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Effects of Dietary Calcium Levels on Productive Performance, Eggshell Quality and Overall Calcium Status in Aged Laying Hens

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ABSTRACT: This study was conducted to investigate the effects of diets with varying levels of calcium on egg production, shell quality and overall calcium status in aged laying hens. A total of five hundred 70-wk-old Hy-Line Brown layers were divided five groups and fed one of the five experimental diets with 3.5%, 3.8%, 4.1%, 4.4%, or 4.7% Ca, for 10 weeks. There were no significant differences in feed intake, egg production and egg weight among groups. The cracked eggs were linearly reduced as dietary Ca levels increased to 4.7% ($p<0.01$). A significant linear improvement for eggshell strength and thickness were determined with increasing dietary Ca levels ($p<0.01$). The contents of serum Ca and phosphorus were not affected by dietary Ca levels. With increase in dietary Ca levels, the tibial breaking strength slightly increased. There were no significant differences in the tibial contents of ash, Ca and phosphorus among groups. In conclusion, eggshell quality, as measured by appearance, strength and thickness of eggshell, were influenced by dietary Ca content as expected ($p<0.05$). These results suggested that aged laying hens require relatively higher level of Ca than required levels from current Korean feeding standards for poultry. (**Key Words:** Dietary Calcium Levels, Cracked Eggs, Eggshell Strength, Tibial Breaking Strength, Aged Laying Hens)

INTRODUCTION

The eggshell quality continues to be a major concern of the egg industry. Eggs with inferior shell quality are a leading economical loss to poultry producers (Roberts, 2004). It has been reported that the average of eggs cracked and lost prior to point of consumption ranged from 13% to 20% (Roland, 1988).

The increased incidence of cracked eggs occurs mainly in late laying period. Decrease in eggshell quality of aged laying hens might be attributed to reduced intestinal Ca uptake and increased egg size (Al-Batshan et al., 1994). Egg size and weight increased with increasing hen age, but it is generally not accompanied by a proportional increase in shell weight, which leads to a decrease in the shell weight to egg weight ratio. Elaroussi et al. (1994) suggested that the increase in cracked eggs seen in aged layers could be a result of disturbances related with the Ca homeostasis.

Calcium is one of the key nutrients required for production and optimal eggshell quality of laying hens (Ahmed et al., 2013). Most research reported that a linear improvement in eggshell quality was evident with increasing dietary Ca levels. Roland (1987) also suggested that a linear increase in eggshell quality when feeding dietary Ca above 4.35 g/d. On the other hands, Leeson et al. (1993) did not find any effect of higher levels of dietary Ca on eggshell quality and concluded that 3.4 g of daily Ca intake was enough for brown egg layers. These discrepancies may be attributed to differences in strains, environmental factors and other nutrients such as phosphorus, which can affect Ca requirement (Garlich et al., 1984).

To our knowledge, a considerable amount of research has been conducted on the effect of feeding various Ca levels during early, mid or total laying stage, but only limited information is available on overall Ca requirement

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in aged laying hens. This experiment was conducted to investigate the effects of dietary Ca levels with equal in the contents of energy and other nutrients, including available phosphorus, on eggshell quality and overall Ca status in aged laying hens.

MATERIALS AND METHODS

Animals, diets and managements

Five hundred 70-wk-old Hy-Line Variety Brown hens were used and allotted in the experimental windowless house. The layers were divided into five dietary treatments with 10 replicates of 10 birds per each and two hens at a time were put into one wire cage (35×40 cm). The layers were fed one of the five experimental diets with 3.5%, 3.8%, 4.1%, 4.4%, or 4.7% Ca, respectively. All diets were formulated to meet and exceed the nutrients requirements of NRC (1994) and Korean Feeding Standard for poultry (2012), except for Ca, as shown in Table 1. The level of available phosphorus was equally set at 0.23% due to addition of conventional phytase. Proximal composition of formulated diets are shown in Table 2. The analyzed values of Ca were slightly lower than calculated composition. The experiment lasted 10 wk and during which diets and water were provided for *ad libitum* intake. A room temperature of 25°C±5°C and a photoperiod of 16/8 h light/dark cycle were maintained throughout the experimental period. The diets were freshly added everyday and feed intake of each replicate was recorded weekly. The protocol for the experiment was approved by the Institutional Animal Care and Use Committee at Konkuk University.

Table 1. Ingredient composition of experimental diets, as-fed basis

Items	Level of Ca (%)				
	3.5	3.8	4.1	4.4	4.7
Ingredients (%)					
Corn	59.55	60.02	60.5	60.98	61.47
Soybean meal	14.54	14.74	14.95	15.16	15.36
Wheat bran	9.49	7.78	6.06	4.34	2.63
Limestone coarse	8.66	9.44	10.23	11.02	11.80
Canola meal	5.00	5.00	5.00	5.00	5.00
Soybean oil	1.00	1.00	1.00	1.00	1.00
Corn gluten meal	0.37	0.59	0.80	1.01	1.22
Dicalcium phosphate	0.71	0.74	0.76	0.79	0.82
Salt	0.30	0.30	0.30	0.30	0.30
Mineral mixture[1]	0.12	0.12	0.12	0.12	0.12
Vitamin mixture[2]	0.10	0.10	0.10	0.10	0.10
DL-methionine, 98%	0.07	0.07	0.07	0.07	0.07
Phytase	0.05	0.05	0.05	0.05	0.05
$NaHCO_3$	0.03	0.03	0.04	0.04	0.04
Choline-Cl, 50%	0.01	0.02	0.02	0.02	0.02
Calculated nutrient content					
CP (%)	14.50	14.50	14.50	14.50	14.50
Ca (%)	3.50	3.80	4.10	4.40	4.70
Avail. P (%)	0.23	0.23	0.23	0.23	0.23
Total Lys (%)	0.65	0.65	0.65	0.65	0.65
Total Met (%)	0.33	0.33	0.33	0.33	0.33
Total TSAA (%)	0.58	0.58	0.58	0.58	0.58
TMEn (kcal/kg)	2,760	2,760	2,760	2,760	2,760

CP, crude protein; TSAA, total sulfur amino acid; TMEn, nitrogen-corrected true metabolizable energy.

[1] Mineral mixture provided following nutrients per kg of diet: Fe, 56 mg; Zn, 106 mg; Mn, 124 mg; Cu, 11.5 mg; I, 1.7 mg; Se, 0.54 mg; Cr, 0.24 mg.

[2] Vitamin mixture provided following nutrients per kg of diet: vitamin A, 8,666 IU; vitamin D_3, 2,666 IU; vitamin E, 20 IU; vitamin K_3, 2 mg; vitamin B_1, 2 mg; vitamin B_2, 4.6 mg; vitamin B_6, 3.3 mg; vitamin B_{12}, 0.013 mg.

Egg production and qualities

In this experiment, the egg production was recorded daily and mean egg weight was determined by daily average weight of egg, excluding abnormal eggs. The percentages of cracked eggs were calculated by replicate (number of soft-shell and broken eggs/number of eggs produced×100). At 6, 8, and 10 wks of experiment, five eggs from each replicate were collected, weighed individually and stored overnight at room temperature for subsequent measurements.

The breaking strength of uncracked eggs was measured with an eggshell strength tester (FHK, Fujihara Ltd., Tokyo, Japan). Eggshell thickness without shell membrane was tested by micrometer (Digimatic micrometer, Series 293-330, Mitutoyo, Japan). Eggshell color and albumin height were measured by using Egg multi tester made by TSS (Technical Services and Supplies Ltd., York, England). Haugh unit, along with albumen height and egg weight, was calculated as previously described (An et al., 2010). Egg yolk color was measured by comparing with Roche yolk color fan (Hoffman-La Roche Ltd., Basel, Switzerland).

Sampling and measurements

At the end of experiment, 10 birds were randomly selected from each treatment. Thereafter, the blood was

Table 2. Analyzed nutrient composition of formulated diet, as-fed basis[1]

Composition (%)	Level of Ca (%)				
	3.5	3.8	4.1	4.4	4.7
Dry matter	91.1	91.6	91.8	91.7	92.1
Crude protein	15.1	15.7	15.3	15.3	15.1
Ether extract	2.1	2.2	3.7	4.1	3.7
Crude fiber	3.1	3.0	2.8	2.7	2.6
Crude ash	7.3	8.4	8.6	8.7	9.4
Ca	3.1	3.3	3.8	4.0	4.3
Total P	0.45	0.47	0.47	0.46	0.45

[1] Data are the mean of duplicate analysis of each diet.

drawn from wing vein and analyzed for concentrations of Ca and phosphorus. The concentrations of serum Ca and phosphorus were measured according to the colorimetric method using biochemical analyzer (Hitachi modular system, Hitachi Ltd., Tokyo, Japan). At euthanasia, the right legs were immediately collected and stored in the refrigerator for the determination of mechanical property and chemical composition of tibias. Bone breaking strength was measured on fresh tibias using an Instron (Model 3342, Instron Universal Testing Machine, Instron Corp., Norwood, MA, USA) with 50-kg-load cell as 50-kg load range with a crosshead speed of 50 mm per min with tibia supported on a 3.35 cm span. The graphs showed the plateau curve of applied maximal force (KN) to measure the tibial strength as expressed as energy stored in the bone. The sheared tibia pieces were collected and deffatted, after which the tibia samples were oven-dried at 100°C for 24 h and then weighted to obtain the dry weight. The tibia samples were ashed in a muffle furnace (Isotemp muffle furnace, Fisher Scientific, Pittsburgh, PA, USA) at 600°C for 24 h in crucibles. The contents of Ca and phosphorus in tibia were determined using AOAC methods (AOAC, 1995).

Statistical analysis

Data were analyzed using the general linear model procedures of SAS 9.2 (SAS Inst. Inc., Cary, NC, USA). The cage was considered the experimental unit. Linear, quadratic, or both compared using the orthogonal contrast coefficients. The NLIN procedure of SAS according to Robbins et al. (2006) was used to find optimum breakpoint of Ca level whenever linear and or quadratic effects were significant.

However, all variables only showed the linear effect that is cannot account for optimum breakpoint of Ca level and therefore proc NLIN procedure was not included in the predictive model. Results were considered significant if their p-values were <0.05.

RESULTS AND DISCUSSION

Egg production

The feed intake and egg production in aged laying hens fed diets with varying levels of Ca are presented in Table 2. There were no significant linear and quadratic trends of dietary Ca levels affecting feed intake, egg production and egg weight. With increasing dietary Ca levels from 3.5% to 4.7%, cracked eggs linearly reduced (p<0.01) from 3.6% to 2.1%.

A number of studies with laying hens have reported that laying performance was not influenced by dietary Ca levels. Cufadar et al. (2011) did not find any significant differences in egg production and egg weight among the Ca levels of 3.0%, 3.6%, or 4.2% of diets in aged laying hens. Frost and Roland (1991) and Keshavarz and Nakajima (1993) also reported that different levels of dietary Ca had no significant effect on egg production, egg weight and egg mass. However, an excess of dietary Ca exerted a negative effect on egg production as a result of reduced feed intake (Ousterhout, 1980; Pelicia et al., 2009).

In present study, the average daily feed intake ranged from 117.1 g to 120.5 g. The dietary Ca did not affect the total feed intake in aged layers and had no negative effects on laying performance. There have been contradictory findings in the relationship to feed intake after feeding diets with varying levels of Ca. Olver and Malan (2000) observed that the dietary Ca levels did not influence total feed intake during 16 to 80 wks of age. Contrary to this, Narvaez-Solarte et al. (2006) reported that daily feed intake was decreased as dietary Ca levels increased. While Chandramoni et al. (1998) found that with increasing dietary Ca levels, the daily feed intake tended to be increase, but not significantly. This discrepancy may be attributed to differences in age of bird, dietary energy density and feeding levels of Ca.

A significant linear decrease in incidence of cracked egg was evident with increasing dietary Ca (Table 3). This decrease in incidence of cracked egg might be associated with improvement in eggshell strength and thickness seen according to increasing dietary Ca. The intake of insufficient amounts of Ca may cause poor shell quality, leading to higher incidence of cracked eggs (Jiang et al., 2013). In this study, the feed intake was not affected by dietary Ca levels during overall experimental period, whereas the tentative total Ca intake was increased as

Table 3. Effect of graded levels of dietary calcium on production performance in the aged laying hens[1,2]

Item	Level of Ca (%)					SEM	p-value	
	3.5	3.8	4.1	4.4	4.7		Linear	Quadratic
Feed intake (g/d/bird)	120.5	117.5	117.8	117.1	118.6	2.06	0.517	0.319
Egg production (%)	75.1	76.0	75.2	75.0	79.1	1.43	0.134	0.197
Egg weight (g/egg)	60.4	60.3	61.0	60.8	61.2	0.64	0.223	0.486
Cracked egg (%)	3.6	3.4	2.3	2.2	2.1	0.46	0.007	0.475

SEM, standard error of the means.

[1] Data are least square of mean of 10 replicate with 5 cages with 2 birds per cage.

[2] Mean values from the overall experimental period.

Table 4. Effect of graded levels of dietary calcium on egg and eggshell qualities in aged laying hens[1,2]

Item	Level of Ca (%)					SEM	p-value	
	3.5	3.8	4.1	4.4	4.7		Linear	Quadratic
Eggshell color[3]	38.7	38.4	38.5	38.3	37.6	0.71	0.312	0.704
Yolk color[4]	5.0	5.1	5.3	5.4	5.5	0.14	<0.001	0.429
Eggshell strength[5]	2.25	2.31	2.37	2.37	2.46	0.05	0.003	0.994
Eggshell thickness[6]	34.8	35.1	35.1	36.6	36.0	0.52	0.006	0.890
Haugh unit[7]	76.55	72.64	75.07	72.59	73.89	1.44	0.237	0.305

SEM, standard error of the means; TSS, Technical Services and Supplies Ltd., York, England.

[1] Data are least square of mean of 10 replicate with 5 cages with 2 birds per cage. [2] Mean values from the 76 or 78 to 80 weeks of age.

[3] Eggshell color is measured by Egg multi tester made by TSS. [4] Yolk color is measured by Roche yolk color fan.

[5] Eggshell strength measurement is expressed as kg/cm^2. [6] Eggshell thickness measurement is expressed as 0.01 mm.

[7] Haugh unit value is determined using the procedure described by Haugh (1937). HU = $100\times\log(H-1.7\times w^{0.37}+7.6)$, where: H = albumen height, mm; w = egg weight, g.

dietary Ca increased. To minimize the incidence rates of cracked eggs in aged layers, the diet must supply enough Ca due to the effect being linear.

Eggshell qualities

The egg and eggshell qualities in aged laying hens fed diets with varying levels of Ca are presented in Table 4. There were no significant linear and quadratic trends of dietary Ca levels affecting eggshell color and Haugh unit. Yolk color score was linearly increased as dietary Ca level increased, although the reason for the difference was not explainable. The eggshell quality was influenced by dietary Ca, as expected. The strength and thickness of eggshell were significantly increased ($p<0.01$) by dietary Ca levels in a linear manner (Table 4).

The available results about effect of dietary Ca levels on eggshell qualities are somewhat inconsistent. Jiang et al. (2013) found that layers on a diet with 2.62% Ca had a weaker eggshell breaking strength than those on a diet with 3.7% or 4.4% Ca. Roland (1987) suggested that the eggshell quality was linearly increased when dietary Ca levels were above 4.35 g per day. On the other hands, Keshavarz and Nakajima (1993) reported that increasing levels of dietary calcium from 3.5% to 5.5% did not have any beneficial effects on eggshell qualities in a long-term experiment. Cufadar et al. (2011) also noted that the level of dietary Ca had no significant effect on eggshell breaking strength and eggshell thickness.

The adequacy of recommended amounts of dietary Ca for optimal eggshell qualities is still being studied. But, based on the results obtained from previous studies, a constant increase in the level of dietary Ca has been associated with improvement of laying performance. Castillo et al. (2004) reported that the biological optimum level for maximum eggshell quality (as specific gravity) was 4.62% Ca in diet. An increase in Ca intake from 4.08 to 4.64 g/d improved the eggshell weight and eggshell thickness in aged Brown layers (Safaa et al., 2008), which is consistent with results of this study. Also, the research results led to the definition of a linear effect on dietary Ca with the eggshell quality. Pelicia (2009) reported that using 90 and 108 weeks of age laying hens, there was no effects of dietary Ca on eggshell strength and thickness; but the eggshell percentage and eggshell weight per surface area (ESWSA) was increased by increasing Ca concentration in the diet. And they obtained linear regression equation $y = 0.119x+8.9985$; $R^2 = 0.899$ in eggshell percentage and $y = 1.5879x+78.556$; $R^2 = 0.886$ in ESWSA. Likewise, the present study showed linear effect in the eggshell strength and eggshell thickness. The determined linear regression equations of the effect of dietary Ca on eggshell strength $y = 0.16x+1.70$; $R^2 = 0.941$ and on eggshell thickness $y = 1.31x+30.14$; $R^2 = 0.656$ showed that both eggshell strength and thickness linearly increase as dietary Ca intake increased. Through these results, we consider that dietary Ca has a strong linear relationship to eggshell strength.

The NRC (1994) suggested the Ca requirement of Brown layers to be 3.4% of dietary Ca for 110 g/d feed intake regardless of age, which seems inadequate for optimal eggshell qualities. More recently, the Korean feeding standard for poultry (2012) proposed the Ca requirement for aged Brown layers up to 4.1% at a feed intake of 110 g/d. The maximum requirement for calcium based on eggshell qualities is uncertain due to the effect being linear in present study. Obviously, aged Brown layers require considerably higher level of Ca to optimize eggshell quality than suggested levels in previous studies.

The inclusion of conventional phytase in layer diets has been greatly increased, in response to reduce the feed and production costs and to minimize phosphorus excretion. There is evidence that phytase positively influences the digestion and absorption of Ca, although the available results about dietary phytase did not have any consistent effects on the eggshell qualities. Punna and Roland (1999) observed a beneficial effect on eggshell quality of phytase inclusion, but others did not find any effect (Parsons, 1999). The possibility of positive effect by dietary phytase should not be precluded and further study is needed to clarify

Table 5. Effect of graded levels of dietary calcium on overall calcium status in serum and tibia[1,2]

Item	Level of Ca (%)					SEM	p-value	
	3.5	3.8	4.1	4.4	4.7		Linear	Quadratic
Serum (mg/dL)								
Calcium	28.9	30.2	29.6	27.2	29.0	1.20	0.488	0.561
Phosphorus	6.55	6.53	6.37	5.99	6.11	0.41	0.274	0.815
Tibia								
Length (cm)	11.61	11.88	11.78	11.66	11.98	0.13	0.233	0.876
Strength[3]	16.15	17.50	17.47	17.58	18.43	0.99	0.148	0.818
Ash (%)	48.83	45.85	45.86	46.57	46.84	1.24	0.408	0.912
Calcium (%)	17.79	17.26	17.68	18.16	18.25	0.48	0.234	0.917
Phosphorus (%)	9.19	8.69	8.55	9.27	9.33	0.24	0.253	0.317

SEM, standard error of the means.
[1] Data are least square of mean of 10 replicate with 1 hen per each replicate. [2] Mean values at 80 weeks of age.
[3] Breaking strength measurements is expressed as kg/mm^2.

dietary Ca levels on eggshell quality, depending on whether or not conventional phytase.

Overall calcium status

There were no significant linear and quadratic trends of dietary Ca levels affecting concentration of serum Ca and phosphorus (Table 5). Contrary to this, Frost and Roland (1991) reported that the level of plasma ionized Ca was significantly increased in a linear manner by increasing dietary Ca levels from 2.75% to 4.25%, but not plasma total calcium.

With increase in dietary Ca levels, the tibial breaking strength tended to be increased, but not significantly. There were no significant linear or quadratic trends of dietary Ca affecting ash, Ca and phosphorus contents in tibia among groups (Table 5). This result is consistent with that of Jiang et al. (2013), who reported that the hens fed diet with 4.4% Ca had similar bone density and strength as compared with those of diet with 3.7% Ca. Contrary to these results, a study has shown that increasing dietary Ca level linearly increased bone strength (Roland et al., 1996). Koutoulis et al. (2009) also suggested that increasing dietary Ca levels from 3.5% to 4.0% significantly increased tibial breaking strength in Brown layers at 72 wks of age. The reason for this discrepancy among authors with respect to bone status is not apparent, but might be attributed to differences in age, strain, dietary Ca levels and nutrient specification of experimental diets.

On the basis of present results, the dietary Ca levels did not affect on the total feed intake and laying performance in aged laying hens. But, the eggshell quality can be improved by ingesting more Ca, up to 4.7%, during last third of total laying period. In summary, our results indicate that aged Brown layers require relatively higher level of Ca to reduce cracked eggs and to maximize eggshell qualities than required levels, 4.1% of diet, from current Korean feeding standards for poultry.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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