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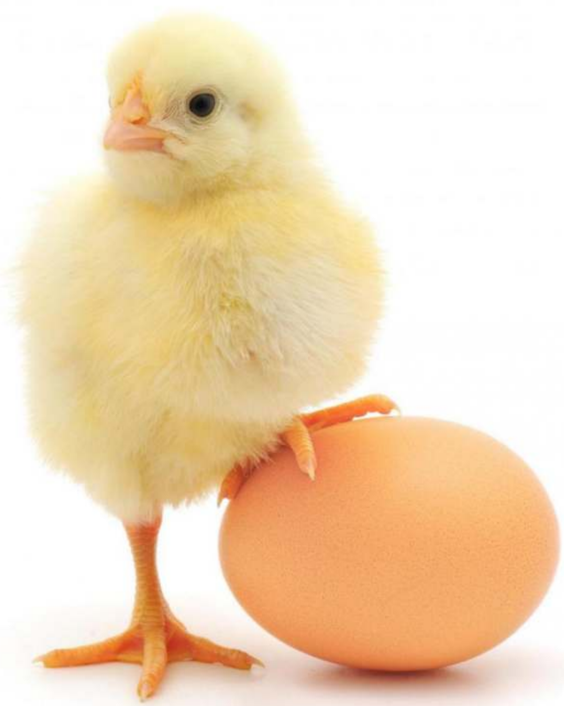

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Short Communication
Effect of Inclusion of Blood Meal in Diets on Eggshell Color of Brown-egg Laying Hens

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Abstract

Background and Objective: The main brown-colored pigment in eggshells is protoporphyrin IX (PP9) produced from the hen's eggshell gland. Blood Meal (BM), which is a by-product of animal processing industry, contains high amounts of Fe and PP9. Thus, the current experiment was conducted to investigate the effect of BM inclusion in diets on eggshell color of brown-egg laying hens. **Materials and Methods:** A total of one hundred twenty 43-week-old Hy-line brown laying hens were randomly allotted to one of three dietary treatments with five replicates. A commercial-type control diet was formulated. Two additional diets were prepared by adding 0.5 or 1.0% BM to the control diet. Productive performance was summarized for 6 weeks. At the end of the 6-week feeding trial, four eggs per replicate were collected to assess the egg quality trait. **Results:** Productive performance was not affected by increasing levels of BM in diets. However, increasing levels of BM in diets decreased egg yolk color (Linear, $p = 0.01$) and eggshell hunter b* value (Quadratic, $p<0.01$) in brown-egg laying hens. **Conclusion:** Dietary supplementation of BM negatively affects egg yolk color and brown coloration of eggshells in brown-egg laying hens.

Key words: Blood meal, brown-egg laying hen, eggshell color, egg yolk color, hunter color value, productive performance, protoporphyrin IX

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The eggshell color is an important measure of egg quality for brown-egg laying hens because consumers have low preferences on light and pale brown-colored eggs. It is suggested that impaired eggshell coloration in brown eggs is often associated with an increase in age, stress and respiratory disease[1]. Therefore, appropriate management practices to reduce stress and improve health of laying hens are required to prevent the production of poor eggshell color as hens become aged. Additionally, various nutritional strategies should be developed and implemented to increase brown eggshell coloration because dietary regimens may be easier to be adapted in a practical circumstance.

The main brown-colored pigment in eggshells is protoporphyrin IX (PP9) produced from the hen's eggshell gland[1]. The PP9 in eggshells was derived from aging or spent erythrocytes during heme metabolism[1]. It can be expected, therefore, that dietary factors affecting heme metabolism such as PP9 synthesis may modify eggshell coloration of brown-egg laying hens. Seo *et al.*[2] reported that increasing levels of proteinated iron (Fe) in diets improved eggshell color of brown-egg laying hens and they speculated that, this improvement may be due to increasing erythrocytes' turnover rates. In addition, dietary supplementation of δ-aminolevulinic acid, which is a precursor molecule for PP9 synthesis in the body, was reported to improve eggshell color of brown-egg laying hens, possibly due to increasing supply of PP9 precursors[3]. Thus, increasing supply of Fe and PP9 may have a beneficial effect on brown eggshell coloration. However, to our knowledge, the information for such a dietary treatment to improve eggshell color of brown-egg laying hens is currently scarce.

Blood Meal (BM) is a by-product of meat industry used as a feed ingredient. The BM contains high amounts of both Fe and PP9; thus, it can be hypothesized that inclusion of BM in diets may increase the intensity of brown eggshell color in brown-egg laying hens, probably due to increasing supply of Fe and PP9. However, this hypothesis has not been tested previously.

Thus, the objective of the current experiment was to investigate the effect of inclusion of BM in diets on eggshell color of brown-egg laying hens.

MATERIALS AND METHODS

A total of one hundred twenty, 43-weeks-old Hy-Line Brown laying hens were randomly allotted to one of three dietary treatments with five replicates per treatment. Each replicate consisted of four consecutive cages with two hens per cage. A commercial-type control diet was formulated (Table 1). Two additional diets were prepared by adding 0.5 or 1.0% BM to the control diet. All nutrients and energy were formulated to meet or exceed the NRC[4] requirement estimates for laying hens. Hens were fed the experimental diets on an ad libitum basis for 6 weeks. A 16 h lighting schedule was used throughout the experiment. The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at Chung-Ang University (IACUC No. 2016-00109).

Data regarding productive performance and egg quality were collected according to the procedure described by Shin *et al.*[5]. In short, productive performance including hen-day egg production, egg weight and egg mass was recorded daily. Feed Intake (FI) and Feed Conversion Ratio (FCR) were recorded weekly. The data for productive performance were then summarized for 6 weeks of feeding trial. Egg quality was assessed with four eggs per replicate randomly collected at the conclusion of the experiment. Eggshell strength was determined using the texture analyzer (Model TAHDi 500, Stable Micro System, Godalming, UK), which displayed compression force (kg cm^{-2}) of breaking eggshells at the given surface area of the large end of eggs. Eggshell color was determined using the eggshell color fan (Samyangsa, Kangwon-do, Republic of Korea; 15 = Very dark brown and 1 = Very light and pale). Egg yolk color was estimated by the Roche color fan (Hoffman-La Roche Ltd., Basel, Switzerland; 15 = Dark organge and 1 = Light pale). The hunter values for lightness (L*), redness (a*) and yellowness (b*) in eggshells were determined using the Minolta Chorma Meter CR-400 (Minolta, Osaka, Japan).

All data were analyzed using one-way ANOVA with a completely randomized design using the PROC MIXED procedure (SAS Institute Inc., Cary, NC). The replicate was used as an experimental unit. The LSMEANS procedure was used to calculate treatment means. The orthogonal polynomial contrast test was performed to determine linear and quadratic effects of increasing levels of BM in diets. Significance for statistical tests was set at $p<0.05$.

RESULTS AND DISCUSSION

Productive performance including hen-day egg production, egg weight, egg mass, FI and FCR in brown-egg laying hens was not influenced by increasing levels of BM in diets (Table 2). For egg quality, eggshell strength was not

Table 1: Composition and nutrient content of the experimental diets

Items (%, unless noted)	Dietary treatments* NC	BM0.5	BM1.0
Ingredients			
Corn	61.10	61.61	62.11
Soybean meal (46% CP)	25.00	24.00	23.00
Soybean oil	2.00	2.00	2.00
Blood Meal (BM)	0.00	0.50	1.00
Mono-dicalcium phosphate	1.02	1.05	1.07
Limestone	10.00	10.00	10.00
Salt	0.10	0.10	0.10
DL-methionine	0.27	0.27	0.27
L-lysine-HCl	0.08	0.05	0.03
L-threonine	0.08	0.07	0.07
Choline	0.05	0.05	0.05
Sodium bicarbonate	0.10	0.10	0.10
Vitamin premix	0.10	0.10	0.10
Mineral premix	0.10	0.10	0.10
Total	100.00	100.00	100.00
Nutrient content**			
AME_n (kcal kg^{-1})	2.784	2.793	2.803
Crude protein	16.24	16.25	16.27
Lysine	0.80	0.80	0.80
Methionine+cysteine	0.71	0.71	0.71
Calcium	3.98	3.98	3.98
Non-phytate phosphorus	0.38	0.38	0.38

Vitamin premix (per kg of the complete diet): vitamin A: 13,000 IU (retinyl acetate), Vitamin D3: 5,000 IU, Vitamin E: 80 IU (DL-alpha-tocopheryl acetate), Vitamin K3: 4.0 mg (menadione dimethpyrimidinol), Vitamin B1: 4.0 mg, Vitamin B2: 10.00 mg, Vitamin B6: 6.0 mg, Vitamin B12: 20 µg, Folic acid: 2.0 mg, Biotin: 200 µg, Niacin: 60 mg, Calcium-pantothenate: 20 mg, Mineral premix (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 [$Ca(IO_3)_2$], *Dietary treatments = NC: Negative control, BM0.5: NC+0.5% blood meal (BM), BM1.0: NC+1.0% blood meal (BM), **Calculated values[2]

Table 2: Effects of inclusion of Blood Meal (BM) in diets on productive performance and egg quality in laying hens

Items	Dietary treatments# NC	BM0.5	BM1.0	SEM	p-value## L	Q
Productive performance						
Hen-d egg production, egg (%)	96.6	95.3	95.4	1.04	0.44	0.59
Egg weight (g)	69.9	68.9	67.9	0.84	0.11	0.99
Egg mass (g day^{-1})	67.6	65.7	64.8	1.32	0.16	0.77
Feed intake (g day^{-1})	132	129	127	2.0	0.13	0.73
Feed conversion ratio (g g^{-1})	1.95	1.96	1.97	0.035	0.79	0.98
Egg quality						
Eggshell strength, (kg cm^{-2})	3.3	3.5	3.1	0.14	0.49	0.18
Egg yolk color (Color fan)	8.5	8.1	7.9	0.17	0.01	0.52
Eggshell color (Color fan)	11.5	10.9	10.7	0.38	0.14	0.67
Eggshell color (Hunter values)						
L*	54.3	54.7	55.5	0.41	0.06	0.68
a*	20.7	20.5	20.1	0.40	0.29	0.80
b*	28.2	26.9	27.9	0.29	0.41	<0.01

#Dietary treatments, NC: Negative control, BM0.5: NC+0.5% blood meal (BM), BM1.0: NC+1.0% blood meal (BM), ##L: Linear effects of increasing levels of BM in diets, Q: Quadratic effects of increasing levels of BM in diet

affected by increasing levels of BM in diets; however, egg yolk color was decreased (linear, $p = 0.01$) with increasing levels of BM in diets. Eggshell color measured by the eggshell color fan was not affected by increasing levels of BM in diets. However, increasing levels of BM in diets decreased (quadratic, $p<0.01$) hunter yellowness (b*) values for eggshell colors without affecting hunter lightness (L*) and redness (a*) values.

The observation for no differences in productive performance agreed with Namgung *et al.*[6], who reported that 2.5 or 5.0% inclusion of BM in diets did not influence productive performance of brown-egg laying hens. These results indicate that inclusion of BM in layer diets has no detrimental effects on productive performance of brown-egg laying hens if diets are formulated based on the current nutrient recommendation[4]. However, egg yolk color was decreased with increasing levels of BM in diets. Similar results for decreased egg yolk color were observed by feeding diets containing BM or spray-dried blood cells to brown-egg laying hens[6,7]. The yellow pigments of egg yolk are xanthophylls that originate primarily from the diet[4,5]. Thus, decreased egg yolk color by feeding diets containing BM is likely caused by decreased absorption and utilization of xanthophylls in diets[4,5]. It was reported that some unknown components in blood cells may reduce absorption and utilization of carotenoids such as xanthophylls in animals[8]. However, the unknown components in blood cells are not elucidated.

Increasing levels of BM in diets decreased hunter yellowness (b*) values for eggshell colors without affecting hunter lightness (L*) and redness (a*) values. Although the significance was not detected, eggshell colors measured by the eggshell color fan also were decreased with increasing levels of BM in diets. These results were in contrast to our hypothesis that BM contains high amounts of PP9, which is the main pigment for brown-color in eggshells and therefore, increasing intake of BM may improve brown eggshell coloration by increasing PP9 accumulation in eggshells. In addition, BM contains high amounts of Fe and Seo *et al.*[2] reported that supplementation of additional Fe in diets improved brown eggshell coloration. Thus, our observation for impaired eggshell coloration was unexpected and difficult to be explained because no previous experiments have reported the negative effect of dietary BM on eggshell coloration of brown-egg laying hens. The reason for our observation is not clear; however, it may be related to very high intake of Fe from BM in diets because very high intake of Fe can accelerate oxidative stress[9,10], which is known to decrease the brown coloration of eggshells[1]. To our knowledge, the current experiment is the first to report the decreased brown eggshell coloration by feeding diets containing BM to brown-egg laying hens. Further research is required to find physiological reasons why dietary BM decreases brown eggshell coloration of brown-egg laying hens.

CONCLUSION

Dietary supplementation of BM negatively affects egg yolk color and brown eggshell coloration of brown-egg laying hens.

SIGNIFICANCE STATMENT

This study discovers the negative effect of dietary BM on egg yolk color and brown eggshell coloration although BM contains high amounts of Fe and PP9. This study will help the researchers who want to modify heme metabolisms with nutritional regimens for the purpose of improving eggshell color of brown-egg laying hens.

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