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Dietary supplementation with protected calcium effects production and egg quality of Hy-line brown laying hens

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Abstract

Context. Currently, the commonly used dietary calcium sources are not sufficiently bioavailable to be used for eggshell formation or bone health in laying hens. Protected calcium, a blend of calcium and medium-chain fatty acids with matrix coating, may exert an effective absorption and metabolism ability for calcium consumption in the laying hen.

Aims. The present study was conducted to evaluate the effects of protected-calcium supplementation on egg production, egg quality, and serum calcium and phosphorus concentrations in laying hens.

Methods. In total, 144 Hy-line brown laying hens (25 weeks old) were randomly allotted to three treatments (8 replicates with 6 hens, 1 hen per cage) in a 10-week trial. Treatments consisted of corn–wheat–soybean meal-based basal diet with limestone (coarse limestone : fine limestone = 50 : 50) as a calcium source (CON) or basal diet supplemented with 0.5% (P1) or 1% (P2) protected calcium in substitution for an equal quantity of coarse limestone. Data were statistically analysed using linear and quadratic contrast with the GLM procedure of SAS. Probability values of <0.05 indicate significance.

Key results. At Weeks 31–33 and 35, the cracked-egg rates were lower in the P1 and P2 groups than in the CON group ($P < 0.05$; linear, $P < 0.05$) and, at Weeks 29 and 34, the cracked-egg rates in the P2 group were also significantly ($P < 0.05$) decreased. At Weeks 26, 30 and 32, the Haugh units in the P2 group were significantly ($P < 0.05$) higher than those in the CON group. In addition, eggshell strength was increased significantly ($P < 0.05$) in the P2 group at Weeks 26, 27 and 31–35. The serum calcium concentration of the P2 group was significantly ($P < 0.05$) greater than that of the CON group in the morning.

Conclusions. In conclusion, a replacement of limestone with 1% protected calcium can increase eggshell quality.

Implications. Protected-calcium supplementation can be used in practice for decreasing the breakage of eggs.

Additional keywords: calcium concentration, cracked-egg rate, phosphorus concentration.

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Introduction

It is well known that dietary calcium (Ca) concentration directly influences egg quality. Supplementation of sufficient Ca contributes to improved egg quality. However, because the laying hen derives 25–40% of Ca required for eggshell formation from the medullary bone and deposits Ca in bone every day, it is necessary to provide dietary Ca to meet the requirement of eggshell formation and bone health (Chen and Chen 2004; Silversides *et al*. 2006; Cufadar *et al*. 2011; Leblebicioglu *et al*. 2013). Several studies have indicated that sources and particle size of Ca are of great interest in increasing the bioavailability and digestibility in laying hens (Pelicia *et al*. 2011; Zhang *et al*. 2017). Calcium deficiency can cause poor eggshell quality (Ahmad and Balander 2003), and, more seriously, it can cause some metabolic diseases, especially those related to bone, such as, for example, osteoporosis and osteomalacia (Whitehead 2004). In addition, excess Ca may lead to metabolic diseases such as urate accumulation, which causes diarrhoea and avian gout (Leeson and Summers 1987; Tang *et al*. 2005). Intestinal absorption of dietary Ca has a central role in the regulation of Ca homeostasis, and approximately 90% of Ca is absorbed in the small intestine (Wasserman 2004; Rivoira *et al*. 2012). Commercial layers transfer about 10% of their total body Ca volume daily to the shell production in the oviduct; of this, only about 50% originates from dietary sources (Kerschnitzki *et al*. 2014).

In poultry rations, Ca carbonate is the main source of Ca and is usually supplied as limestone or marine shells (Ganjigohari *et al*. 2017). Different Ca sources, particle size, as well as hen age affect shell deposition in laying hens (Roland 1986; Pavlovski *et al*. 2003; Mazzuco and Hester 2005). Thus, adequate Ca concentrations, and optimum Ca source and particle size are essential factors

for eggshell formation and bone health, helping minimise cracked-egg rate and production costs (Keshavarz 2003; Saunders-Blades *et al*. 2009). The commonly used dietary Ca sources are not optimally bioavailable to be used for eggshell formation or bone health in laying hens (Lukić *et al*. 2009). Further research remains to be conducted on new sources of Ca for laying hens. Thus, reduction in Ca supplementation by the use of a modified form such as coated or protected Ca for effective absorption and metabolism can be a viable strategy.

With the development of coating or encapsulation technologies, the targeted delivery of feed additives to different gut segments has gained considerable attention (Upadhaya *et al*. 2014). The protected Ca used in the current study is a blend of Ca and medium-chain fatty acids with matrix coating. The beneficial mechanism of protected Ca is characterised by prolonged retention times in the small intestine, and it dissolves more slowly. A slower solubilisation of protected Ca would make Ca available during the eggshell calcification and prevent the mobilisation of bone Ca and P reserves (Skřivan *et al*. 2010), and would supply the hen more evenly with Ca, which may positively influence eggshell quality. We hypothesised that protected Ca, which is used to substitute traditional Ca sources, may be readily available for production of improved-quality eggs. Therefore, a preliminary assessment of protected Ca (PCa) was conducted to evaluate egg production performance, egg quality and serum Ca and phosphorus (P) concentrations in laying hens.

Materials and methods

The experimental protocol of the present study was subjected to approval by the Animal Care and Use Committee of Dankook University, Choongnam, South Korea. The PCa used in the current study was manufactured by a commercial company (Morningbio Co., Cheonan, South Korea) and consisted of 45% Ca carbonate, 1.2% medium-chain fatty acids (6 g/kg capric and 6 g/kg caprylic acids), and a carrier (hydrogenated palm oil).

Experiment design, diets and management

In total, 144 Hy-line brown laying hens at 25 weeks of age were randomly assigned into three dietary treatments (CON, P1 and P2, see below) with three levels of PCa substitute for dietary Ca. Each treatment contained eight replicates with six hens in each, in a 10-week feeding trial. Each replicate consisted of six adjacent cages, with one hen per cage. All diets were formulated to meet or exceed the NRC (1994) recommendations (shown in Table 1). The dietary treatments were as follows: CON, basal diet with limestone (coarse limestone : fine limestone = 50 : 50; the particle size of coarse limestone: 0.8–2 mm, the particle size of fine limestone: <0.5 mm) as a Ca source; P1, basal diet with 0.5% PCa (the particle size: 0.5–1.0 mm) as a substitute for an equal quantity of coarse limestone; P2, basal diet with 1% PCa as a substitute for an equal quantity of coarse limestone. The doses of PCa were set as a preliminary assessment in the current study. Feed and water were provided *ad libitum* by an individual feeder and nipple drinker. During the experimental period (25–35 weeks), the hens were maintained in an environmentally controlled house and caged in a three-tiered battery cage. The replicates were equally distributed into the upper and lower cages to minimise the effect of cage level. A photoperiod of 17 h light–7 h darkness was provided, with 5.2 lx artificial-light intensity daily, and the temperature was maintained at approximately 23°C.

Table 1. Basal diet composition (as-fed basis)
Ca, calcium; CP, crude protein; P, phosphorus

Item	Concentration
Ingredients (g/kg)	
Corn	562.8
Soybean meal, 48% CP	155.3
Wheat grain	100.0
Corn gluten meal, 60% CP	20.0
Wheat bran	50.0
Tallow	17.0
Limestone	75.2
Dicalcium phosphate, 18% P	13.7
Salt	3.0
DL-methionine, 50%	1.0
Vitamin premix[A]	1.0
Mineral premix[B]	1.0
Calculated values	
Metabolisable energy (kcal/kg)	2700
Available P (g/kg)	3.7
Analysed nutrient concentration (g/kg)	
Crude protein	170.4
Ether extract	39.8
Lysine	7.8
Methionine+cysteine	6.3
Ca	34.5
Total P	6.1

[A]Provided the following per kilogram of diet: vitamin A, 10000 IU; vitamin D_3, 3000 IU; vitamin E, 10 mg; vitamin K_3, 2 mg; vitamin B_1, 1 mg; vitamin B_2, 5 mg; vitamin B_6, 1 mg; vitamin B_{12}, 0.015 mg; folic acid, 0.5 mg; niacin, 35 mg; Ca-pantothenate acid, 10 mg; and biotin, 0.05 mg.

[B]Provided the following per kilogram of diet: manganese (as MnO2), 80 mg; zinc (as $ZnSO_4$), 60 mg; coper (as $CuSO_4{\cdot}5H_2O$), 5 mg; iron (as $FeSO_4{\cdot}7H_2O$), 40 mg; cobalt (as $CoSO_4{\cdot}5H_2O$), 0.3 mg; iodine (as KI), 1.5 mg; and selenium (as $Na_2SeO_3{\cdot}5H_2O$), 0.15 mg.

Sampling and measurements

Eggs were collected and counted on a daily basis, cracked eggs (shell-less, soft-shelled, and crack eggs) were recorded on a daily basis; egg production was calculated as an average hen-day on a weekly basis, and egg quality was evaluated weekly. In total, 30 eggs (except for cracked eggs) were randomly collected from each treatment at 1700 hours, and the egg quality was measured at 2000 hours on the same day of the week. The egg weight, albumen height, yolk colour, Haugh unit and eggshell strength were evaluated (Digital Egg Tester, Nabel Co., Kyoto, Japan), and the eggshell thickness was measured at the blunt end, the equatorial region, and the sharp end (Digimatic Pipe Gage, Mitutoyo Co., Sakado, Japan). Blood samples were randomly collected

from eight laying hens per treatment from the wing vein, by using a sterilised syringe, in the morning and afternoon from the same bird for each treatment at 25, 30 and 35 weeks. The blood samples were transferred into clot-activator vacuum tubes, and then centrifuged at 3000 rpm at 4 °C for 10 min to obtain the plasma (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). The serum Ca concentrations were determined by the methyl thymol blue colorimetry method and P concentrations were determined by the phosphomolybdic acid colorimetry method.

Statistical analyses

All data were statistically analysed with ANOVA, using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, USA). Orthogonal comparisons were conducted using polynomial regression to measure the linear and quadratic effects of supplemental PCa at different concentrations. Differences among the treatment means were determined by Duncan's multiple-range tests, with $P < 0.05$ indicating significance.

Results

Egg production and cracked-egg rate

The effects of supplemental PCa on egg production and cracked-egg rates are shown in Table 2. At Weeks 29 and 34, the cracked-egg rates in P2 diets were decreased significantly ($P < 0.05$; linear, $P < 0.05$), and at Weeks 31–33 and 35, the cracked-egg rates in P1 and P2 groups were significantly ($P < 0.05$; linear, $P < 0.01$) lower than those in the CON group.

Egg quality

The addition of PCa affected certain indices of egg quality, as presented in Table 3. At Weeks 27 and 29, a significant ($P < 0.05$) increase in egg weight was observed in the P2 group, as compared with the CON group; at Weeks 26, 27 and 29, egg weight increased linearly with an increasing level of PCa supplementation ($P < 0.05$). In comparison with the P1 group, the albumen height of the P2 group was increased at Week 30 ($P < 0.05$; quadratic, $P < 0.05$). At Week 33, supplementation with 1% PCa decreased yolk colour in comparison with that in the CON group (quadratic, $P < 0.05$). At Weeks 26, 30 and 32, the Haugh units in the P2 group were significantly ($P < 0.05$; linear, $P < 0.05$) higher than those in the CON group. At Weeks 27 and 33–35, the eggshell strength was significantly increased in birds fed the P1 and P2 diets. Supplementation with 1% PCa also increased eggshell thickness at Weeks 26 and 28 ($P < 0.05$) compared with other treatments and there was a significant ($P < 0.05$) linear increase in eggshell thickness at Weeks 26 and 28 with an increase in the PCa concentration.

Serum Ca and P concentrations

Supplementing the diet of laying hens with PCa affected the blood concentrations of Ca and P, as shown in Table 4. At Week 30, the serum P concentration of birds fed 1% PCa diet was higher than that of the birds fed the CON and P1 diets in the morning ($P < 0.05$; linear, $P < 0.01$); in the afternoon, a significant ($P < 0.05$; linear, $P < 0.01$) increase was observed in the P2 group, as compared with the CON. At Week 35, the serum Ca concentration in the P2 group was significantly ($P < 0.05$; linear, $P < 0.01$) greater than that in the CON group in the morning; and the P concentration of the P2 group was the highest in the afternoon ($P < 0.05$; linear, $P < 0.01$).

Table 2. The effects of dietary supplementation of protected calcium on egg production and cracked-egg rate in laying hens

The Hy-line brown laying hens at 25 weeks of age were used in this 10-week feeding trial. CON, basal diet with limestone as a calcium source; P1, basal diet with 0.5% protected calcium as a substitute for an equal quantity of coarse limestone; P2, basal diet with 1% protected calcium as a substitute for an equal quantity of coarse limestone. s.e.m., standard error of the mean. Means within a row with different lowercase letters differ significantly (at $P \leq 0.05$)

Hen age (weeks)	CON	P1	P2	s.e.m.	*P*-value	
					Linear	Quadratic
			Egg production (%)			
25	96.4	96.5	96.9	0.6	0.593	0.852
26	96.7	97.0	97.9	0.8	0.324	0.770
27	96.0	96.7	96.1	0.8	0.922	0.624
28	88.5	89.9	90.2	1.2	0.765	0.912
29	91.1	94.6	96.4	1.4	0.063	0.630
30	94.1	94.7	94.6	3.2	0.921	0.946
31	92.9	93.5	94.1	2.1	0.763	1.000
32	92.3	93.5	94.6	1.3	0.069	1.000
33	92.9	93.5	94.1	1.6	0.455	1.000
34	91.1	93.5	94.0	1.6	0.156	0.547
35	91.7	93.5	94.1	2.3	0.543	0.856
			Cracked-egg rate			
25	2.18	1.23	2.15	0.48	0.972	0.146
26	3.10	1.20	1.20	0.60	0.052	0.228
27	2.50	1.53	1.23	0.74	0.257	0.717
28	2.90	1.31	0.98	1.08	0.239	0.644
29	2.00a	1.30ab	0.60b	0.24	0.003	1.000
30	2.75	1.30	1.90	0.53	0.293	0.151
31	2.60a	1.25b	0.65b	0.40	0.008	0.459
32	2.60a	1.30b	1.30b	0.17	<0.0001	0.012
33	3.25a	1.25b	0.65b	0.41	0.002	0.200
34	2.55a	1.25ab	0.65b	0.56	0.040	0.628
35	2.60a	1.25b	0.60b	0.41	0.007	0.497

Discussion

In contrast to fine limestone, its coarse form can be ground slowly in the gizzard, thus facilitating a slow release of Ca for the requirement of egg formation. Normally, fine limestone and coarse limestone are fed simultaneously, so that the larger particles of limestone are retained in the gizzard, and require a longer time to be dissolved ready for absorption; meanwhile, the small particles of limestone are quickly absorbed to meet the short-term needs. The PCa used in the current study has a mechanism of action similar to that of coarse limestone, which can be slowly released into the intestine to satisfy the Ca requirements of laying hens.

The current study showed that PCa supplementation at different concentrations did not significantly influence the rate of egg production. These results are in accordance with the studies of Pelicia *et al*. (2009) and Valdés *et al*. (2011),

Table 3. The effects of dietary supplementation of protected calcium on egg quality in laying hens

The Hy-line brown laying hens at 25 weeks of age were used in this 10-week feeding trial. CON, basal diet with limestone as a calcium source; P1, basal diet with 0.5% protected calcium as a substitute for an equal quantity of coarse limestone; P2, basal diet with 1% protected calcium as a substitute for an equal quantity of coarse limestone. s.e.m., standard error of the mean. Means within a row with different lowercase letters differ significantly (at $P \leq 0.05$)

Items	CON	P1	P2	s.e.m.	*P*-value	
					Linear	Quadratic
		25 weeks				
Egg weight (g)	55.87	56.06	55.71	0.81	0.89	0.79
Albumen height (mm)	8.3	8.4	8.4	0.08	0.80	0.81
Yolk colour	6.1	6.0	5.9	0.08	0.17	0.87
Haugh unit	87.70	88.13	89.30	0.90	0.21	0.74
Eggshell strength (kg/cm^2)	3.428	3.396	3.438	0.07	0.92	0.68
Eggshell thickness (10^{-2} mm)	42.79	42.36	42.54	0.71	0.80	0.73
		26 weeks				
Egg weight (g)	55.63	57.39	58.38	0.94	0.04	0.74
Albumen height (mm)	8.3	8.3	8.4	0.08	0.91	0.81
Yolk colour	6.2	6.0	6.1	0.08	0.56	0.18
Haugh unit	87.98b	88.87ab	91.56a	0.94	0.01	0.43
Eggshell strength (kg/cm^2)	3.404	3.469	3.602	0.07	0.01	0.61
Eggshell thickness (10^{-2} mm)	42.78	43.12	44.67	0.68	0.04	0.46
		27 weeks				
Egg weight (g)	56.32b	58.20ab	59.33a	0.85	0.01	0.71
Albumen height (mm)	8.3	8.4	8.3	0.10	0.93	0.69
Yolk colour	6.1	6.0	5.9	0.08	0.23	1.00
Haugh unit	86.80	87.56	88.02	0.89	0.35	0.89
Eggshell strength (kg/cm^2)	3.315b	3.522a	3.536a	0.05	0.002	0.119
Eggshell thickness (10^{-2} mm)	44.12	45.08	45.25	0.56	0.15	0.56
		28 weeks				
Egg weight (g)	56.88	58.18	58.31	0.95	0.30	0.62
Albumen height (mm)	8.1	8.3	8.3	0.12	0.19	0.27
Yolk colour	6.0	6.2	6.1	0.16	0.53	0.40
Haugh unit	87.42	87.04	86.73	0.94	0.56	0.98
Eggshell strength (kg/cm^2)	2.931	2.863	3.155	0.11	0.21	0.20
Eggshell thickness (10^{-2} mm)	36.67b	39.08ab	41.45a	1.23	0.01	0.99
		29 weeks				
Egg weight (g)	56.91b	60.71ab	61.39a	1.31	0.03	0.38
Albumen height (mm)	8.0	8.3	8.3	0.12	0.22	0.28
Yolk colour	6.2	6.0	5.9	0.16	0.22	0.81
Haugh unit	86.78	88.27	88.42	0.92	0.20	0.55
Eggshell strength (kg/cm^2)	3.519	3.552	3.623	0.07	0.31	0.83
Eggshell thickness (10^{-2} mm)	45.16	45.39	45.49	0.57	0.76	0.95
		30 weeks				
Egg weight (g)	58.89	61.21	61.33	1.18	0.16	0.47
Albumen height (mm)	8.10ab	7.93b	8.38a	0.12	0.12	0.04
Yolk colour	5.8	6.2	6.1	0.15	0.25	0.23
Haugh unit	84.29b	85.42ab	86.88a	0.64	0.003	0.828
Eggshell strength (kg/cm^2)	3.694	3.703	3.768	0.05	0.32	0.65
Eggshell thickness (10^{-2} mm)	45.29	44.87	45.44	0.61	0.86	0.44
		31 weeks				
Egg weight (g)	60.38	61.09	60.74	1.16	0.82	0.70
Albumen height (mm)	7.98	8.17	8.26	0.13	0.13	0.75
Yolk colour	6.0	5.8	5.8	0.16	0.53	0.54
Haugh unit	85.15	85.84	85.99	0.52	0.23	0.65
Eggshell strength (kg/cm^2)	3.704b	3.855ab	4.009a	0.05	<0.0001	0.98
Eggshell thickness (10^{-2} mm)	44.44	45.03	45.72	0.75	0.23	0.95
		32 weeks				
Egg weight (g)	61.56	61.25	61.50	0.92	0.97	0.82
Albumen height (mm)	7.99	8.07	8.25	0.14	0.16	0.77
Yolk colour	6.0	6.0	6.0	0.16	0.83	0.90

(*continued next page*)

Table 3. (*continued*)

Items	CON	P1	P2	s.e.m.	*P*-value	
					Linear	Quadratic
Haugh unit	85.10b	86.08ab	87.18a	0.50	0.01	0.92
Eggshell strength (kg/cm^2)	3.843	3.886	3.908	0.05	0.40	0.88
Eggshell thickness (10^{-2} mm)	44.41	44.26	45.03	0.86	0.60	0.66
		33 weeks				
Egg weight (g)	61.54	62.60	63.58	0.74	0.10	0.84
Albumen height (mm)	8.39	8.34	8.36	0.11	0.90	0.81
Yolk colour	5.6	5.3	5.5	0.12	1.00	0.07
Haugh unit	86.89	86.63	87.19	0.43	0.61	0.41
Eggshell strength (kg/cm^2)	3.885c	4.023b	4.131a	0.03	<0.0001	0.33
Eggshell thickness (10^{-2} mm)	45.82	45.84	45.80	0.82	0.79	0.86
		34 weeks				
Egg weight (g)	61.66	62.41	63.03	0.80	0.29	0.95
Albumen height (mm)	8.65	8.77	8.69	0.07	0.65	0.26
Yolk colour	5.5	5.6	5.6	0.11	0.53	0.72
Haugh unit	86.71	86.97	87.57	0.33	0.08	0.69
Eggshell strength (kg/cm^2)	3.902b	4.075a	4.117a	0.04	0.0001	0.15
Eggshell thickness (10^{-2} mm)	45.30	45.70	45.19	0.75	0.92	0.62
		35 weeks				
Egg weight (g)	61.62	62.19	62.79	0.79	0.37	0.99
Albumen height (mm)	8.70	8.76	8.74	0.07	0.70	0.66
Yolk colour	5.4	5.5	5.6	0.11	0.36	0.86
Haugh unit	86.62	87.15	87.43	0.34	0.09	0.76
Eggshell strength (kg/cm^2)	3.986b	4.112a	4.119a	0.04	0.011	0.182
Eggshell thickness (10^{-2} mm)	44.26	44.81	45.16	0.74	0.42	0.92

[A] The Hy-line brown laying hens at 25 weeks of age were used in this 10 weeks feeding trial.

Table 4. The effects of dietary supplementation of protected calcium on serum calcium and phosphorus concentrations in laying hens

The Hy-line brown laying hens at 25 weeks of age were used in this 10-week feeding trial. CON, basal diet with limestone as a calcium source; P1, basal diet with 0.5% protected calcium as a substitute for an equal quantity of coarse limestone; P2, basal diet with 1% protected calcium as a substitute for an equal quantity of coarse limestone. s.e.m., standard error of the mean. Means within a row with different lowercase letters differ significantly (at $P \leq 0.05$)

Item	CON	P1	P2	s.e.m.	*P*-value	
					Linear	Quadratic
			Initial			
Calcium (mg/dL)						
Morning	19.00	19.13	19.13	0.12	0.478	0.644
Afternoon	19.00	19.20	19.15	0.16	0.534	0.550
Phosphorus (mg/dL)						
Morning	6.58	6.75	6.78	0.17	0.432	0.730
Afternoon	6.68	6.70	6.78	0.08	0.404	0.806
			30 weeks			
Calcium (mg/dL)						
Morning	19.00	19.03	19.18	0.17	0.511	0.784
Afternoon	18.95	19.18	19.08	0.20	0.672	0.527
Phosphorus (mg/dL)						
Morning	6.45b	6.73b	7.08a	0.10	0.001	0.739
Afternoon	6.53b	6.90ab	7.00a	0.12	0.018	0.360
			35 weeks			
Calcium (mg/dL)						
Morning	18.30b	19.08a	19.20a	0.20	0.006	0.171
Afternoon	19.00	19.23	19.38	0.18	0.166	0.866
Phosphorus (mg/dL)						
Morning	6.60	6.73	6.95	0.22	0.215	0.831
Afternoon	6.45b	6.90ab	7.05a	0.14	0.009	0.368

who fed different levels of limestone and demonstrated that there were no significant effects in commercial laying hens. Also Cufadar *et al*. (2011) reported similar findings. However, Nascimento *et al*. (2014) found a significant change in egg-production rate when different concentrations of Ca were provided to laying hens. In our study, no significant changes were found in egg production, although the values were slightly higher in birds fed diets with PCa. The possible reason for a lack of a significant effect may be that the concentrations of PCa were not high enough, or that Ca absorption was restricted in the laying hens. Therefore, further study is needed to assess the optimal range of PCa concentration and the absorbable amounts of PCa in laying hens.

The eggshell comprises 95% Ca carbonate; Ca is the most essential nutrient determining the eggshell quality in laying hens. A majority of eggshell problems are related to different nutrient sources and levels, feed condition, egg-production period and production cycle in laying hens (Arpasova *et al*. 2010; Pavlovski *et al*. 2012). Providing an optimal Ca supplementation level is a crucial step to ensure proper eggshell calcification and formation. However, Keshavarz (2003) and Pastore *et al*. (2012) demonstrated that there were hardly any positive effects on egg quality from increasing the Ca supplementation level. Larger eggs are associated with a higher breaking rate, lower eggshell strength and thickness, and lower egg quality (Pizzolante *et al*. 2009). Unlike in other studies, despite the increase in egg weight, the eggshell strength and thickness were increased, and the cracked-egg rate was decreased, which indicated that PCa supplementation had positive effects on eggshell strength, thickness and egg weight in the current study. The eggshell quality also might be affected by the solubility of limestone from different origins, particle size and Ca concentration (Saunders-Blades *et al*. 2009). However, different particle sizes of limestone have yielded conflicting results. Calcium sources with different particle sizes showed no effect on eggshell strength and eggshell thickness (Kuhl *et al*. 1977; Keshavarz 1998). Cufadar *et al*. (2011) also reported that there were no significant effects on eggshell strength and eggshell thickness when different particle sizes of limestone were fed to laying hens. In contrast, our study showed that the PCa supplied in the laying hen's diet had a positive effect on the eggshell strength and thickness, and the cracked-egg rates decreased significantly at Weeks 29 and 31–35. This may be due to the fact that PCa can be slowly released into the intestine (thus being absorbed more evenly), which is similar to coarse limestone, maintaining the serum Ca concentration and satisfying the process of calcification to increase eggshell strength and thickness, thereby decreasing the cracked-egg rate, as was demonstrated in our study.

During the dynamic biological process of egg formation, it takes 20 h to form the eggshell in the eggshell gland, which means that eggshell formation is the most important stage of egg production. Lichovnikova (2007) showed that when fine limestone was replaced with coarse limestone, this prolonged the retention time in the gizzard and led to a slow release of Ca into the intestine for absorption, thereby maintaining the Ca concentration of the serum to ensure calcification of the eggshell, and achieving an improvement in egg quality. Eggshell strength is one of the most important indices for the evaluation of optimum Ca and P concentrations in the diet of laying hens. The Ca and effective P concentrations in the diet determine the eggshell strength and toughness to resist breakage (An *et al*. 2016). However, insufficient or excessive P in the diet may cause an imbalance problem in the Ca : P ratio, hindering the absorption of Ca and resulting in a decrease in egg quality (Roberts 2004). Calcium absorption is also influenced and regulated by hormones, such as vitamin D, calcitonin and parathyroid hormone (Bronner and Pansu 1999). In the current study, the serum Ca and P concentrations were increased in birds fed the P2 diet compared with those in the CON and P1 groups, indicating that PCa supplemented at 1% was available to the birds for proper calcification of eggs, thereby increasing egg quality. Therefore, supplementation of 1% PCa produced better results than did supplementation of 0.5% or no PCa in terms of increasing egg quality, Ca retention and utilisation, and increasing eggshell thickness of laying hens.

Conclusions

Supplementing the hen diet with PCa increased the value of Haugh unit, and eggshell strength and thickness, indicating that egg quality was increased. It also increased serum Ca and P concentrations. In conclusion, 1% PCa supplementation can be used in practice for decreasing the breakage of eggs and as a partial replacement of limestone, to increase the eggshell quality.

Conflicts of interest

The authors declare no conflicts of interest.

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