Research Note: Improvement of eggshell strength and intensity of brown eggshell color by dietary magnesium and δ-aminolevulinic acid supplementation in laying hens

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ABSTRACT The objective of the current experiment was to investigate the effect of dietary magnesium (**Mg**) and δ-aminolevulinic acid (**ALA**) supplementation on productive performance and egg quality in laying hens. The present experiment was performed using a completely randomized design with 2 × 2 factorial arrangements including 2 levels of supplemental Mg (0 and 0.12%) and ALA (0 and 0.25%) in diets. A total of 192 43-wk-old Hy-Line Brown laying hens were allotted to 1 of 4 dietary treatments with 6 replicates. Each replicate consisted of 4 consecutive cages with 2 hens per cage. Treatment diets were fed to hens for 6 wk. Results indicated that dietary Mg supplementation improved ($P < 0.01$) eggshell strength but decreased ($P < 0.05$) eggshell color score, regardless of dietary ALA supplementation. However, the decreased eggshell color by dietary Mg supplementation was relieved by dietary ALA supplementation, which led to a significant interaction ($P < 0.05$). Likewise, an interaction ($P < 0.05$) was also observed for eggshell lightness (L*) because dietary ALA supplementation decreased eggshell L* values only when Mg was supplemented to diets. In conclusion, dietary Mg supplementation improves eggshell strength but decreases the intensity of brown eggshell color in laying hens. However, this negative effect of dietary Mg supplementation on eggshell color is likely ameliorated by dietary ALA supplementation.

Key words: eggshell quality, laying hen, magnesium, performance, δ-aminolevulinic acid

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INTRODUCTION

Eggshell strength is one of the most important egg quality variables in the layer industry because increased eggshell strength minimizes the incidence of egg breakages, which can prevent economic losses of layer production. Many nutritional studies have been performed to improve eggshell strength in laying hens, especially by the modification of dietary calcium and phosphorus concentrations (Bello et al., 2020). However, limited information for other dietary strategies has been available. Several experiments have reported that additional supplementations of magnesium (**Mg**) in diets improved the eggshell strength of laying hens (Seo et al., 2010; Kim et al., 2013a,b). Kim et al. (2013a) observed that the effects of increasing inclusion levels from 0 to 0.14% Mg in diets linearly increased eggshell strength of laying hens. Similarly, Kim et al. (2013b) also reported that laying hens fed diets supplemented with increasing levels of Mg in diets from 0 to 0.24% with 0.12% Mg providing the greatest eggshell strength. Therefore, it is suggested that additional supplementation of 0.12% Mg in diets can be a potential means to improve eggshell strength in laying hens. This improvement has been associated with the fact that Mg is required for the proper development and formation of eggshells in the uterus (Waddell et al., 1991). Moreover, Mg is the second most abundant mineral in eggshells, suggesting that additional supplementation of Mg in diets may increase Mg deposition in eggshells (Kim et al., 2013a,b). Notwithstanding this benefit on eggshell strength, dietary Mg supplementation was reported to decrease the intensity of brown eggshell color (i.e., paler egg production), possibly due to increasing Mg deposition in eggshells and its white element color (Kim et al., 2013a,b). The intensity of brown eggshell color is another important aspect of egg quality because it influences consumer preferences for eggs in several countries. Thus, a possible way of preventing the negative effect of dietary Mg supplementation on the brown eggshell coloration should be developed.

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Protoporphyrin IX (**PP9**) is the primary brown pigment in brown eggshells (Samiullah et al., 2015). The δ-aminolevulinic acid (**ALA**) is a precursor molecule of PP9 biosynthesis in the uterus (Fujino et al., 2016). Accordingly, it is hypothesized that increasing supply of dietary ALA as a PP9 precursor may aid in improving the intensity of brown eggshell color. However, besides a report that dietary supplementation of 5 to 10 mg/kg ALA increased the intensity of brown eggshell color of laying hens (Wang et al., 2011), there has been a lack of evidence about the effect of dietary ALA supplementation on brown eggshell color of laying hens. Therefore, the objective of the current experiment was to test the hypothesis that dietary ALA supplementation ameliorates the decrease in brown eggshell color of laying hens fed diets containing supplemental Mg.

MATERIALS AND METHODS

Birds, Diets, and Experimental Design

All experimental procedures in the current study were reviewed and approved by the Animal Care and Use Committee at Chung-Ang University. The present experiment was performed using a completely randomized design with 2 × 2 factorial arrangements including 2 levels of supplemental Mg (0 and 0.12%) and ALA (0 and 0.25%; 90.0% purity) in diets. Magnesium oxide (MgO, 60.3% Mg) was chosen as the source of supplemental Mg because dietary supplementation of MgO in layer diets showed the positive effects on eggshell strength in previous experiments (Seo et al., 2010; Kim et al., 2013a,b). A total of 192 43-wk-old Hy-Line Brown laying hens were allotted to 1 of 4 dietary treatments with 6 replicates. Each replicate consisted of 4 consecutive cages with 2 hens per cage (30 × 37 × 40 cm = width × length × height). All nutrients and energy in the basal diet were formulated to meet or exceed the requirement estimates for Hy-Line Brown laying hens (Hy-Line, 2016; Table 1). The MgO and ALA were supplemented to the basal diet at the expense of celite (Celite 545, Samchun Pure Chemical Co., Ltd., Pyeongtaek, Republic of Korea). Diets were fed to laying hens on an ad libitum basis for 6 wk. All hens were provided free access to water during the entire experiment. The room temperature and relative humidity were maintained at 20°C and 60%, respectively. A 16-h lighting schedule was used throughout the experiment.

Table 1. Composition and nutrient concentrations of the basal diet (as-fed basis).

Items	Inclusion (%)
Ingredients	
Corn grains	59.60
Soybean meal (46% CP)	25.00
Tallow	2.00
Mono and dicalcium phosphate	1.02
Limestone	9.82
Cornstarch	1.00
Celite[1]	0.68
L-Lysine-HCl (78%)	0.08
L-Threonine (98.5%)	0.08
DL-methionine (98%)	0.27
Choline	0.05
Sodium bicarbonate	0.10
Salt	0.10
Mineral premix[2]	0.10
Vitamin premix[3]	0.10
Total	100.00
Calculated energy and nutrient concentrations[4]	
AME_n (kcal/kg)	2,772
Crude protein (%)	16.13
Lysine (%)	0.80
Methionine + cysteine (%)	0.71
Methionine (%)	0.48
Threonine (%)	0.60
Tryptophan (%)	0.15
Calcium (%)	3.91
Available phosphorus (%)	0.38
Magnesium (%)	0.14

[1]Magnesium oxide (60.3% Mg) and δ-aminolevulinic acid (90.0% purity) were supplemented to the diet by replacing the celite.

[2]Provided per kg of the complete diet: iron, 50 mg ($FeSO_4$); zinc, 60 mg ($ZnSO_4$); manganese, 50.0 mg (MnO); copper, 6.00 mg ($CuSO_4$); cobalt, 250 μg ($CoSO_4$); selenium, 150 μg (Na_2SeO_3); iodine, 1 mg [$Ca(IO_3)_2$].

[3]Provided per kg of the complete diet: vitamin A, 13,000 IU (Retinyl acetate); vitamin D_3, 5,000 IU; vitamin E, 80 IU (DL-alpha-tocopheryl acetate); vitamin K_3, 4.0 mg (menadione dimethpyrimidinol); vitamin B_1, 4.0 mg; vitamin B_2, 10.0 mg; vitamin B_6, 6.0 mg; vitamin B_{12}, 20 μg; folic acid, 2.0 mg; biotin, 200 μg; niacin, 60 mg; calcium-pantothenate, 20 mg.

[4]Calculated values from Hy-Line (2016).

Productive Performance and Egg Quality

Productive performance including hen-day egg production, egg weight, egg mass, and broken and shell-less egg production rate were recorded daily. Feed intake (**FI**) and feed conversion ratio (**FCR**) were calculated weekly. The FCR was calculated by dividing FI with egg mass. The data for productive performance were then summarized for 6 wk, from 43 to 48 wk of age. Six eggs per replicate were collected at the end of the experiment to measure egg quality. Eggshell strength was measured by the compression test cell with Texture systems (model T2100C, Food Technology Co. Ltd., Rockville, MD) and was expressed as unit of compression force exposed to unit of eggshell surface area. Eggshell thickness was measured from three different regions (top, middle, and bottom) without inner and outer shell membranes using a dial pipe gauge (model 7360, Mitutoyo Co., Ltd., Kawasaki, Japan). Egg yolk color was determined by using the Roche color fan (Hoffman-La Roche Ltd., Basel, Switzerland; where 15 = dark orange and 1 = light pale). Eggshell color fan (Samyangsa, Kangwon-do, Republic of Korea; where 15 = very dark brown and 1 = very light and pale) and color reader (model CR-10, Konica Minolta Optics Inc., Japan) were used to measure the eggshell color score and CIE Lab value, respectably. The eggshell color for color reader was expressed as lightness (L*), redness (a*) and yellowness (b*) values. The Haugh Unit (**HU**) values were calculated from egg weight (W) and albumen height (H), considering the following equation: $HU = 100 \log (H - 1.7\ W^{0.37} + 7.6)$, as demonstrated by Eisen et al. (1962).

Statistical Analysis

All data were analyzed by 2-way ANOVA as a completely randomized design using the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC). The replicate was considered an experimental unit for all measurements. Outlier data were checked using the PROC UNIVARIATE procedure of SAS. The main effects in the model included dietary Mg supplementation, ALA supplementation, and their interaction. The LSMEANS procedure was used to calculate treatment means. The PDIFF option of SAS was used to separate the means when the interaction was significant. Significance and tendency for statistical tests were considered at $P < 0.05$ and $0.05 \leq P \leq 0.10$, respectively.

RESULTS AND DISCUSSION

No significant interactions were observed between dietary supplementation of Mg and ALA on productive performance of laying hens (Table 2). As the main effects, dietary Mg supplementation did not affect hen-day egg production, egg weight, egg mass, FCR, and broken and shell-less egg production, except for a tendency ($P = 0.09$) toward increased FI when Mg was supplemented to diets. Likewise, dietary ALA supplementation did not influence productive performance of laying hens.

Dietary Mg supplementation improved ($P < 0.01$) eggshell strength but decreased ($P < 0.05$) eggshell color score, regardless of dietary ALA supplementation (Table 2). However, decreased eggshell color of laying hens fed diets containing supplemental Mg was ameliorated by dietary ALA supplementation, which led to a significant interaction ($P < 0.05$). Moreover, an interaction ($P < 0.05$) was also observed for eggshell lightness (L* values). Dietary ALA supplementation decreased eggshell L* values when Mg was supplemented to diets, whereas this effect was not identified for diets containing no supplemental Mg. Interestingly, dietary Mg supplementation improved ($P < 0.05$) egg yolk color, regardless of dietary ALA supplementation.

The current observation for the significant increase in eggshell strength by dietary Mg supplementation agreed with previous experiments reporting that increasing supplementation of Mg in diets from 0 to 0.12% linearly increased eggshell strength of 72-wk-old laying hens (Kim et al., 2013a). Similar improvements in eggshell strength were also observed for 46-wk-old laying hens fed diets supplemented with increasing levels of Mg in diets from 0 to 0.24% with 0.12% Mg providing the greatest eggshell strength (Kim et al., 2013b). Furthermore, Seo et al. (2010) also observed positive effects of dietary supplementation of 0.18% Mg on eggshell strength of 24-wk-old laying hens. These improvements in eggshell strength with dietary Mg supplementation have been attributed to the role of Mg in promoting the proper development of eggshells in the uterus (Waddell et al., 1991) and its positive effect on Mg deposition in eggshells (Kim et al., 2013a,b).

Although dietary Mg supplementation improved eggshell strength of laying hens, the current experiment revealed a decrease in the intensity of brown eggshell color with decreased eggshell color scores and increased L* values. This result was expected because of increasing Mg deposition in eggshells and white element color of Mg as shown in previous experiments (Kim et al., 2013a,b). Therefore, dietary ALA supplementation was hypothesized to alleviate the adverse effect of dietary Mg supplementation on brown eggshell color based on the

Table 2. Effects of dietary magnesium (Mg) and δ-aminolevulinic acid (ALA) supplementation on productive performance and egg quality of laying hens.[1]

		Treatments[2]							
		Mg-		Mg+			*P*-value		
Items		ALA-	ALA+	ALA-	ALA+	SEM	Mg	ALA	Mg × ALA
Productive performance									
Hen-day egg production (eggs/hen/d)		0.93	0.92	0.94	0.93	0.02	0.51	0.54	0.87
Egg weight (g)		65.9	66.3	66.2	66.8	0.83	0.60	0.54	0.94
Egg mass (g)		61.0	60.9	62.3	62.0	1.63	0.47	0.88	0.95
Feed intake (g/hen/d)		128	129	132	136	3.05	0.09	0.53	0.68
Feed conversion ratio (g/g)		2.10	2.13	2.13	2.19	0.05	0.39	0.40	0.69
Broken and shell-less eggs (%)		0.3	0.2	0.3	0.2	0.01	0.88	0.30	0.66
Egg quality									
Eggshell thickness (μm)		429	434	437	428	4.92	0.83	0.68	0.19
Eggshell strength (kg/cm^2)		2.92	3.00	3.30	3.36	0.07	<0.01	0.33	0.89
Haugh Unit[3]		98.9	96.6	97.5	97.5	0.80	0.18	0.77	0.18
Egg yolk color (Roche color fan)[4]		7.6	7.6	8.0	8.0	0.15	0.01	0.71	0.97
Eggshell color (Eggshell color fan)[5]		11.3[a]	11.3[a]	10.1[b]	11.4[a]	0.26	0.03	0.02	0.03
Eggshell color (CIE Lab value)[6]	L*	52.4[b]	52.8[ab]	54.3[a]	52.4[b]	0.57	0.21	0.20	<0.05
	a*	19.5	19.8	18.9	19.3	0.31	0.10	0.20	0.91
	b*	25.4	25.9	24.8	25.4	0.39	0.16	0.17	0.84

[a-b]Means with different superscripts within a row differ ($P < 0.05$).
[1]Data are least squares means of 6 replicates per treatment.
[2]Treatments included 2 supplemental levels of dietary Mg (0 and 0.12%) and ALA (0 and 0.25%).
[3]Haugh Unit was calculated based on the equation (H = albumen height; W = egg weight): Haugh unit = 100 log (H − 1.7W$^{0.37}$ + 7.6).
[4]Egg yolk was evaluated by the Roche color fan (15 = dark orange and 1 = light pale).
[5]Eggshell color was evaluated by the eggshell color fan (15 = dark brown and 1 = light white).
[6]L* = lightness; a* = redness; b* = yellowness.

assumption that dietary ALA supplementation may facilitate the synthesis of PP9 (i.e., eggshell brown pigment) in the uterus, and thereby, increase the intensity of brown eggshell color. Accordingly, brown eggshell color was improved by dietary ALA supplementation when Mg was supplemented to the diet. However, this improvement in brown eggshell color was not observed for hens fed diets containing no supplemental Mg. The reason for this observation is not clear, but it may be associated with impaired ALA synthesis by Mg supplementation in diets. Therefore, it appears that dietary ALA supplementation may compensate for decreased PP9 synthesis in the uterus due to Mg supplementation in diets. It may be speculated that decreased intensity of brown eggshell color by dietary Mg supplementation may result from both increasing deposition of white-colored Mg in eggshells and decreasing ALA synthesis in the uterus. However, this speculation should be verified by further researches on interactive effects between dietary Mg and ALA on eggshell pigmentation of laying hens.

In conclusion, dietary Mg supplementation improves eggshell strength but decreases the intensity of brown eggshell color of laying hens. This decreased intensity of eggshell color is likely ameliorated by dietary ALA supplementation. Therefore, dietary supplementation of Mg and ALA can be a potential solution to improve eggshell strength of laying hens with no reduction in brown eggshell color.

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DISCLOSURES

The authors declare no conflict of interest for the data presented in this experiment.

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