Research Note

Effect of feeding duration of diets containing corn distillers dried grains with solubles on productive performance, egg quality, and lutein and zeaxanthin concentrations of egg yolk in laying hens

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ABSTRACT This experiment was conducted to investigate the effect of feeding duration of diets containing corn distillers dried grains with solubles (DDGS) on productive performance, egg quality, and lutein and zeaxanthin concentrations of egg yolk in laying hens. A total of 300 57-week-old Hy-Line Brown laying hens were randomly assigned to one of 5 treatment groups (feeding duration) with 6 replicates consisting of 5 consecutive cages with 2 hens per cage. Diets were formulated to contain either 0% (the control diet) or 20% DDGS. Experimental diets were fed to hens for 12 wk. The feeding duration of diets containing 20% DDGS was 0, 3, 6, 9, or 12 wk before the conclusion of the experiment. Feeding the diet containing 20% DDGS for 3, 6, or 9 wk followed feeding the control diet for 9, 6, or 3 wk, respectively. The data for productive performance were summarized for 12 wk of the feeding trial. Results indicated that increasing feeding duration of diets containing 20% DDGS had no effects on productive performance of laying hens, but increased egg yolk color (linear, $P < 0.01$), hunter a* value (linear and quadratic, $P < 0.01$), and b* values (linear, $P < 0.05$) with a decrease in hunter L* value (linear and quadratic, $P < 0.05$). Lutein and zeaxanthin concentrations of egg yolks also were increased (linear, $P < 0.01$) by increasing the feeding duration of diets containing 20% DDGS. In conclusion, feeding diets containing 20% DDGS to laying hens has no adverse effects on productive performance. Increasing the feeding duration of diets containing 20% DDGS improves egg yolk coloration with a concomitant increase in lutein and zeaxanthin concentrations of egg yolks in laying hens.

Key words: corn distillers dried grains with solubles, egg yolk, laying hen, lutein, zeaxanthin

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INTRODUCTION

Lutein and zeaxanthin are carotenoid pigments as the subclass of xanthophylls (Krinsky, 1993). Lutein and zeaxanthin are the isomers of each other and differ only in the position of double bond at the end ring (Nolan et al., 2013). These xanthophylls have gained great attention as dietary phytonutrients for human health, especially for the protection of certain eye disease from harmful wavelengths of visible light (Leeson and Caston, 2004). In addition, there has been increasing evidence that these xanthophylls show diverse beneficial functions such as antioxidant activity, immune modulation, and anticancer action (Krinsky, 1993). Among various food sources high in lutein and zeaxanthin for humans, eggs have been known to contain a relatively high amount of available lutein and zeaxanthinin in egg yolks (Perry et al., 2009). Therefore, various dietary means to fortify egg yolks with these xanthophylls have been investigated (Leeson and Caston, 2004). However, most of the previous experiments have studied lutein in egg yolks and dietary interventions for lutein-enriched egg production (Leeson and Caston, 2004; Sun et al., 2013), whereas the information regarding zeaxanthin has been limited despite its health benefits similar to lutein.

Corn distillers dried grains with solubles (**DDGS**) is a co-product of corn fermentation from the ethanol industry and has been widely used as a feed ingredient for poultry diets. It has been reported that increasing inclusion of DDGS at a high level of up to 20% in diets fed to laying hens improved egg quality such as egg yolk color with no detrimental effects on productive performance although the recommended upper limit in the inclusion levels is still debatable (Świąkiewicz and Koreleski, 2008; Salim et al., 2010). This improvement in egg yolk color often has been associated with increased concentrations of xanthophylls in egg yolks (Salim et al., 2010; Masa'deh et al., 2011). Sun et al.

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(2013) also observed increased concentrations of lutein in egg yolks by feeding diets containing increasing amounts of DDGS to hens and increasing the feeding duration of the diets. Although zeaxanthin is present at a similar or even greater level than lutein in egg yolks (Romanoff and Romanoff, 1949; Leeson and Caston, 2004), few experiments have focused on the effects of feeding diets containing DDGS to hens and different feeding durations of the diets on the concentrations of zeaxanthin in egg yolks.

Therefore, the objective of the current experiment was to investigate the effects of feeding duration of diets containing DDGS on productive performance, egg quality, and lutein and zeaxanthin concentrations of egg yolks in laying hens.

MATERIALS AND METHODS

The protocol for this experiment was reviewed and approved by the Institutional Animal Care and Use Committee at Chung-Ang University.

Animals, Diets, and Experimental Design

A total of 300 57-week-old commercial Hy-Line Brown laying hens (initial BW = 2.3 ± 0.13 kg) in the first laying cycle were randomly assigned to one of 5 treatment groups (i.e., feeding duration) with 6 replicates. All hens were fed a commercial layer diet for 4 wk prior to the experiment. Each replicate consisted of 5 consecutive cages with 2 hens per cage (30 × 37 × 40 cm, width × length × height). The corn-soybean meal-based control diet was prepared and one additional diet was formulated by adding 20% DDGS to the control diet in the partial replacement of soybean meal, rapeseed meal, and wheat that contained very low amounts of lutein and zeaxanthin. The inclusion level of the corn was maintained at 40% to minimize its contribution of lutein and zeaxanthin in the diets. The nutrient composition of DDGS used in this experiment is presented in Table 1. The analyzed concentrations of lutein and zeaxanthin in DDGS were 104.1 and 141.2 μg/g, respectively. The analyzed concentrations of lutein and zeaxanthin in diets were increased from 5.2 to 24.0 μg/g for lutein, and from 12.6 to 40.3 μg/g for zeaxanthin by inclusion of 20% DDGS in diets (Table 2). The inclusion level of DDGS was chosen based on the results of our previous study (Shin et al., 2015) and those of other studies (Salim et al., 2010; Purdum et al., 2014). All nutrients and energy were included to meet or exceed nutrient requirement estimates for laying hens (NRC, 1994). Experimental diets were formulated on a total amino acid basis. Diets were fed to hens for 12 wk. The feeding duration of diets containing 20% DDGS was 0, 3, 6, 9, or 12 wk before the conclusion of the experiment. Feeding diets containing 20% DDGS for 3, 6, or 9 wk followed by feeding the control diet for 9, 6, or 3 wk, respectively. Hens had free access to the diet and were provided with a 16L:8D photoperiod. The temperature was set at 15.0 ± 1.3°C during the experiment.

Table 1. Nutritional composition of corn distillers dried grains with solubles (DDGS) used in this experiment (as-fed basis).

Items	Value
AME_n,[1] kcal/kg	2,820
Nutrient[2]	
DM, %	88.90
CP, %	26.34
Ether extract, %	8.70
Ash, %	5.75
Neutral detergent fiber, %	34.81
Acid detergent fiber, %	13.80
Total lysine, %	0.81
Total methionine, %	0.55
Total cysteine, %	0.60
Total threonine, %	0.97
Calcium, %	0.23
Total phosphorus, %	0.82
Available phosphorus,[3] %	0.67
Lutein,[4] μg/g	104.1
Zeaxanthin,[4] μg/g	141.2

[1]The value was adopted from Batal and Dale (2006).
[2]Nutrients except for lutein and zeaxanthin were analyzed by AOAC methods (AOAC International, 2006).
[3]Calculated from total phosphorus with the assumption of 82% availability (Martinez Amezcua et al., 2004).
[4]Lutein and zeaxanthin concentrations were analyzed by the method as described by Schlatterer and Breithaupt (2006) and Perry et al. (2009).

Sample Collection and Analysis

Detailed procedures for sample collection and data analyses were described by Kim et al. (2013). In short, hen-day egg production, broken-shell less egg production, egg weight, and egg mass were recorded daily, whereas feed intake and feed conversion ratio (**FCR**) were recorded weekly. The data for productive performance were summarized for 12 wk of the feeding trial. At the end of the experiment, a total of 120 eggs (4 eggs per replicate) were randomly collected to measure eggshell strength, eggshell thickness, eggshell color, egg yolk color, and Haugh unit (**HU**). 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 eggshell surface area (in kilograms per square centimeter). Eggshell thickness (without inner and outer shell membrane) was determined at 3 different points (top, middle, and bottom) using a dial pipe gauge (model 7360, Mitutoyo Co., Ltd., Kawasaki, Japan) without cracking the eggshell. Color fans were used to measure eggshell color (Color Fan of Samyang Feed Co. Ltd., Seoul, Korea). Egg yolk color was evaluated by the Roche color fan (Hoffman-La Roche Ltd., Basel, Switzerland; 15 = dark orange; 1 = light pale). The hunter values for lightness (L^*), redness (a^*), and

Table 2. Composition and nutrient content of experimental diets (as-fed basis).[1]

	Control	20% DDGS[2]
Ingredients (%)		
Corn	40.00	40.00
Soybean meal	16.93	8.35
Rapeseed meal	5.00	3.90
Wheat	18.27	9.04
DDGS[2]	0.00	20.00
Tallow	6.81	5.92
Mono-dicalcium phosphate	1.52	0.95
Limestone	10.59	10.85
L-lysine HCl	0.15	0.31
L-threonine	0.08	0.09
DL-methionine	0.25	0.19
$NaHCO_3$	0.10	0.10
Salt	0.10	0.10
Vitamin-mineral premix[3]	0.20	0.20
Total	100.0	100.0
Energy and nutrient content[4]		
AME_n, kcal/kg	2,900	2,900
CP, %	14.8	14.8
Total lysine, %	0.83	0.83
Total methionine + cysteine, %	0.74	0.74
Total threonine, %	0.63	0.63
Calcium, %	4.29	4.29
Available phosphorus, %	0.37	0.37
Lutein, μg/g	5.2	24.0
Zeaxanthin, μg/g	12.6	40.3

[1]Diet formulation was provided partly in our previous experiment (Shin et al., 2015).

[2]Corn distillers dried grains with solubles.

[3]Provided per kilogram of the complete diet: Vitamin A, 12,500 IU (retinyl acetate); vitamin D_3, 2,500 IU; vitamin E, 20 IU (DL-α-tocopheryl acetate); vitamin K_3, 2 mg (menadione dimethpyrimidinol); vitamin B_1, 2 mg; vitamin B_2, 5 mg; vitamin B_6, 3 mg; vitamin B_{12}, 18 μg; folic acid, 1 mg; biotin, 50 μg; niacin, 24 mg; zinc, 60 mg; manganese 50 mg; iron, 50 mg; copper, 6 mg; cobalt, 250 μg; iodine, 1 mg; Selenium, 150 μg.

[4]All values were calculated except that lutein and zeaxanthin contents were analyzed (Schlatterer and Breithaupt, 2006; Perry et al., 2009).

yellowness (b*) in egg yolks also were determined using the Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan). The HU was measured using the micrometer (model S-8400, Ames, Waltham, MA), and the HU values were calculated from egg weight (W) and albumen height (H), considering the following formula: $HU = 100 \log (H - 1.7W^{0.37} + 7.6)$ as described by Eisen et al. (1962). For the analyses of lutein and zeaxanthin in egg yolks, 3 eggs from each replicate were separated into yolk and albumen, and then the 3 yolks were pooled in a tray, freeze-dried, and ground for lutein and zeaxanthin analyses.

Lutein and Zeaxanthin Analyses

Lutein and zeaxanthin analyses for DDGS, diets, and egg yolks were performed as described previously (Schlatterer and Breithaupt, 2006; Perry et al., 2009; Shin et al., 2015). In short, the samples of DDGS, experimental diets, and egg yolks were extracted with acetone for 24 h and removed in vacuo to obtain crude extracts. The resultant solutions were filtered through a Whatman 0.45-μm PVDF syringe filter (Cat No. 6779, Whatman, Marlborough, MA) for HPLC analysis. The quantitative analysis of lutein and zeaxanthin in samples was performed using a reverse phase system. A reverse phase column (Discovery C18, 5 μm, 250 × 4.6 mm, Supelco Analytical, Bellefonte, PA) was used, and a gradient solvent system (acetonitrile:ethyl acetate, vol/vol) was employed as the mobile phase. The gradient solvent system was 100:0 initially and was increased in linear gradients to 80:20 for 35 min. The flow rate was kept constant at 0.5 mL/min, and the peaks were identified using UV absorbance at 445 nm. The injection volume was 10 μL of prepared acetone solution. HPLC analyses were performed in triplicate. Calibration standard solutions were prepared at 5 different concentration levels (1.0, 0.5, 0.25, 0.125, and 0.06 μg/mL) and injected in triplicate. Calibration curves were constructed by linear regression of the peak area-ratios (Y) of lutein and zeaxanthin versus the concentration (X, mg/mL); the relative standard deviation was used as a measure of repeatability.

Statistical Analysis

All data were analyzed as a completely randomized design using the Proc Mixed procedure in SAS (SAS Institute, Cary, NC). Replicate was an experimental unit for all data analysis. Outlier data were identified by the UNIVARIATE procedure of SAS, but no outliers were detected. Dietary treatment (i.e., feeding duration) was a fixed effect in the model. The LSMEANS procedure was used to calculate mean values. The orthogonal polynomial contrast test was performed to determine linear and quadratic effects of increasing the feeding duration of diets containing DDGS on laying performance, egg quality, and lutein and zeaxanthin concentrations of egg yolks. Significance for statistical tests was set at $P < 0.05$.

RESULTS

Increasing the feeding duration of diets containing 20% DDGS from 0 to 12 wk had no significant linear or quadratic effects on productive performance including hen-day egg production, broken and shell-less egg production, egg weight, egg mass, feed intake, and FCR of laying hens (Table 3).

Eggshell quality such as eggshell strength, eggshell thickness, and eggshell color was not affected by increasing the feeding duration of diets containing 20% DDGS (Table 4). However, increasing the feeding duration of diets containing 20% DDGS increased Roche color score (linear, $P < 0.01$), hunter a* values (linear and quadratic, $P < 0.01$), and hunter b* values (linear, $P < 0.05$) in egg yolks. However, hunter L* values in egg yolks were decreased (linear and quadratic, $P < 0.05$) with increasing feeding duration of diets containing 20% DDGS. There was a quadratic relationship ($P < 0.05$) between HU values and feeding duration of

Table 3. Effect of feeding duration of diets containing corn distillers dried grains with solubles (DDGS) on productive performance of laying hens.[1,2]

Items	Feeding duration of diets containing 20% DDGS					SEM	P-value[3]		
	0 wk	3 wk	6 wk	9 wk	12 wk		Duration	Linear	Quadratic
Hen-day egg production, %	88.0	85.9	89.3	87.6	88.3	1.79	0.75	0.68	0.94
Broken-shell less egg production, %	0.04	0.05	0.01	0.02	0.04	0.019	0.58	0.61	0.33
Egg weight, g	66.6	66.8	66.1	67.7	65.9	0.39	0.03	0.69	0.31
Egg mass, g/d	58.7	57.4	59.0	59.3	58.2	1.31	0.85	0.80	0.85
Feed intake, g/d	122	122	121	124	120	1.39	0.36	0.61	0.57
Feed conversion ratio, g of feed /g of egg	2.10	2.15	2.05	2.10	2.07	0.05	0.74	0.51	0.90

[1]Diets containing 20% DDGS were fed to hens during 0, 3, 6, 9, or 12 wk before the end of the experiment.
[2]Data are least squares means of 6 replicates per treatment. Data were summarized for 12 wk of feeding trial.
[3]Duration = Fixed effects (i.e., feeding duration) of the model; linear and quadratic effects of increasing feeding duration.

Table 4. Effect of feeding duration of diets containing corn distillers dried grains with solubles (DDGS) on egg quality of laying hens.[1,2]

Items		Feeding duration of diets containing 20% DDGS					SEM	P-value[3]		
		0 wk	3 wk	6 wk	9 wk	12 wk		Duration	Linear	Quadratic
Eggshell strength, kg/cm^2		2.84	2.96	2.83	2.78	3.07	0.15	0.36	0.57	0.44
Eggshell thickness, μm		421	431	436	425	441	6.39	0.09	0.10	0.86
Eggshell color (color fan)		12.2	12.0	12.3	12.1	12.3	0.31	0.54	0.80	0.78
Egg yolk color (Roche color fan)		8.3	8.9	9.0	9.3	9.5	0.11	<0.01	<0.01	0.13
Egg yolk color (Hunter color)	L*	57.1	55.4	55.5	55.4	55.2	0.36	<0.01	<0.01	0.03
	a*	– 3.5	– 1.7	– 1.8	– 1.6	– 1.4	0.15	<0.01	<0.01	<0.01
	b*	26.6	27.6	27.2	27.5	27.5	0.27	0.04	0.04	0.15
Haugh unit		95.9	96.8	94.4	93.5	98.0	0.99	0.12	0.78	0.03
Lutein in egg yolks, μg/g		39.1	67.6	70.9	73.3	102.6	5.26	<0.01	<0.01	0.98
Zeaxanthin in egg yolks, μg/g		61.2	103.7	109.3	119.3	174.2	9.40	<0.01	<0.01	0.41

[1]Diets containing 20% DDGS were fed to hens during 0, 3, 6, 9, or 12 wk before the end of the experiment.
[2]Data are least squares means of 6 replicates per treatment. Four eggs per replicate for egg quality were analyzed, whereas 3 eggs per replicate were assessed for lutein and zeaxanthin concentrations of egg yolks.
[3]Duration = Fixed effects (i.e., feeding duration) of the model; linear and quadratic effects of increasing feeding duration.

diets containing 20% DDGS. Increasing the feeding duration of diets containing 20% DDGS increased (linear, $P < 0.01$) lutein and zeaxanthin concentrations of egg yolks in laying hens.

DISCUSSION

The results for the recommended maximal inclusion levels of DDGS in layer diets have been inconsistent. Some researchers have recommended an inclusion level of up to 15% DDGS for satisfactory performance (Lumpkins et al., 2005; Roberson et al., 2005; Świąkiewicz and Koreleski, 2006, 2008), whereas others have reported that laying hens showed no negative performance when diets were included with DDGS at the level of 20% (Cheon et al., 2008; Wu-Haan et al., 2010; Purdum et al., 2014) or even greater than 25% (Loar II et al., 2010; Masa'deh et al., 2011; Sun et al., 2012). In this experiment, feeding diets containing 20% DDGS up to the 12 wk of the feeding trial had no adverse effects on productive performance of laying hens. This variation among experiments is likely a consequence of differences in the ages of hens, feeding duration, energy and nutrient composition, and experimental conditions among experiments (Świąkiewicz and Koreleski, 2008; Salim et al., 2010). Among those factors, available amino acid concentrations and their balance in diets have been considered as the major factors (Masa'deh et al., 2011). It is suggested, therefore, that if a good quality of DDGS with desirable color, no mycotoxin contamination, and few anti-nutrients was used and the diets containing DDGS were balanced, especially for available essential amino acids, a greater amount of DDGS can be included in the diets fed to laying hens (Salim et al., 2010; Masa'deh et al., 2011; Sun et al., 2012), which appears to be the reason no negative effects on productive performance was observed in this experiment.

The lack of an effect of 20% DDGS inclusion in diets on eggshell color, eggshell thickness, and eggshell strength indicates that inclusion of 20% DDGS in layer diets does not affect eggshell quality. This observation is in agreement with previous experiments (Świąkiewicz and Koreleski, 2006; Cheon et al., 2008; Deniz et al., 2013). However, the interior quality of eggs was affected by feeding diets containing 20% DDGS and its different feeding duration in the current experiment. Previous experiments reported that laying hens fed diets containing 20% DDGS had improved egg yolk color compared with those fed the control diet (Cheon et al., 2008; Wu-Haan et al., 2010; Masa'deh et al., 2011). This result is in good agreement with the results of the current experiment. As egg yolk color was improved by increasing the feeding duration of diets containing 20% DDGS, hunter a* (redness) and b* (yellowness) values were increased, whereas hunter L* (lightness) was decreased in this experiment. Similar changes in hunter color values also were observed by Roberson et al. (2005) and Loar II et al. (2010). These improvements in egg yolk color by DDGS inclusion in diets have been associated with the greater concentrations of xanthophylls in DDGS than substituted feed ingredients such as corn and soybean meal, and the subsequently increased accumulation of xanthophylls in egg yolks (Salim et al., 2010; Masa'deh et al., 2011; Sun et al., 2012). Lutein has been considered the xanthophyll of main interest in egg yolks (Leeson and Caston, 2004; Sun et al., 2013). In this experiment, we found increased concentrations of lutein in egg yolks by feeding diets containing 20% DDGS to laying hens in a linear feeding duration-dependent manner. A similar increase in the concentrations of zeaxanthin in egg yolks was observed in this experiment, and this result was expected because zeaxanthin is an isomer of lutein, and its utilization is likely close to the utilization of lutein for laying hens (Leeson and Caston, 2004). To our knowledge, however, the present experiment is the first that has reported increased concentrations of zeaxanthin in egg yolks as affected by feeding diets containing DDGS to laying hens with different feeding durations. Interestingly, a previous experiment reported that feeding diets containing DDGS increased the concentrations of lutein in egg yolks during the first 5-wk feeding period, but thereafter the concentrations of lutein tended to be decreased with increasing the feeding duration (Sun et al., 2013), which was contradictory to our observation for both lutein and zeaxanthin in this experiment. The reason for this variable result is not clear; however, it may be related to the differences in the experimental conditions, DDGS sources, and genotype of hens, which affects feed intake, egg production, and egg weight. For instance, we used the Hy-line Brown commercial laying hens, whereas Sun et al. (2013) used the Hy-line White Leghorn laying hens (W-36). The values for feed intake and egg weight observed in this experiment were greater than those observed by Sun et al. (2013). In addition, the ages of the hens may be a potential factor, but the starting age of hens in both experiments was close at 54 wk old (Sun et al., 2013) vs. 57 wk old in this experiment. Therefore, there may be interactive effects of genotype and age on the transfer rate of lutein and zeaxanthin in diets to those in egg yolks.

In conclusion, feeding diets containing 20% DDGS to laying hens improves egg yolk color with no negative effects on laying performance and other egg quality. Increasing the feeding duration of diets containing DDGS linearly improves egg yolk color with a concomitant increase in lutein and zeaxanthin concentrations of egg yolks in laying hens. Therefore, DDGS can be a potential ingredient for the production of eggs enriched with lutein and zeaxanthin.

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