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H. Sun
Iowa State University

E. J. Lee
University of Wisconsin–Stout

H. Samaraweera
Iowa State University

M. Persia
Iowa State University

Dong U. Ahn
Iowa State University, duahn@iastate.edu

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Abstract

The objective of this study was to determine the effects of feeding high concentrations of corn distillers dried grains with solubles (DDGS) on chemical composition and selected nutrient content of egg yolk. Four isocaloric diets were formulated to contain 0, 17, 35, or 50% corn DDGS. A total of two hundred forty 54-wk-old Single-Comb White Leghorn laying hens were randomly allotted to 2 birds per cage with 3 consecutive cages representing an experimental unit (EU). Each EU was randomly assigned to 1 of 4 dietary treatments according to a completely randomized design. Hens were fed for a 24-wk experimental period after transition feeding to gradually increase corn DDGS inclusion over a 4-wk period. Two sets of experimental diets were formulated to meet or exceed the NRC nutrient recommendations for laying hens. Each diet formula was fed for 12 wk. Chemical composition and nutritional components in egg yolk were measured every 2 wk. The results showed that egg yolk from hens fed a DDGS-containing diet tended to have higher fat content and lower protein content. Total polyunsaturated fatty acids were significantly increased by the DDGS diet. The contents of choline and cholesterol were initially higher in the 50% DDGS treatment group, but were not different in the later period, especially during the last 4 wk. Lutein content increased linearly as DDGS level increased. The results indicated that feeding a high level of DDGS can increase the content of lutein and polyunsaturated fatty acids in egg yolk, but may not affect the content of cholesterol and choline.

Keywords

corn distillers dried grains with solubles, egg yolk, choline, lutein, cholesterol

Disciplines

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Effects of increasing concentrations of corn distillers dried grains with solubles on chemical composition and nutrient content of egg

H. Sun,* E. J. Lee,† H. Samaraweera,* M. Persia,* and Dong U. Ahn*‡[1]

*Department of Animal Science, Iowa State University, Ames 50011; †Department of Food and Nutrition, University of Wisconsin–Stout, Menomonie, 54751; and ‡Department of Agricultural Biotechnology, Major in Biomodulation, Seoul National University, Seoul 151-921, Korea

ABSTRACT The objective of this study was to determine the effects of feeding high concentrations of corn distillers dried grains with solubles (DDGS) on chemical composition and selected nutrient content of egg yolk. Four isocaloric diets were formulated to contain 0, 17, 35, or 50% corn DDGS. A total of two hundred forty 54-wk-old Single-Comb White Leghorn laying hens were randomly allotted to 2 birds per cage with 3 consecutive cages representing an experimental unit (EU). Each EU was randomly assigned to 1 of 4 dietary treatments according to a completely randomized design. Hens were fed for a 24-wk experimental period after transition feeding to gradually increase corn DDGS inclusion over a 4-wk period. Two sets of experimental diets were formulated to meet or exceed the NRC nutrient recommendations for laying hens. Each diet formula was fed for 12 wk. Chemical composition and nutritional components in egg yolk were measured every 2 wk. The results showed that egg yolk from hens fed a DDGS-containing diet tended to have higher fat content and lower protein content. Total polyunsaturated fatty acids were significantly increased by the DDGS diet. The contents of choline and cholesterol were initially higher in the 50% DDGS treatment group, but were not different in the later period, especially during the last 4 wk. Lutein content increased linearly as DDGS level increased. The results indicated that feeding a high level of DDGS can increase the content of lutein and polyunsaturated fatty acids in egg yolk, but may not affect the content of cholesterol and choline.

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INTRODUCTION

Distillers dried grains with solubles (**DDGS**) have been available for feed industry for many years, and have been considered a nutritional and economical feed ingredient. As a by-product of ethanol industry, DDGS is the leftover product after the grain fermentation process and ethanol distillation. After starch is used during the fermentation process of ethanol production, other nonfermentable nutrients (protein, fiber, fat, vitamins, and minerals) are concentrated around 3-fold in the final DDGS product (NRC, 1994; Weigel et al., 1997). As the production of bioethanol has increased rapidly in recent years, an increased quantity of DDGS has become available for the feed industry (Licht, 2011).

The DDGS are often used at low concentrations (10 or 15%) as a feed ingredient for laying hens without affecting laying performance and egg quality (Lumpkins et al., 2005; Roberson et al., 2005; Swiatkiwicz and Koreleski, 2006). As large amounts of DDGS become available in feed market, the possibility of using a higher DDGS inclusion rate in poultry feed has become an interest for many researchers and poultry producers. A previous study (Pineda et al., 2008) showed that DDGS could be incorporated at the 69% level in laying hen diets for the short term without negative effects on egg production, metabolic conditions, and egg quality.

The DDGS contain high levels of protein, fiber, and fat and also contain considerable amounts of other important nutrients, such as lutein, choline, and long-chain unsaturated fatty acids. The differences in component and nutrient concentration of DDGS diet may influence the chemical composition and nutrient content of eggs, especially when DDGS are used at high levels in the diet. Yolk is the most nutritive part of the egg and contains many functional nutrients such as choline and lutein. Phosphatidylcholine (**PC**) constitutes around 80% of the total phospholipids in the egg yolk and has many physicoshemical functions. Choline is very important for brain development, liver function, and cognitive function for cellular membranes (Shaw et al., 2004). Deficiency in choline may result in a higher

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[1]Corresponding author: duahn@iastate.edu

risk of cancer (Xu et al., 2008, 2009) and neural tube defects (Shaw et al., 2004).

Egg yolk carotenes are classified as xanthophylls and carotenes. Xanthophylls include lutein, zeaxanthin, and cryptoxanthin, and are present at the level of 0.1, 0.2, and 0.03% of egg yolk, respectively (Romanoff and Romanoff, 1949). Lutein has been used in the poultry diet for a long time. This pigment can provide desirable yellow color in egg yolk and chicken skin, which consumers prefer (Pérez-Vendrell et al., 2001; Leeson and Caston, 2004). Moreover, lutein plays an important role in preventing age-related macular degeneration (Moeller et al., 2000). Avian and mammalians cannot synthesize xanthophyll pigments, and thus fully depend on dietary sources for color absorption (Goodwin, 1984). The DDGS contain high levels of xanthophyll, and thus diets containing DDGS can increase lutein content in egg yolk.

Cholesterol content of egg yolk can range from 11 to 15 mg/g of yolk, which is around 5% of total yolk lipids (Kudchodkar et al., 1976; Vargas et al., 1986). Free cholesterol is about 84% of the total cholesterol, and the remaining 16% is cholesterol esters. The cholesterol of egg yolk can come from the feed ingredient such as animal fat (Basmacioglu and Ergul, 2005), with the major part synthesized when egg yolk is formed in the ovary (Griffin, 1992). High dietary fiber content in DDGS diets may have a positive effect on controlling cholesterol levels in eggs because many researchers have found a positive relationship between high-fiber diets and low serum cholesterol in human (Lairon et al., 2005; Bruckert and Rosenbaum, 2011).

Egg is a very important component of human food, and it is important to evaluate its chemical composition and the content of important nutrients in egg yolk from a high level DDGS diet. Interest in increasing DDGS content in the laying hen diet has been rekindled; however, little work has been done to determine the effect of high inclusion rate of DDGS on egg yolk composition and nutrient content of egg yolk. The objective of this study is to investigate the changes of chemical composition and nutrient content of egg yolk by diets with high DDGS inclusion rates.

MATERIALS AND METHODS

DDGS Diets

The corn DDGS used in this study was obtained from a local ethanol plant (Lincolnway Energy, Nevada, IA). Diets for laying hens were formulated based on NRC nutrient recommendations for laying hens (NRC, 1994) and the current DDGS nutrient profiles on the website of University of Minnesota (http://www.ddgs.umn.edu/profiles-current.htm), and the DDGS data from the University of Arkansas (Table 1) were used to formulate DDGS diets. Isocaloric diets were formulated with 4 levels of corn DDGS (0, 17, 35, and 50%) by replacing increasing amounts of corn, soybean meal, dicalcium phosphate, and DL-methionine, with DDGS, animal-vegetable blended fat, limestone, and lysine. The highest inclusion level of corn DDGS was chosen on the basis of the results of Pineda et al. (2008). The diets were formulated to be isocaloric based on energy values for feed ingredients published by the NRC. The diets were formulated on a total amino acid basis for arginine, histidine, isoleucine, leucine, lysine, total sulfur amino acids, phenylalanine, and tyrosine, threonine, valine, and tryptophan. As DDGS increased in the diet, total CP of the diets increased.

Table 1. Nutrient analysis[1] of corn distillers dried grains with solubles (DDGS) used in the study (%, as is)

Item	Value	Item	Value
Amino acid profile (%)		Nutrient[2] (%)	
Aspartic acid	1.91	DM	87.60
Threonine	0.97	Protein	27.30
Serine	1.13	Ash	4.35
Glutamic acid	4.46	Fat	10.67
Glycine	1.13	Mineral (mg/kg)	
Alanine	2.01	P	9,296
Cysteine	0.76	K	11,578
Valine	1.46	Ca	312
Methionine	0.81	Mg	3,532
Isoleucine	1.08	S	4,871
Leucine	3.19	Na	2,171
Tyrosine	0.89	Fe	68.90
Phenylalanine	1.32	Mn	8.97
Lysine	0.91	Zn	62.60
Histidine	0.75	Cu	6.24
Arginine	1.16	Al	30.70

[1]Analyzed at Central Analytical Laboratory, Poultry Science Center, University of Arkansas.

[2]Nutrient values expressed as grams per 100-g sample on weight/weight percentage.

After the first 12-wk experiment period, diet formulas were modified by adding additional amounts of lysine and methionine in an attempt to meet the production requirements of laying hens fed the 50% DDGS diet. The composition and nutrient contents of the 2 DDGS diet formulas are shown in Tables 2 and 3. The 2 formulas had the same concentrations of ME, Ca, P, most amino acids except lysine and methionine, and very similar protein levels in the treatment with the same DDGS level.

The DDGS samples were prepared for proximate and amino acid content. The nutrient composition of corn DDGS used in this study was analyzed at the Poultry Science Center, University of Arkansas using the AOAC methods (AOAC, 1990; AOAC International, 2000; Table 1).

Birds and Experimental Design

The experiment was conducted in the poultry research and teaching farm at Iowa State University. The study was approved by the Institutional Animal Care and Use Committee at Iowa State University (approval # 4–09–6732-G). A total of two hundred forty 54-wk-old Single-comb White Leghorn laying hens (Hy-Line

Table 2. Composition and calculated analysis of experimental diet (1 to 12 wk) with various levels of corn distillers dried grains with solubles (DDGS; %, as is)

	% DDGS			
Item	0	17	35	50
Ingredient				
Corn	59.20	51.37	36.62	26.80
Soybean meal	25.00	15.00	10.00	3.50
DDGS[1]	0.00	17.00	35.00	50.00
Blended fat[2]	2.70	3.68	5.65	7.00
Limestone[3]	11.02	11.22	11.46	11.65
Dical phosphate	1.05	0.80	0.45	0.18
Salt	0.38	0.29	0.16	0.07
V and M mix[4]	0.50	0.50	0.50	0.50
L-Thr	0.01	0.05	0.00	0.00
Bio-Lys[5]	0.00	0.03	0.16	0.30
DL-Met	0.14	0.06	0.00	0.00
Calculated (%)				
CP	16.70	16.50	18.30	19.00
ME (kcal/kg)	2,825	2,825	2,825	2,825
Ca	4.50	4.50	4.50	4.50
P	0.30	0.30	0.30	0.30
Na	0.17	0.18	0.17	0.17
Arg	1.10	0.95	0.97	0.91
His	0.46	0.45	0.51	0.53
Ile	0.70	0.67	0.74	0.75
Leu	1.53	1.69	2.00	2.20
Lys	0.89	0.81	0.81	0.81
Met	0.41	0.39	0.35	0.39
Cys	0.29	0.31	0.36	0.39
SAA[6]	0.70	0.70	0.72	0.78
Phe + Tyr	1.35	1.38	1.60	1.70
Thr	0.63	0.62	0.68	0.71
Val	0.71	0.75	0.88	0.95
Trp	0.22	0.18	0.18	0.16

[1]DDGS was donated by LincolnWay Energy, Nevada, IA.

[2]Blended fat contained vegetable and animal fat, donated by Feed Energy Company, Des Moines, IA.

[3]Limestone was donated by Iowa Limestone Company, Des Moines, IA.

[4]V and M mix = vitamin and mineral premix: contained the following per kilogram of diet: selenium, 0.2 mg/kg; vitamin A, 6,608 IU; vitamin D_3, 2,203 ICU; vitamin E, 14 IU; menadione, 0.88 mg; vitamin B_{12}, 9.35 μg; biotin, 33 μg; choline, 358 mg; folic acid, 1.1 mg; niacin, 33 mg; pantothenic acid, 8.8 mg; pyridoxine, 0.88 mg; riboflavin, 4.4 mg; thiamine, 1.1 mg.

[5]Bio-Lys (78.8% lysine).

[6]SAA = sulfur amino acids, including Met and Cys.

Table 3. Composition and calculated analysis of experimental diet (13 to 24 wk) with various levels of corn distillers dried grains with solubles (DDGS; %, as is)

	% DDGS			
Item	0	17	35	50
Ingredient				
Corn	59.13	50.87	36.38	26.62
Soybean meal	25.00	15.00	10.00	3.50
DDGS[1]	0.00	17.00	35.00	50.00
Blended fat[2]	2.72	3.88	5.75	7.07
Limestone[3]	11.02	11.22	11.46	11.65
Dicalcium phosphate	1.05	0.80	0.45	0.18
Salt	0.38	0.29	0.16	0.07
V and M mix[4]	0.50	0.50	0.50	0.50
L-Thr	0.01	0.05	0.00	0.00
Bio-Lys[5]	0.00	0.24	0.26	0.41
DL-Met	0.19	0.15	0.04	0.00
Calculated				
CP	16.70	16.60	18.30	19.00
ME (kcal/kg)	2,825	2,825	2,825	2,825
Fat	5.16	7.26	10.22	12.46
Fiber	2.28	3.26	4.38	5.28
Ca	4.50	4.50	4.50	4.50
P	0.30	0.30	0.30	0.30
Na	0.17	0.18	0.17	0.17
Arg	1.10	0.95	0.97	0.91
His	0.46	0.45	0.51	0.53
Ile	0.70	0.67	0.73	0.75
Leu	1.53	1.68	2.00	2.20
Lys	0.89	0.86	0.86	0.87
Met	0.41	0.39	0.35	0.39
Cys	0.29	0.31	0.36	0.39
SAA[6]	0.70	0.70	0.72	0.78
Phe + Tyr	1.34	1.38	1.59	1.70
Thr	0.63	0.65	0.68	0.71
Val	0.71	0.75	0.88	0.95
Trp	0.22	0.18	0.18	0.16

[1]DDGS was donated by LincolnWay Energy, Nevada, IA.

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[5]Bio-Lys (50.7% lysine).

[6]SAA = sulfur amino acids, including Met and Cys.

W-36) were placed in 120 cages (2 birds/cage) with 96 square inches (0.06 m^2) per bird. Three consecutive cages were considered an experiment unit, and were assigned to 1 of 4 diets containing 0, 17, 35, or 50% DDGS using a completely randomized design. Every diet was fed to 10 experimental units of hens. Hens had free access to the diet and were provided with 16L:8D per day. The temperature was maintained at 26°C throughout the study.

Prior to the experiment, the hens (except for control birds) were fed a corn-soybean meal-based diet containing 10% corn DDGS for 2 wk, and the hens were acclimated to the inclusion rates of corn DDGS by gradually introducing the treatment diets over the next 4-wk period (a total of 6 wk of acclimation period was given before the experiment).

Chemicals and Reagents

For choline analysis, choline standard (choline bitartrate) and enzymes, including phospholipase D, choline oxdase, and peroxidase, were purchased from Sigma Chemical Co. (St. Louis, MO). Phospholipase D (type VI, 4,750 units/mg) was produced from *Streptomyces chromofusus*, choline oxidase (12 units/mg) was produced from *Alcaligenes* species, and peroxidase (type I, 116 purpurogallin units/mg) was produced from horseradish. Chromogenic reagent was prepared by dissolving 100 units of phospholipase D, 120 units of choline oxidase, 280 units of peroxidase, 15 mg of 4-aminoantipyrine, and 50 mg of phenol added in 100 mL of 0.05 *M* Trizma buffer (pH 8.0). The chemicals used

for lutein extraction were reagent grades. Lutein (xanthophyll) standard was purchased from Sigma Chemical Co. Folch's solution (chloroform: methanol = 2:1) for fat extraction was prepared using reagent grade chloroform and methanol, and BF_3-methanol (boron-trifluoride methanol, 14%, Sigma Chemical Co.) was used as a methylating reagent for fatty acids. For cholesterol analysis, ascorbic acid and cholestane were purchased from Sigma Chemical Co. Pyridine was from Fisher Scientific (Anthem, AZ). Sylon BFT [BSTFA (bis trifluoroacetamide) + TMCS (trimethylchlorosilane), 99:1] was purchased from Sigma-Aldrich-Supelco Chemical.

Sample Preparation

Every 2 wk, a total of 80 eggs (20 eggs for each treatment) were randomly selected to prepare yolk samples. Each treatment had 4 replications (5 eggs for each replication), and the age of hens at the beginning of dietary treatments was 60 wk old and that at the end of the work was 84 wk old. The eggs from each treatment were broken to separate yolk and albumen, and then 5 yolks from the same treatment were mixed in a beaker, transferred to scintillation vials, and used as a replication. All the eggs and samples were stored at refrigerated temperature until use.

Analyses of Yolk Composition (Lipid, Moisture, and Protein Contents)

Lipid Extraction. Yolk lipids were extracted using Folch's solution (Folch et al., 1957). Three grams of yolk and 30 mL of Folch's solution ($CHCl_3$: CH_3OH = 2:1) were added in a 50-mL test tube and homogenized at high speed for 30 s using a Polytron (type PT 10/35, Brinkman Instruments Inc., Westbury, NY). After standing overnight, the sample was filtered through a Whatman #1 filter paper into a 100-mL graduated cylinder (with glass stopper). Test tube was washed twice with 8 mL (4+4 each side) and 4 mL (2+2 each side) of Folch's solution, and the filter paper was washed with 10 mL (5+5 each side) of Folch's solution.

Ten milliliters (equal to one quarter of filtrate volume) of 0.88% NaCl solution was added to the filtrate and mixed well. The inside of the cylinder was washed with 2 mL of Folch's solution. After overnight standing for phase separation, the lower layer (lipid and chloroform) volume was recorded, and the top layer (methanol and water) of the solution was carefully siphoned off to avoid contaminating the $CHCl_3$ layer. Ten milliliters of $CHCl_3$ layer was transferred to a preweighted weighing dish (42 mL, low form aluminum, Fisher Scientific) and weighed after chloroform was evaporated in a fume hood. Total crude lipids were calculated. The remaining $CHCl_3$ layer was transferred into a glass scintillation vial and used for other analysis.

Moisture and Protein Measurements. Moisture was determined using the AOAC method (AOAC, 1980). One gram of yolk sample was transferred to a preweighted weighing dish and kept in an oven at 105 to 110°C for 12 h. After cooling, the weight loss was determined and used to calculate moisture content. Yolk protein content was measured using the micro-Kjeldahl method 990.03 (AOAC International, 2006).

Fatty Acid Composition. One milliliter methylating reagent (boron-trifluoride methanol, Sigma Chemical Co.) and 0.4 mL of lipid extract were added into a 20-mL test tube, capped tightly, and incubated in a 90°C water-bath for 1 h. After cooling to room temperature, 3 mL of hexane and 5 mL of water were added, mixed thoroughly, and left at room temperature overnight for phase separation. The top hexane layer containing methylated fatty acids (1.5 mL) was collected in a GC vial and analyzed using a GC (HP 6890, Hewlett Packard Co.). The HP-wax column (30 m × 0.25 mm i.d., 0.25 μm nominal) was used to separate fatty acid methylates. Increased oven temperature conditions (from 180°C, increased to 200°C at 5°C/min, held at 200°C for 6 min, to 220°C at 10°C/min, to 230°C at 5°C/min, and then held at 230°C for 7.0 min) were used. Temperatures of the inlet and detector were 230°C and 280°C, respectively. Helium was the carrier gas at linear flow of 0.9 mL/min. Detector (flame ionization detector; **FID**) air, H_2, and make-up gas (He) flows were 350, 35, and 42.1 mL/min, respectively. Fatty acids were identified by comparing the retention times of known standards. Relative quantities were expressed as weight percentage of total fatty acids (Nam et al., 2001).

Cholesterol. Five milliliters of chloroform layer from lipid extraction was transferred to a 50-mL test tube and evaporated under nitrogen gas. Ten milliliters of saponification reagent (ethanol: 33% KOH = 94:6), 0.5 mL of 20% ascorbic acid, and 100 μL of 5 α-cholestane solution (10 mg/μL in chloroform) were added to the sample, and the sample was incubated in a waterbath at 50°C for 1 h. After cooling to room temperature, 8 mL of water and 3 mL of hexane were added to the sample, mixed thoroughly by vigorous shaking, and kept overnight for phase separation. One milliliter of the top layer (hexane) was transferred to a scintillation vial, dried under nitrogen flow, and then added with 200 μL of pyridine and 100 μL of Sylon BFT (99% BSTFA+1% TMCS). After being kept overnight at room temperature, the sample was transferred to a gas chromatography (**GC**) vial for GC analysis. Cholesterol was analyzed using a HP 6890 GC equipped with an autosample injector and an FID (Hewlett Packard Co., Wilmington, DE). A 0.25-mm i.d. 30-m HP-5MS column with 0.25-μm film thickness was used. A splitless inlet (5 μL) was used to inject samples into the capillary column using an autosampler (model 7683, Hewlett Packard Co.). An increased oven temperature was used (from 180°C increased to 260°C at 8°C/min, increased to 280°C at 2°C/min, and held for 13 min).

Temperature of the inlet was 290°C, and detector temperature was 320°C. Helium was the carrier gas at a constant flow of 1.2 mL/min. Detector (FID) air, H_2, and make-up gas (He) flow rates were 400 mL/min, 35 mL/ min, and 40 mL/min, respectively. The area of cholesterol peak (pA·s) was integrated using the ChemStation software (Hewlett Packard Co.), and the amount of cholesterol was calculated using an internal standard (5-cholestane).

Lutein. A 0.5-g yolk sample was weighed in a 50-mL conical centrifuge tube and homogenized with 10 mL of 30% methanolic potassium hydroxide (wt/vol) and 50-μL 10% methanolic butylated hydroxytoluene using a Brinkman Polytron (Type PT 10/35, Brinkman Instrument Inc., Westbury, NY) for 15 s at high speed. The homogenate was heated for 1 h at 50°C to saponify the lipids and hydrolyze the carotenol esters. After heating, the sample was kept in the dark. The carotenoids were extracted with 10 mL of ether: hexane (1:1, vol:vol) by homogenizing with a Brinkman Polytron. After that 10 mL of water was added to the sample and homogenized again. The mixture was centrifuged at 4,000 × *g* for 30 min at 4°C for phase separation. Three milliliters of the top layer (hexane-ether layer) was collected in a scintillation vial, and the solvents were evaporated to dryness using N_2 gas. The dried carotenoids residue was dissolved in 1 mL of methanol, filtered through a 0.45-μm polytetrafluoroethylene membrane filter, and then analyzed using an HPLC. An Agilent 1100 Series HPLC equipped with binary pump, micro-vacuum degasser, micro-autosampler, column compartment with temperature controller, and diode-array detector was used. A reversed-phase HPLC column (Zobax Eclipse XDB-C18, 2.1 mm × 100 mm, 3.3-μm particle size) was used to separate lutein. The column flow rate and injection volume were 0.5 mL/min and 10 μL, respectively. Two mobile phases (solvent A: prepared with 75% acetonitrile, 15% methanol, 10% water, 0.04% ammonium acetate, and solvent B: 85% methanol with 2% 1 *M* ammonium acetate pH 4.6, 15% methyl-tert-butylether) were used with a step-gradient from 100% solvent A to 100% solvent B at 15 min. Column temperature was maintained at 15°C (Handelman et al., 1999; Bonora et al., 2000).

Choline. Choline was determined using the AOAC official method 999.14. Sample (1 g) in a 50-mL tube was homogenized with 15 mL of 1 *N* sodium hydroxide (NaOH) solution using a Brinkman Polytron (Type PT 10/35, Brinkman Instrument Inc.) for 15 s at high speed. The homogenate was heated for 3 h at 70°C, cooled to room temperature, pH adjusted to pH 3.5 to 4.0 with 6 *N* HCl, net volume adjusted to 25 mL, and then homogenized using a Polytron for 5 s at high speed. The homogenate was filtered through a Whatman #41 filter paper and the filtrate was collected. The collected filtrate was refiltered through a glass microfiber syringe filter (25 mm, Whatman).

For each sample, two 10-mL test tubes were labeled as sample test solution (tube 1) and sample blank (tube 2). An aliquot (100 μL) of the final filtrate was transferred to each tube. Water (3 mL) was added to each sample blank (tube 2) and 3 mL of chromogenic reagent was added to each sample test solution (tube 1), the standards, and the reagent blank. All test tubes were incubated in covered water bath at 37°C for 15 min to develop color. The absorbance of samples was measured at 505 nm against a reagent blank containing 100 μL water and 3 mL of chromogenic reagent. The amount of choline was expressed as milligrams of choline hydroxide per 100 g of sample.

Statistical Analyses

This experiment used a completely randomized design. The data were analyzed by one-way ANOVA with the GLM procedure of SAS by 1 variation (treatment or week; SAS 9.2, 2008, SAS Institute Inc., Cary, NC), and Duncan's multiple range test was used to separate means. In addition, data were analyzed as repeated measure, employing split-plot mixed procedure of SAS after sorting the data by week, with main effects (treatment and week) and random effect (treatment × week interaction). Statistical significance was assumed at $P < 0.05$ to determine the differences among treatments.

RESULTS AND DISCUSSION

During the 24-wk experiment period, no difference in moisture content of egg yolk among 4 DDGS treatments was found, and the moisture content of egg yolk ranged from 48.54 to 48.74% (Table 4). However, the diet with highest DDGS inclusion rate (50%) affected the fat and protein content of egg yolk. Fat content of egg yolk in the 50% DDGS group was significantly higher than that of the other 3 groups, and protein content of egg yolk in the 50% DDGS group was significantly lower than that of the other 3 groups (Table 4). Fat and protein levels of DDGS diets increased as DDGS inclusion rate increased, and fat content of DDGS diets differed greatly ranging from 5.16 to 12.46%. The diet with higher fat content resulted in greater levels of yolk lipids. The difference in protein and fat content of egg yolk may come from the different protein and fat level in the DDGS diets. But this effect was only observed in the diet with 50% DDGS concentration.

The fatty acid composition of 4 DDGS diets was different, and resulted in the difference in fatty acid composition of egg yolk (Table 5). All fatty acids but margaroleic acid and DHA in egg yolk were influenced by DDGS diets. Plamitoleic acid and oleic acid decreased linearly as DDGS level increased. On the contrary, margaric acid and linoleic acid increased linearly as DDGS levels increased as reported by Cheon et al. (2008). Total saturated and unsaturated fatty acids among 4 DDGS treatments were not significantly different; however, total polyunsaturated fatty acids increased linearly as dietary DDGS level increased. This result agrees with the study of Rew et al. (2009) who reported that

Table 4. Fat, moisture, and protein content of egg yolk from hens fed diets with various levels of corn distillers dried grains with solubles (DDGS)[1]

Chemical component (%)	DDGS diet (%)				
	0	17	35	50	SEM
Fat	32.13[b]	32.53[b]	32.31[b]	33.41[a]	0.24
Moisture	48.54	48.74	48.66	48.61	0.08
Protein	16.89[a]	16.88[a]	17.02[a]	16.24[b]	0.09

	P-value		
	Fat	Moisture	Protein
Treatment (T)	0.0125	0.3749	0.0041
Week (W)	<0.001	<0.0001	<0.0001
T × W	0.0895	0.0039	0.1172

[a,b]Means with no common superscript in the same row differ significantly ($P < 0.05$).

[1]Values are means of 24-wk sampling period.

20% DDGS diet resulted in increased polyunsaturated fatty acids in egg yolk. Schilling et al. (2010) reported a linear increase in linoleic and polyunsaturated fatty acids in breast and thigh meat as dietary DDGS increased.

Dietary fatty acid composition was considered as the most important factor that influence the fatty acid composition of broiler meat and hen eggs (Cortinas et al., 2004). The current study shows that different DDGS inclusion rates can influence the fatty acid composition of egg yolk due to the differences in fatty acid composition in DDGS diets (mainly palmitic, oleic, and linoleic acids). Among those polyunsaturated fatty acids in egg yolk, linoleic and arachidonic acids belong to n-6 fatty acids, and linolenic acid, docosapentaenoic acid, and docosahexaenoic acid belong to n-3 fatty acids. Both n-3 fatty acids (linolenic acid and eicosapentaenoic acid) and n-6 fatty acids (linoleic acid) were increased by DDGS diets.

All diets (0, 17, 35, and 50% DDGS) contained similar levels of choline (77.7, 77.4, 76.9, and 77.3 mg/100 g, respectively). As shown in Figure 1A, choline content in yolk (mg of choline/100 g of egg yolk) of 50% DDGS treatment was higher than that of the other 3 groups (0, 17, and 35%) most of the times during the study. The differences in choline content in egg yolk at 3, 7, 11, 13, 15, and 17 wk of study were significant ($P < 0.05$), and the differences were mainly due to higher choline content in 50% DDGS group. However, choline content among the 4 DDGS treatments was not different during the last 6-wk period. The higher choline content in eggs fed with 50% DDGS diet could be related to smaller egg size in that group, so the choline content was higher when expressed as milligrams of choline/100 g of yolk.

Table 5. Fatty acid composition (weight percent of total fatty acids) of diet formula and egg yolks from hens fed diets with various levels of corn distillers dried grains with solubles (DDGS)

Fatty acid (%)	DDGS diet[1]				Egg yolk[2]				SEM[3]
	0% DDGS	17% DDGS	35% DDGS	50% DDGS	0% DDGS	17% DDGS	35% DDGS	50% DDGS	
Myristic acid	0.8	0.5	0.55	0.6	0.36[a]	0.35[ab]	0.32[c]	0.33[bc]	0.01
Palmitic acid	22.8	18.25	17.55	18.6	31.59[a]	30.15[ab]	28.77[b]	28.69[b]	0.52
Palmitoleic acid	0.65	0.55	0.6	0.6	2.08[a]	1.52[b]	1.09[c]	0.95[d]	0.02
Margaric acid	0.4	0.25	0.25	0.25	0.27[d]	0.30[c]	0.38[b]	0.43[a]	0.005
Margaroleic acid	0.1	0.1	0.1	0.1	0.14	0.13	0.15	0.14	0.006
Stearic acid	8.2	6.2	5.95	6	12.71[b]	13.05[ab]	13.26[ab]	13.62[a]	0.23
Oleic acid	28.15	25.65	26.4	25.6	33.25[a]	31.42[b]	29.41[c]	27.60[d]	0.3
Linoleic acid	36.2	45.4	45.15	45.25	14.65[d]	18.01[c]	21.44[b]	22.76[a]	0.4
Linolenic acid	2.5	2.9	3.35	2.9	0.55[b]	0.54[b]	0.65[a]	0.68[a]	0.02
Arachidonic acid	0.2	0.1	0.15	0.1	3.25[ab]	3.36[ab]	3.09[b]	3.48[a]	0.11
EPA[4]	0	0	0	0	0.04[b]	0.05[b]	0.05[b]	0.11[a]	0.005
DHA[4]	0	0	0	0	1.12	1.15	1.1	1.23	0.05
SFA[5]	32.1	25.25	24.35	25.4	44.56	43.49	42.42	42.73	0.75
USFA[5]	67.9	74.75	75.65	74.6	55.44	56.51	57.59	57.27	0.75
PUFA[5]	38.95	48.45	48.6	48.25	19.61[d]	23.09[c]	26.34[b]	28.26[a]	0.55

[a–d]Means with no common superscript in the same row differ significantly ($P < 0.05$).

[1]Fatty acid composition of DDGS diets, values are means of 4 replications (average of the 2 formulas, 2 analyses for each formula. Analyzed at 1, 6, 13, 19 wk).

[2]Fatty acid composition of egg yolk from DDGS diets, values are means of 4 replications.

[3]Standard error of mean of fatty acid composition of egg yolk from laying hens fed DDGS diets. Values are means of 24-wk sampling period.

[4]EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

[5]SFA: saturated fatty acid, USFA: unsaturated fatty acid, PUFA: polyunsaturated fatty acid.

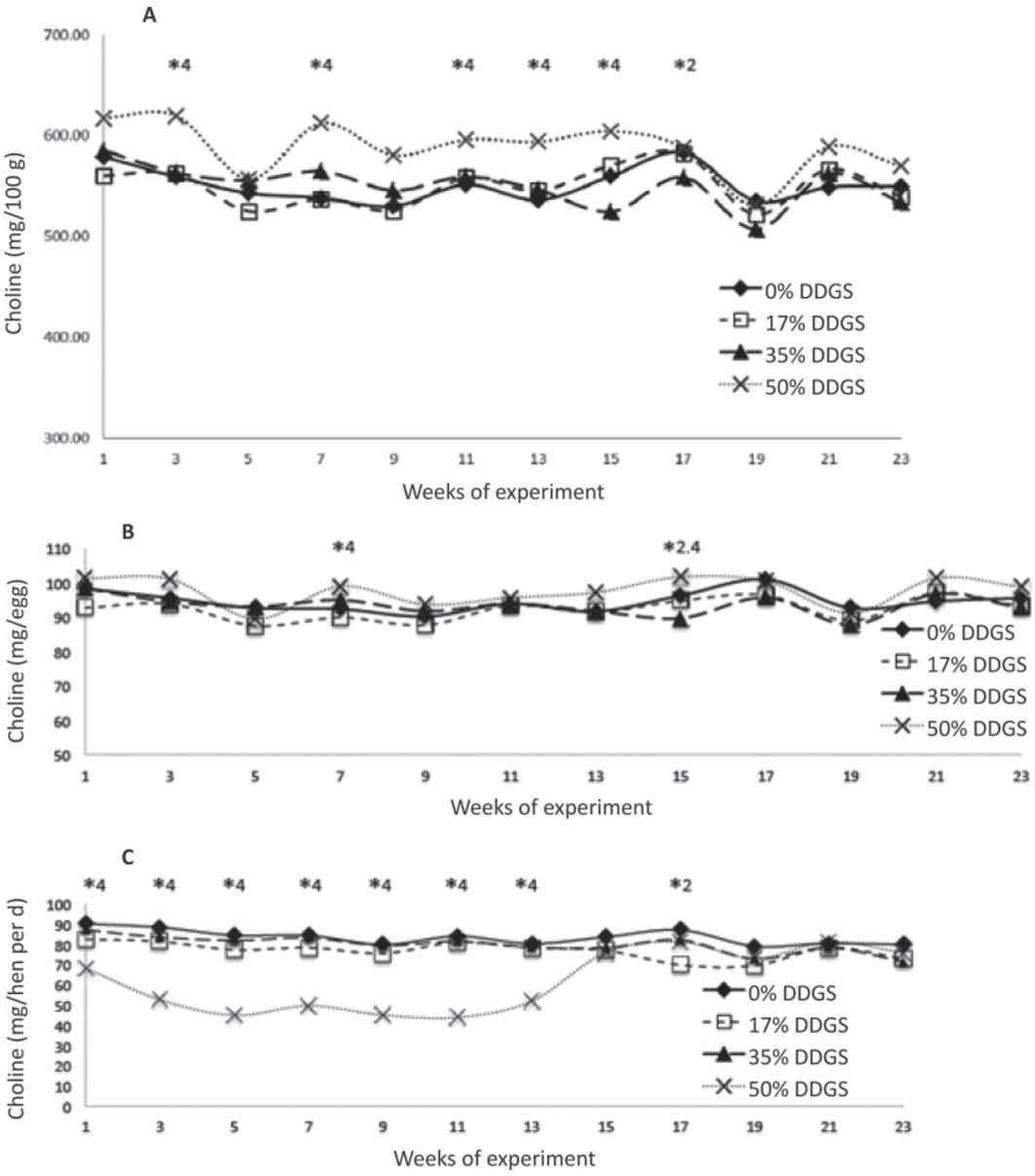

Figure 1. Effect of diets with various levels of corn distillers dried grains with solubles (DDGS) on choline content of egg yolk during the 24-wk-long period. Panel A is based on choline content of 100 g of egg yolk, panel B is based on choline content per egg, and panel C is based on choline content per hen per day. *[4]Denotes that 50% DDGS dietary treatment is significantly higher than the 0% DDGS dietary treatment ($P < 0.05$). *[2]Denotes that 17% DDGS dietary treatment is significantly different from the 0% DDGS dietary treatment ($P < 0.05$). *[2.4]Denotes that 17 and 50% DDGS dietary treatments are significantly different from the 0% DDGS dietary treatment ($P < 0.05$). The ratio of corn and soybean meal base in diet reduced as DDGS level increased. Each data point means averaged choline content on a 2-wk basis. Diet was changed to second formula at 13 wk of age. n = 4.

When the egg size was not different among 4 treatments during the last 6 wk, the choline content among 4 treatments was not significantly different. As shown in Figure 1B, when choline content is expressed as milligrams of choline per egg, there were only differences at wk 7 and 15 ($P < 0.05$) among 4 DDGS treatments. Choline content in each egg from 4 treatments was not influenced by DDGS addition. When choline content is expressed as milligrams per hen per day (Figure 1C), the choline pattern is very similar to that of egg production, feed intake, and egg weight (data not shown). Dietary reference intake for choline recommends 425 mg/d for adult women and 550 mg/d for adult men and children (Institute of Medicine, 1997). In this study, choline content in egg yolk from 50% DDGS treatment was higher than those of other treatments, but DDGS diets did not affect the choline content in egg, especially when the egg size was the same among the 4 DDGS treatments.

Cholesterol level in egg yolk from hens fed with 50% DDGS diet was continuously higher than that of the other 3 DDGS treatment groups during 5 to 13 wk feeding period, but the difference became small at the later period (Figure 2). The cholesterol content of eggs from hens fed with 50% DDGS diet showed an inconsistent response, which could be related to the size of eggs

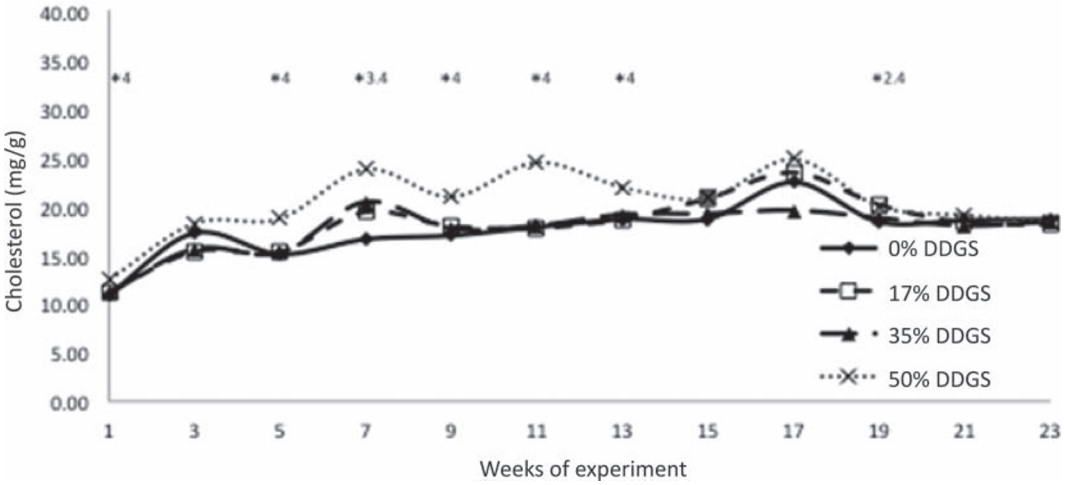

Figure 2. Effect of diets with various levels of corn distillers dried grains with solubles (DDGS) on cholesterol content of egg yolk during the 24-wk-long period. *[4]Denotes that 50% DDGS dietary treatment is significantly higher than the 0% DDGS dietary treatment ($P < 0.05$). *[2.4]Denotes that 17 and 50% DDGS dietary treatments are significantly higher than the 0% DDGS dietary treatment ($P < 0.05$). *[3.4]Denotes that 35 and 50% DDGS dietary treatments are significantly higher than the 0% DDGS dietary treatment ($P < 0.05$). The ratio of corn and soybean meal base in diet decreased as DDGS level increased. Each data point means averaged cholesterol content of egg yolk on a 2-wk basis. Diet was changed to second formula at 13 wk of age. n = 4.

that were used for analysis. Larger eggs tend to contain lower cholesterol concentration than smaller eggs. The inconsistent response observed in 50% DDGS treatment may also be related to its lower egg production because lower egg production allows cholesterol to be condensed more in the yolk. When the egg production and egg size of 4 DDGS treatments became similar during the last 6 wk, the difference in yolk cholesterol from 4 DDGS groups was not significant. A slight increase of cholesterol content in 0, 17, and 35% DDGS groups was observed over time, and that could be due to the decreased egg production as the hen's age increased.

Cholesterol content in yolk is relatively resistant to change, and only slightly differ by cholesterol levels in the feed (Fenton and Sim, 1991). Increased cholesterol content in yolk was observed when high cholesterol diets were fed (Harris and Wilcox, 1963; Weiss et al., 1967), especially when total fat in a diet was high. In current study, fat level in the diets increased as DDGS level increased (Table 3). Because the fat in the DDGS diet was from vegetable and animal sources, the cholesterol content from animal fat in the diet would be higher in the DDGS diet with higher fat levels. Cholesterol intake might be higher in 50% DDGS group and may have resulted in higher cholesterol content in egg yolk. In addition, fatty acid composition in the diet may have some effect on cholesterol level. Several studies found that feeding polyunsaturated fatty acids would increase yolk cholesterol level (Summers et al., 1966; Weiss et al., 1964). The content of polyunsaturated fatty acids in egg yolk was the highest with 50% DDGS treatments in this study, which may have contributed to the increased cholesterol level in egg yolk. Furthermore, dietary fiber has been found to have cholesterol-lowering effect (Kirby et al., 1981), and soluble fiber is the main component to have this effect (Glassman et al., 1990; Williams et al., 1991). Bruckert and Rosenbaum (2011) found that increased fiber intake can significantly lower cholesterol concentration of serum. Higher dietary fiber consumption is often associated with lower total serum cholesterol (Lairon et al., 2005). However, high fiber content in DDGS diets did not show any cholesterol-lowering effect, which would be due to the low amount of soluble fiber present in the DDGS diets. Because multiple factors would influence the cholesterol content in egg yolk, the effect of DDGS diet on cholesterol content would be compromised, and did not show a significant difference.

Egg yolk is rich in color pigments, such as carotenes and xanthophylls, which are responsible for yolk color. They cannot be synthesized by hen and must be obtained from feed. Xanthophylls include lutein, zeaxanthin, and cryptoxanthin, and present at levels of 0.1, 0.2, and 0.03% of egg yolk, respectively (Romanoff and Romanoff, 1949). The lutein content of DDGS diets increased as DDGS level increased and was 4.86, 6.80, 8.15, and 9.10 μg/g of feed, respectively (Figure 3). The lutein content of egg yolk from 4 DDGS treatments were significantly different ($P < 0.05$), and increased linearly as DDGS level increased. This suggests that dietary lutein can be digested and absorbed very well by layers. The DDGS, as a good source of xanthophylls, was reported to contain 2.37 to 34.00 mg/kg in several DDGS products (NRC, 1981; Sauvant and Tran, 2004). Moeller et al. (2000) found that lutein from food sources can reduce the risk of age-related macular degeneration up to 40% and reduce the risk of cataract up to 20%. Seddon et al. (1994) reported that lutein and zeaxanthin are prominent macular pigments that can reduce the risk of age-related macular degeneration. Even though egg is not the richest source of xanthophylls, the xanthophylls in egg are highly di-

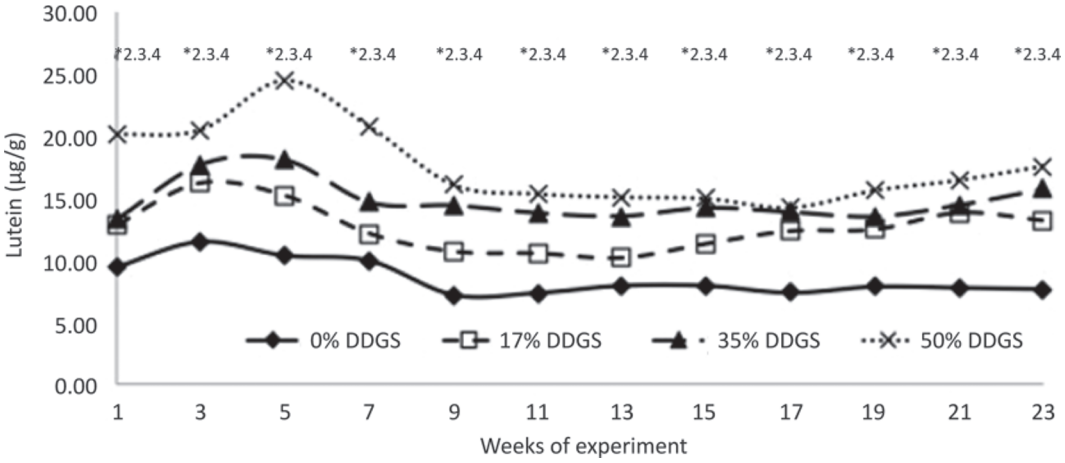

Figure 3. Effect of diets with various levels of corn distillers dried grains with solubles (DDGS) on lutein content of egg yolk during the 24-wk-long period. $^{*2.3.4}$Denotes that 17, 35, and 50% DDGS dietary treatments are significant higher than 0% DDGS dietary treatment ($P < 0.05$). The ratio of corn and soybean meal base in the diet decreased as DDGS level increased. Each data point means averaged lutein content of egg yolk on a 2-wk basis. Diet was changed to second formula at 13 wk of age. n = 4.

gestible and absorbable (Moeller et al., 2000). Thus, DDGS could be used as a good lutein source for eggs, and lutein-enriched eggs could have great potential to lower the risk of eye diseases.

In summary, DDGS diet can influence egg yolk composition and other important nutrients content in egg yolk. Fat and protein content of egg yolk was affected by 50% DDGS treatment. The proportion of polyunsaturated fatty acids in egg yolk increased significantly by DDGS diet. The contents of choline and cholesterol were initially higher with 50% DDGS treatment, but were not affected by the DDGS treatments in the later period, especially during last 4 wk. Lutein content increased linearly as DDGS level increased. These results indicated that feeding high levels of DDGS can increase the content of lutein and polyunsaturated fatty acids in egg yolk, but may not affect the content of cholesterol and choline.

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