quality, i.e. eggshell percent, thickness, density and breaking strength, in aged laying hens irrespective of Ca dietary level.

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