Effects of corn dried distillers' grains with solubles on performance, egg quality, yolk fatty acid composition and oxidative status in laying ducks

D. Ruan,[*,†] A. M. Fouad,[*,‡] Q. L. Fan,[*] W. Chen,[*] W. G. Xia,[*] S. Wang,[*] Y. Y. Cui,[*] Y. Wang,[*] L. Yang,[†,1] and C. T. Zheng[*,1]

[*]*Institute of Animal Science, Guangdong Academy of Agricultural Sciences, Key Laboratory of Animal Nutrition and Feed Science (South China) of Ministry of Agriculture, State Key Laboratory of Livestock and Poultry Breeding, Guangdong Public Laboratory of Animal Breeding and Nutrition, Guangdong Key Laboratory of Animal Breeding and Nutrition, Guangzhou 510640, P. R. China;* [†]*College of Animal Science, South China Agricultural University, Guangzhou 510640, P. R. China; and* [‡]*Department of Animal Production, Faculty of Agriculture, Cairo University, Giza 12613, Egypt*

ABSTRACT The study investigated the effects of increasing content of corn distillers' dried grains with solubles (DDGS) in the diets of laying ducks on oxidative status, laying performance, egg quality, and egg yolk fatty acid composition. Longyan females (1080) with similar BW at 17 wk of age were randomly assigned to 6 treatment groups, each consisting of 6 replicates of 30 birds. The basal diet (I) was a typical corn-soybean ration while the experimental diets (II to VI) substituted corn DDGS for soybean meal and wheat bran and a small reduction of corn. The level of substitution in diets (II to VI) was 6%, 12%, 18%, 24% and 30%. The experiment lasted for 18 wk. Average egg weight decreased linearly as the level of corn DDGS inclusion increased ($P < 0.001$). Haugh unit, albumen weight, and proportion declined as linear responses to corn DDGS substitution ($P < 0.05$), but yolk color linearly increased ($P < 0.001$); the proportions of oleic (C18:1) and total monounsaturated fatty acids in egg yolk linearly decreased with increasing corn DDGS and many of the key polyunsaturated fatty acids (PUFAs) like linoleic (C18:2n-6), arachidonic (C20:4n-6) and α-linolenic (C18:3n-3) acids linearly increased ($P < 0.001$), but not those of eicosapentaenoic (C20:5n-3) and docoshexaenoic (C22:6n-3) acids. The PUFAs n-6/n-3 ratio linearly increased with increasing corn DDGS level ($P < 0.001$). Increasing corn DDGS linearly increased hepatic expression of *GPX1*, *HO-1*, and *Nrf2* and hepatic activity of GSH-Px and the liver content of MDA ($P < 0.001$). There were no treatment effects on egg production, egg mass, feed conversion ratio, eggshell thickness, strength, and yolk cholesterol content ($P > 0.05$). In conclusion, the current study indicates that the use of corn DDGS is possible as a replacement, primarily for soybean meal at levels up to 18% in the diets of laying ducks without affecting laying performance, egg quality, and antioxidant status. Increasing amounts of corn DDGS linearly increased egg yolk concentrations of key fatty acids like like C18:2n-6 and C18:3n-3 and the antioxidant enzyme activity of GSH-Px through the Nrf2 pathway to avoid oxidative stress.

Key words: corn DDGS, performance, egg quality, fatty acids, oxidative status

2017 Poultry Science 0:1–10
http://dx.doi.org/10.3382/ps/pex331

INTRODUCTION

Corn distillers' dried grains with solubles (**DDGS**) is a by-product of corn fermentation from the ethanol industry and has been widely used as a feed ingredient for poultry diets. China ranks as the third-highest producer of DDGS, and it is also one of the largest consumers of DDGS produced in the United States (Jie et al., 2013). Protein, fat, amino acids, essential fatty acids, vitamins, minerals, mannan oligosaccharides, yeast β-glucan, inositol, glutamine, nucleic acids, xanthophylls, lutein, and zeaxanthin are available in corn DDGS (Salim et al., 2010; Min et al., 2015; Trupia et al., 2016; Shin et al., 2016a). Using corn DDGS as an ingredient in poultry diets has reduced feed costs, and environmental additions of nitrogen, phosphorus, NH_3 and H_2S from their manure (Wu-Haan et al., 2010; Masa'deh et al., 2011; Li et al., 2014; Abd El-Hack et al., 2016). In broiler chickens and Chinese native chickens, adding corn DDGS to diets enhanced meat quality through increasing the content of polyunsaturated fatty acids, reducing drip loss and cocking loss and elevated efficiency of the immune system by promoting cytokine secretion

Received January 12, 2017.
Accepted November 9, 2017.
[1]Corresponding authors: zhengcht@163.com; ylin898@126.com

Table 1. Composition and nutrient levels of the 6 diets (g/kg, as fed basis).

Ingredients	I	II	III	IV	V	VI
Corn	560.9	550.3	539.7	529.1	518.5	507.9
Wheat bran	110.5	92.1	73.7	55.3	36.9	18.5
Soybean meal	220.3	189.2	158.1	127.0	95.9	64.8
Corn DDGS	0.0	60.0	120.0	180.0	240.0	300.0
L-Lysine·HCl	0.75	1.35	1.95	2.25	3.15	3.75
DL-Methionine	1.65	1.55	1.45	1.35	1.25	1.15
Limestone	81.7	81.9	82.1	82.3	82.5	82.7
Calcium hydrogen phosphate	11.2	10.6	10.0	9.4	8.8	8.2
Sodium chloride	3.0	3.0	3.0	3.0	3.0	3.0
Premix*	10.0	10.0	10.0	10.0	10.0	10.0
Total	1000	1000	1000	1000	1000	1000
Nutrient level						
AME (MJ/kg)	10.46	10.46	10.46	10.46	10.46	10.46
CP, %#	17.15	17.08	17.12	17.05	16.94	16.91
EE, % #	2.88	3.38	3.95	4.46	5.00	5.50
Ca, %	3.60	3.60	3.60	3.60	3.60	3.60
Total P, %	0.63	0.61	0.58	0.59	0.58	0.57
Available P, %	0.35	0.35	0.35	0.35	0.35	0.35
Total Lys, %	0.85	0.85	0.86	0.86	0.87	0.87
Total Met, %	0.40	0.40	0.40	0.40	0.40	0.40
Total Met+Cys, %	0.68	0.69	0.70	0.71	0.71	0.71
Total Thr, %	0.68	0.69	0.70	0.71	0.71	0.72
Total Trp, %	0.21	0.20	0.19	0.19	0.19	0.18

*The premix provided per kilogram diet: vitamin A, 12,000 IU; vitamin D_3, 2400 IU; vitamin E, 20 mg; vitamin K_3, 2.5 mg; vitamin B_1, 4.0 mg; vitamin B_2, 6.0 mg; vitamin B_6, 6.0 mg; vitamin B_{12}, 0.02 mg; chloride choline, 500 mg; nicotinic acid, 15 mg; D-pantothenic acid, 20 mg; folic acid, 1.0 mg; biotin, 0.2 mg; Fe, 60 mg; Cu, 8 mg; Mn, 100 mg; Zn, 90 mg; I, 0.5 mg; Se, 0.2 mg; Co, 0.26 mg.

#Measured values of CP and EE content. Other nutrient levels are calculated values.

including IL-4 and IL-6, and antibody synthesis (Corzo et al., 2009; Min et al., 2012, 2015; Ruan et al., 2016), whereas in laying hens, corn DDGS enhanced egg quality by increasing yolk color score, polyunsaturated fatty acids, lutein, and zeaxanthin content of egg yolk (Shin et al., 2016b; Trupia et al., 2016). Corn DDGS contains high levels of unsaturated fatty acids, particularly oleic acid and linoleic acid that is prone to oxidation, which may increase oxidative stress in animals consuming the feed and modulate antioxidant defense system, especially through the transcription factor (nuclear factor erythroid 2-related factor 2, **Nrf2**). Therefore, feeding corn DDGS containing oxidized lipids may induce oxidative stress, alter immune function and thus, negatively affect animal growth performance (Song et al., 2012; Hanson et al., 2015; Min et al., 2015). Li et al. (2012) reported that feeding diets containing corn DDGS to geese results in an acceleration of lipid oxidation in muscle, which will cause the rancidity. The optimal dietary levels of corn DDGS that optimize the performance of broiler chickens, Chinese native chickens, laying hens, turkeys, quail, Peking ducks, geese, and rabbits have been established (Świątkiewicz and Koreleski, 2008; Adamski et al., 2011; Kowalczyk et al., 2012; Li et al., 2012; El-Abd, 2013; Alagón et al., 2015; Ruan et al., 2016), but not yet in the diets of laying ducks diet. Therefore, the objectives of this study were to determine the optimal level of corn DDGS in diets of egg-laying ducks based on their productive performance, egg quality, and expression of genes related to the antioxidant defense system.

MATERIALS AND METHODS

Experimental Design, Animals, and Housing

The study was approved by the Animal Care and Use Committee of Guangdong Academy of Agriculture Sciences. Longyan pullets (1,080), of the same genetic background and of comparable body weight (**BW**) at 17 wk of age, were randomly assigned to 6 dietary treatments, each with 6 replicates of 30 birds (each replicate was housed with indoor 3 × 4 m, outdoor 3 × 5 m, and pool 3 × 5 m areas), and they were studied for 18 wk. Fresh drinking water was available ad libitum throughout the study. The daily feed allowance was the maximum without leaving refusals and it was split into 2 feeds, which were provided at 7:00 a.m. and 3:00 p.m. All birds were given 160 g/d feed during the early and peak-laying period. Each replicate groups of ducks had daytime access to the outdoor and pool areas, then they were all housed in doors at night, with 4 h of light (incandescent lighting of 10 lx) from 6:00 p.m. to 10:00 p.m, i.e., a light: dark regime of 16:8 h. Birds in the control group were fed a corn-soybean meal basal diet (I), and the others were fed diets (II to VI) where 6%, 12%, 18%, 24% and 30% corn DDGS was included, as partial substitution of soybean meal and wheat bran and a reduction of corn, to maintain constant energy and CP, as shown in Table 1; dietary nutrient levels were based on previous results from this laboratory for Longyan ducks. Corn DDGS (US origin) was purchased from China SDIC International Trade Co., Ltd and was stored under refrigeration. The measured nutrient

Table 2. Analyzed nutrient composition and some important mycotoxins content of Corn DDGS (g/kg, as-fed basis)*.

Item	Value	Item	Value
Nutrient composition		Amino acid profile	
DM	893.4	Arginine	12.9
CP	300.2	Histidine	6.8
Ether extract	68.5	Isoleucine	7.2
Ca	0.5	Leucine	37.2
P	7.8	Lysine	6.3
Ash	46.4	Methionine	5.3
Crude fiber	76.6	Phenylalanine	15.5
ADF	120.2	Threonine	12.0
NDF	280.8	Tryptophan	1.8
Fatty acid profile		Valine	10.7
Tetradecanoic acid (C14:0)	0.04	Alanine	24.3
Palmitic acid (C16:0)	8.5	Aspartic acid	22.8
Heptadecanoic acid (C17:0)	0.05	Cysteine	5.6
Stearic acid (C18:0)	1.4	Glutamic acid	58.3
Palmitoleic acid (C16:1)	0.07	Glycine	12.6
Oleic acid (C18:1)	20.0	Proline	27.9
Eicosenoic acid (C20:1)	0.22	Serine	17.8
Linoleic acid (C18:2n-6)	38.1	Tyrosine	10.2
α-Linolenic acid (C18:3n-3)	0.83		
Mycotoxins, μg/kg			
Afatoxin B_1	<1.0		
Deoxynivalenol	205.0		
Zearalenone	17.25		

*Determined according to A. O. A. C. (2006).

values and contents of main mycotoxins of the corn DDGS used here are presented in Table 2.

Tissue Sampling and Storage

After 18 wk of feeding, 2 birds were selected at random from each replicate, excluding obvious outliers in BW for the treatment group. Heparinized blood was collected from the wing vein, centrifuged (1,200 × *g*) at 4°C for 10 min and plasma was held at −20°C. The birds were then stunned, exsanguinated, and samples of liver were collected, rinsed quickly with phosphate-buffered saline (**PBS**), snap frozen in liquid nitrogen, and stored at −80°C.

Productive Performance

Feed intake was recorded daily on a per replicate basis. The numbers of total, broken and shell-less eggs were recorded daily by replicate. Eggs produced were individually weighed and graded daily. Egg production, egg weight, egg mass, average daily feed intake, feed conversion ratio (**FCR**, g feed g^{-1} egg) were calculated daily, on a per replicate basis, then presented as the averages for the complete 18-wk study period.

Egg Quality

Egg quality was measured on 4 eggs collected at random from each replicate each month, and the average of these 24 eggs was used. Yolk color, albumen height, and Haugh units were measured on the day of collection using an Egg Analyzer (model EA-01, ORKA Food Technology, Ramat HaSharon, Israel). The strength of the shell was determined on the vertical axis using an Egg Force Reader (model EFR-01, ORKA). Eggshell thickness was measured using a digital micrometer, and egg shape index (**SI**) was determined with a digital caliper and calculated with the formula SI = width × 100/length, the distance between the blunt and pointed ends for length, and the diameter at mid-length for the width. Yolks were separated, weighed and expressed as percentages of egg weight. The shells with membranes were weighed after drying at 105°C.

Biochemical Determinations

The plasma contents of high density lipoprotein cholesterol (**HDL-C**), low density lipoprotein cholesterol (**LDL-C**), total triglyceride (**TG**) and total cholesterol (**TC**) were measured with colorimetric assay kits (Nanjing Jiancheng Institute of Bioengineering, Nanjing, Jiangsu, P. R. China).

Forty milligrams of frozen liver were homogenized on ice in 4 mL of homogenization buffer (0.05 M Tris-HCl, pH 7.4, 1 mM EDTA, 0.25 M sucrose) with an Ultra-Turrax (T8, IKA-Labortechnik, Staufen, Germany) for 5 s at 13,500 rpm. The homogenate was centrifuged at 3,000 × *g* for 10 min at 4°C, and the supernatant was stored at −80°C. The activities of total superoxide dismutase (**T-SOD**), glutathione peroxidase (**GSH-Px**), catalase (**CAT**), total antioxidant capacity (**T-AOC**) and contents of malondialdehyde (**MDA**) were measured with kits (Nanjing Jiancheng Institute of Bioengineering).

All samples were measured in duplicate, at appropriate dilutions, to give activities of the enzymes in the linear range of standard curves constructed with pure enzymes. Protein content of supernatants was determined using the Coomassie Brilliant Blue G250 (Sigma Chemical, St. Louis, MO) with bovine serum albumin standards.

Lipid Analysis

The concentrations TG, TC, HDL-C, and LDL-C were measured with assay kits (Nanjing Jiancheng Institute of Bioengineering) on separated yolks from sampled eggs.

Total lipid was extracted from approximately 0.5 g separated egg yolk, weighed into a 50 mL test tube with 20 mL of chloroform: methanol (2:1, vol/vol), and was homogenized with a Polytron for 5 to 10 s at high speed (Folch et al., 1957). Butylated hydroxyanisole in 98% ethanol was added prior to homogenization. The homogenate was filtered through a Whatman 1 filter paper into a 100-mL graduated cylinder and 5 mL of 0.88% sodium chloride solution was added, stoppered, and mixed. After phase separation, the volume of lipid layer was recorded, and the top

layer was completely siphoned off. Total lipids were converted to fatty acid methyl esters (**FAME**) using a mixture of boron-trifluoride, hexane, and methanol (35:20:45, vol/vol). The FAME were separated and quantified by an automated gas chromatograph equipped with an autosampler and flame ionization detector, using a 30 m × 0.32 mm inside diameter fused silica capillary column, as described (Cherian and Sim, 1991). A Shimadzu EZChrom chromatography (2010 type) data system was used to integrate peak areas. The calibration and identification of fatty acid peaks was carried out by comparison with retention times of known authentic standards. Fatty acid composition is expressed as weight percentages.

Relative Hepatic Expression of Genes Related to Antioxidation

Total RNA was extracted from the frozen liver using Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA) and treated with DNase I (TaKaRa Biotechnology Co. Ltd., Dalian, China), quantified by $OD_{260:280}$, and evaluated after gel electrophoresis.

Total RNA (2.5 μg) was used to generate cDNA in a final volume of 20 μL according to the manufacturer's instructions (Promega, Madison, WI). All primers for the genes of interest were designed with Primer Premier 5.0 from duck sequences in GenBank and obtained from Shanghai ShengGong Biological Company (Shanghai, China). Primer sequences for **SOD1** (GeneBank: XM_01,309,7859.1), GPX1 (GeneBank: KU048803.1), **CAT** (GeneBank: KU048802.1), heme oxygenase 1 (**HO-1**, GeneBank: KU048806.1), nuclear factor erythroid 2 like 2 (**Nrf2**, GeneBank: NM_0,013,10777.1), and β-actin (GeneBank: EF667345.1) are shown as follows: 5′-CC TGTGGTGTCATCGGAATA-3′ and 5′-TTGAACG AGGAAGAGCAAGTA-3′, 5′-CAGTACATCATCTG GTCGCC-3′ and 5′-CCTGGATCTTGATGGTTT CG-3′, 5′-CTGTTGAGGAAGCAGGAAGG-3′ and 5′-GAAAGACCAGGATGGGTAGTTG-3′, 5′-CCCATG CCTACACTCGCTAT-3′ and 5′-GCCTCCTCCAA GACTCGTTT-3′, 5′-GTTGAATCATCTGCCTGTG G-3′ and 5′-TAAGCTAGGTGGTCGAGTGC-3′, 5′-GCTATGTCGCCCTGGATTT-3′ and 5′-GGATGCCACAGGACTCCATAC-3′. The quantity of cDNA was amplified by polymerase chain reaction (**PCR**) under the optimal conditions, which comprised initial 5 min denaturation at 94°C; 35 cycles of 30 s at 94°C, 30 s annealing at X°C, and extension for 30 s at 72°C; with a final 10 min extension at 72°C. Aliquots of PCR products were evaluated by electrophoresis in 1.5% agarose gels and excised products from the gels were sequenced to verify authenticity.

Quantitative real-time PCR (qPCR) was performed using the same primers to quantify mRNA content. Each 20 μL PCR mixture contained 10 μL of 2X iQTM SYBR Green Supermix, 0.6 μL (10 mM) of each primer, and 2 μL of cDNA. Mixtures were incubated in an iCycler iQ Real-time Detection system (Bio-Rad, Hercules, CA) using 40 cycles (95°C for 15 s and 35 s at the T_A). Quantification of the transcripts was performed using a standard curve with 10-fold serial dilutions of cDNA. A melting curve was constructed to verify that only a single PCR product was amplified. Samples were assayed in triplicate with standard deviations of threshold cycle (**Ct**) values not exceeding 0.5. The $2^{-\Delta\Delta Ct}$ method was used (Livak and Schmittgen, 2001) with β-actin as the reference transcript for relative gene expression and then expressed relative to the transcript abundance in birds fed the control diet (I).

Statistical Analysis

Replicate (n = 6) was taken as the experimental unit. Except where noted otherwise, 2 sampled birds per replicate were used. The effect of substituting corn DDGS in the diets was examined by one-way analysis of variance (**ANOVA**) procedure of SAS 9.1 (SAS Institute Inc., 2004). Where appropriate, orthogonal polynomial contrasts were used to estimate the linear and quadratic effects of the increasing levels of corn DDGS and the probability level of 0.05 was applied to test significance. Data for each variable are presented as means, along with the SE for n = 6, based on the ANOVA error mean square.

RESULTS

Productive Performance

The results for performance of the laying ducks are presented in Table 3. The average egg weight during the early and peak period of laying decreased as linear responses to corn DDGS substitution ($P < 0.001$). There were no significant effects of treatment on daily egg production, egg mass, FCR, broken rate, and abnormal eggs.

Egg Quality

Of the indices of egg quality (Table 4), Haugh unit, albumen weight, and albumen proportion decreased as linear responses to corn DDGS substitution ($P < 0.05$), but yolk color linearly increased ($P < 0.001$). There was no effect on eggshell thickness, egg SI, eggshell strength, eggshell weight and yolk weight. Increasing the level of corn DDGS beyond 18% significantly reduced Haugh unit, while more than 24% of corn DDGS significantly reduced albumen weight compared with the control. Inclusion of corn DDGS at levels higher than 6% significantly improved yolk color compared with the controls.

Table 3. Effect of dietary corn DDGS substitution on performance of ducks at early and peak laying period.[1]

Variable	Dietary corn DDGS level (%)						SEM[2]	*P*-value[3]		
	0	6	12	18	24	30		DDGS	Linear	Quadratic
Early laying period (50% < egg production < 80%, 17 to 18 wk of age)										
Egg production (%)	71.6	68.6	70.7	71.9	76.5	74.2	2.81	0.139		
Average egg weight (g)	58.5^{a}	57.9^{a,b}	57.7^{a,b}	57.9^{a,b}	56.9^{a,b}	56.4^{b}	0.39	<0.001	0.050	0.083
Egg mass (g/d)	42.4	39.7	41.1	41.8	43.7	42.5	1.59	0.075		
Feed conversion ratio[4] (g feed g^{-1} egg)	3.88	4.06	3.96	3.94	3.74	3.84	0.16	0.117		
Peak laying period (egg production > 80%, 19 to 35 wk of age)										
Egg production (%)	87.1	88.4	86.7	88.3	86.0	87.8	1.259	0.852		
Average egg weight (g)	62.7^{a}	61.8^{a}	62.3^{a}	62.5^{a}	61.2^{a,b}	60.6^{b}	0.392	0.009	<0.001	0.045
Egg mass (g/d)	54.1	54.0	54.3	54.8	53.1	53.3	0.594	0.922		
Feed conversion ratio[4] (g feed g^{-1} egg)	2.97	2.99	2.99	2.96	3.04	3.03	0.025	0.973		
Whole laying period (17 to 35 wk of age)										
Initial body weight (g)	1471.7	1469.1	1471.7	1468.9	1477.2	1474.5	6.52	0.966		
Final body weight (g)	1498.1	1499.7	1505.4	1484.9	1477.6	1463.3	16.15	0.106		
Body weight gain (g)	27.2	30.6	33.2	16.0	0.5	−11.2				
Egg production (%)	81.5	80.7	81.3	82.6	81.9	82.5	1.334	0.621		
Average egg weight (g)	59.6^{a}	59.5^{a,b}	59.5^{a,b}	59.3^{a}	58.9^{a,b}	57.7^{b}	0.388	0.018	0.049	0.085
Egg mass (g/d)	48.6	48.4	48.0	48.4	48.2	47.9	0.915	0.425		
Feed conversion ratio[4] (g feed g^{-1} egg)	3.34	3.35	3.40	3.41	3.39	3.41	0.047	0.400		
Broken rate (%)	0.01	0.01	0.02	0.03	0.01	0.02	0.007	0.235		
Abnormal rate (%)	0.72	1.03	0.57	0.46	0.76	0.60	0.187	0.376		

[1]Each value represents the mean of 6 replicates (30 birds per replicate).
[2]Derived from ANOVA error mean square.
[3]DDGS, treatment effect; when significant, linear and quadratic effects were tested.
[4]All birds were given 160 g/d during the early and peak-laying period.
[a,b]Means with different letters in the same row differ significantly ($P < 0.05$).

Table 4. Effect of dietary corn DDGS substitution on egg composition and quality of ducks during the laying period (17 to 35 wk of age).[1]

Variable	Dietary corn DDGS level (%)						SEM[2]	*P*-value[3]		
	0	6	12	18	24	30		DDGS	Linear	Quadratic
Egg composition										
Yolk weight, g	20.48	19.76	20.45	20.33	19.85	19.95	0.337	0.946		
Albumen weight, g	36.79^{a}	37.84^{a}	36.70^{a}	36.25^{a}	36.05^{a,b}	34.82^{b}	0.327	0.046	0.020	0.217
Eggshell weight, g	6.32	6.33	6.30	6.29	6.26	6.24	0.038	0.445		
Albumen (%)	58.48^{a,b}	60.95^{a}	58.60^{a,b}	57.96^{a,b}	57.64^{a,b}	57.05^{b}	0.327	0.046	0.018	0.409
Yolk (%)	32.17	32.54	32.61	32.31	31.88	32.71	0.337	0.954		
Eggshell (%)	10.03	10.16	10.07	10.05	10.16	10.24	0.131	0.486		
Egg quality										
Eggshell thickness (mm)	0.339	0.355	0.347	0.342	0.349	0.336	0.004	0.157		
Eggshell strength (N)	38.1	40.9	38.5	40.6	38.9	39.9	1.554	0.619		
Egg shape index	74.4	74.0	74.5	73.6	73.9	73.9	0.517	0.734		
Haugh unit	76.3^{a}	73.2^{a,b}	72.6^{a,b}	72.0^{a,b}	71.6^{b}	71.1^{b}	1.731	0.023	0.048	0.452
Yolk color	6.96^{b}	7.50^{b}	8.38^{a}	8.50^{a}	8.58^{a}	8.79^{a}	0.255	0.001	<0.001	0.049

[1]Each value represents the mean of 6 replicates (4 eggs per replicate).
[2]Derived from ANOVA error mean square.
[3]DDGS, treatment effect; when significant, linear and quadratic effects were tested.
[a,b]Means with different letters in the same row differ significantly ($P < 0.05$).

Egg Yolk Fatty Acid Composition

The fatty acid composition of egg yolk lipids is provided in Table 5. The proportions of oleic acid (C18:1) and total monounsaturated fatty acids (**MUFAs**) decreased linearly with increasing corn DDGS substitution ($P < 0.001$). The contents of many of the polyunsaturated fatty acids (**PUFAs**) especially linoleic (C18:2n-6) and linolenic acid (C18:3n-3) in egg yolk linearly increased ($P < 0.001$) but not those of eicosapentaenoic (C20:5n-3) and docosahexaenoic acid (C22:6n-3). This increase with corn DDGS substitution also occurred in total n-6 fatty acids and n-6/n-3 and a smaller increase in n-3 fatty acids. There were no treatment effects on the crude fat, TCH and TG content of egg yolk.

Biochemical Analyses and Hepatic Expression of Genes Related to antioxidation

As shown in Table 6, the plasma contents of TG, hepatic activity of GSH-Px and the liver content of MDA

Table 5. Effect of dietary corn DDGS substitution on triglyceride and total cholesterol content and fatty acid composition in egg yolks of ducks during the laying period (17 to 35 wk of age).[1]

	Dietary corn DDGS level (%)							*P*-value[4]		
Variable, %[2]	0	6	12	18	24	30	SEM[3]	DDGS	Linear	Quadratic
TC, mg/g	26.8	26.8	26.2	27.0	26.6	26.3	0.485	0.926		
TG, mg/g	255.9	259.4	252.2	258.3	272.7	269.1	4.993	0.134		
Total lipids	30.9	31.0	30.0	31.1	31.2	31.4	0.297	0.183		
Tetradecanoic acid (C14:0)	0.32	0.30	0.31	0.31	0.32	0.33	0.012	0.494		
Palmitic acid (C16:0)	24.2	23.7	23.7	23.9	23.8	24.1	0.264	0.489		
Heptadecanoic acid (C17:0)	0.14	0.13	0.14	0.13	0.14	0.13	0.003	0.149		
Stearic acid (C18:0)	5.82	5.90	5.50	5.69	5.81	5.55	0.171	0.619		
Total SFAs	30.5	30.1	29.7	30.0	30.1	30.1	0.342	0.456		
Palmitoleic acid (C16:1)	2.24	2.07	2.19	2.08	1.97	1.94	0.107	0.644		
Oleic acid (C18:1)	47.6^{a,b}	48.1^{a}	46.3^{a-c}	45.6^{b,c}	44.5^{c,d}	43.5^{d}	0.461	<0.001	<0.001	0.345
Eicosenoic acid (C20:1)	0.32	0.32	0.31	0.28	0.28	0.28	0.013	0.136		
Total MUFAs	50.1^{a,b}	50.5^{a}	48.8^{a-c}	48.0b^{c,d}	46.9^{c,d}	45.7^{d}	0.522	0.001	<0.001	0.393
Linoleic acid (C18:2n-6)	9.11^{c}	9.23^{c}	10.77^{c}	12.29^{b}	13.27^{a,b}	14.51^{a}	0.358	<0.001	<0.001	0.440
α-Linolenic acid (C18:3n-3)	0.45^{b}	0.47^{b}	0.51^{a,b}	0.53^{a,b}	0.60^{a}	0.61^{a}	0.002	0.021	<0.001	0.482
cis-11, 14-Eicosenoic acid (C20:2n-6)	0.21^{b}	0.22^{b}	0.24^{b}	0.29^{a}	0.30^{a}	0.32^{a}	0.012	<0.001	<0.001	0.823
cis-8, 11, 14-Eicosenoic acid (C20:3n-6)	0.27^{b,c}	0.25^{c}	0.27^{b,c}	0.31^{a,b}	0.33^{a}	0.34^{a}	0.010	0.001	<0.001	0.495
Arachidonic acid (C20:4n-6)	1.44^{b,c}	1.29^{c}	1.40^{b,c}	1.61^{a,b}	1.72^{a}	1.78^{a}	0.073	0.004	<0.001	0.128
Eicosapentaenoic acid (C20:5n-3)	0.12	0.13	0.11	0.11	0.13	0.13	0.010	0.373		
Docoshexaenoic acid (C22:6n-3)	0.14	0.12	0.13	0.13	0.12	0.13	0.015	0.380		
Total PUFAs	11.5^{c}	11.6^{c}	13.3^{c}	15.2^{b}	16.3^{a,b}	17.7^{a}	0.421	<0.001	<0.001	0.367
Total n-6 fatty acids	10.8^{c}	10.7^{c}	12.4^{c}	14.2^{b}	15.3^{a,b}	16.6^{a}	0.476	<0.001	<0.001	0.366
Totaln-3 fatty acids	0.61^{b,c}	0.57^{c}	0.65^{a-c}	0.66^{a-c}	0.73^{a,b}	0.74^{a}	0.031	0.019	<0.001	0.404
Total n-6/n-3	17.8^{d}	19.1^{c}	19.3^{c}	21.6^{b}	21.9^{b}	22.7^{a}	0.331	<0.001	<0.001	0.568

[1]Each value represents the mean of 6 replicates (4 samples per replicate).
[2]TG, triglycerides; TCH, total cholesterol; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.
[3]Derived from ANOVA error mean square.
[4]DDGS, treatment effect; when significant, linear and quadratic effects were tested.
[a-d]Means with different letters in the same row differ significantly ($P < 0.05$).

Table 6. Effect of dietary corn DDGS substitution on biochemical indices in blood and liver of laying ducks at the end of the study (35 wk of age).[1]

	Dietary corn DDGS level (%)							*P*-value[4]		
Variable[2]	0	6	12	18	24	30	SEM[3]	DDGS	Linear	Quadratic
					Plasma					
HDL-C (mmol/L)	0.48	0.45	0.50	0.44	0.49	0.51	0.209	0.919		
LDL-C (mmol/L)	1.31	1.25	1.22	1.05	1.30	1.38	0.053	0.799		
TG (mmol/L)	2.97^{b}	3.04^{b}	3.41^{b}	3.61^{a,b}	3.78^{a}	4.11^{a}	0.288	<0.001	<0.001	0.524
TCH (mmol/L)	2.39	2.58	2.51	2.44	2.60	2.73	0.274	0.854		
					Liver					
GSH-Px (U/mg prot)	55.1^{b}	55.9^{b}	55.0^{b}	62.2^{a,b}	63.7^{a}	63.9^{a}	2.01	0.035	<0.001	0.543
T-SOD (U/mg prot)	371.3	373.1	374.5	375.3	388.2	376.1	14.05	0.365		
CAT (U/mg prot)	3.78	3.72	3.74	3.70	3.65	3.67	0.132	0.696		
T-AOC (U/mg prot)	1.33	1.27	1.31	1.26	1.23	1.19	0.062	0.268		
MDA (nmol/mg prot)	1.43^{c}	1.48^{c}	1.69^{b,c}	1.66^{b,c}	2.21^{b}	2.92^{a}	0.166	<0.001	<0.001	0.005

[1]Each value represents the mean of 6 replicates (2 birds per replicate).
[2]HDL-C, high density lipoprotein cholestrol; LDL-C, low density lipoprotein cholestrol; TG, total triglyceride; TCH, total cholesterol; GSH-Px, glutathione peroxidase; T-SOD, total superoxide dismutase; CAT, catalase; T-AOC, total antioxidant capacity; MDA, malondialdehyde;
[3]Derived from ANOVA error mean square.
[4]DDGS, treatment effect; when significant, linear and quadratic effects were tested.
[a-c]Means with different letters in the same row differ significantly ($P < 0.05$).

increased as linear responses to corn DDGS substitution ($P < 0.05$). Also, the results in Table 7, showed that increasing corn DDGS substitution linearly increased the expression of *GPX1*, *HO-1*, and *Nrf2* in the liver of laying ducks ($P < 0.001$). There were no significant effects of corn DDGS substitution on plasma contents of HDL-C, LDL-C or TCH, nor hepatic expression of *SOD1* or *CAT* or measured hepatic activities of T-SOD or CAT.

DISCUSSION

Effect of Dietary Corn DDGS Substitution on Performance and Egg Quality of Laying Ducks at Early and Peak Laying Period

Corn DDGS is a high quality protein feed material having a low starch, high protein, fat, fiber and

Table 7. Effects of dietary corn DDGS substitution on relative hepatic expression of genes related to antioxidation in laying ducks at the end of the study (35 wk of age).[1]

	Dietary corn DDGS level (%)							*P*-value[4]		
Gene[2]	0	6	12	18	24	30	SEM[3]	DDGS	Linear	Quadratic
SOD1	1.01	1.00	0.90	0.92	0.93	0.98	0.095	0.812		
GPX1	1.02^{b}	0.97^{b}	1.04^{b}	0.93^{b}	1.12^{b}	1.58^{a}	0.125	0.011	0.009	0.028
CAT	1.05	0.93	0.90	0.92	0.86	0.87	0.113	0.223		
HO-1	1.04^{c}	0.92^{c}	0.95^{c}	1.06^{c}	1.24^{b}	1.47^{a}	0.046	0.008	<0.001	0.008
Nrf2	1.00^{c}	0.99^{c}	0.97^{c}	1.03^{c}	1.19^{b}	1.52^{a}	0.033	0.001	<0.001	<0.001

[1]Each value represents the mean of 6 replicates (2 birds per replicate).
[2]*SOD1,* superoxide dismutase 1; *GPX1*, glutathione peroxidase 1; *CAT,* catalase; *HO-1,* heme oxygenase 1; *Nrf2*, nuclear factor erythroid 2 like 2.
[3]Derived from ANOVA error mean square.
[4]DDGS, treatment effect; when significant, linear and quadratic effects were tested.
[a-c]Means with different letters in the same row differ significantly ($P < 0.05$).

digestible phosphorus content, and is widely used in livestock production. The present study demonstrated increasing corn DDGS can linearly decreased egg weight during the early and peak laying periods. No significant differences were detected in egg production, egg mass and feed conversion ratio between groups of laying ducks fed 0 to 30% corn DDGS during experimental period. Lumpkins et al. (2005), Roberson et al. (2005), Masa'deh et al. (2011) and Deniz et al. (2013) reported that feeding corn DDGS up to 15% had no negative effect on egg production, while 30% was acceptable in other studies (Loar et al., 2010; Awad et al., 2011). In contrast, Pineda et al. (2008), Świątkiewicz and Koreleski (2008), and Deniz et al. (2013) reported reduced laying performance with 20% DDGS, which may be attributed to the high percentage of crude fiber, the low palatability, sulfur content, high variability in nutrients (especially lysine), and high proportion of polyunsaturated fatty acid of the DDGS. Creswell (2006) reported that 20% DDGS diets lacked sufficient starch, so the hens depended upon converting part of the dietary amino acids to glucose to achieve euglycemia and relied increasingly on fatty acid oxidation to supply energy. These changes could have influenced metabolism over time, and ultimately might have affected laying performance.

In Lohman Brown layers, Świątkiewicz and Koreleski (2008) reported that increasing corn DDGS from 0 to 20% had no effects on Haugh unit, egg shell thickness, or eggshell breaking strength, but yolk color improved significantly over controls with inclusion of 5, 10, and 15% corn DDGS. Also, Cheon et al. (2008) observed in Hy-LineLine Brown hens that Haugh unit, eggshell weight, and eggshell breaking strength did not change with increasing corn DDGS to 20%, but 10, 15, or 20% corn DDGS increased yolk color. Masa'deh et al. (2011) similarly noted that feeding Bovans White layers with diets containing up to 25% corn DDGS, or 32% in the case of Loar et al. (2010), had no affect on Haugh unit or eggshell quality, but linearly improved yolk color, while Deniz et al., (2013) showed in Super Nick White hens that yolk color was only enhanced significantly with 20% corn DDGS, but eggshell thickness, eggshell strength, and Haugh unit were not affected by up to 20% corn DDGS. Similar results were obtained by Świątkiewicz et al. (2013), who found that 20% corn DDGS in diets of ISA Brown layers did not affect Haugh unit, yolk proportion, eggshell proportion, eggshell thickness, eggshell breaking strength, but improved yolk color. Moreover, Jiang et al. (2013) reported that yolk color and eggshell thickness enhanced, whereas eggshell strength, relative weight of yolk and albumen and Haugh unit did not influence by consuming Hy-Line Brown hens diets including 20% corn DDGS, but Shin et al. (2016b) reported that inclusion of 5, 10, 15, or 20% corn DDGS in Hy-Line Brown hens diets increased linearly yolk color and reduced numerically Haugh unit without effect on eggshell thickness or strength, while Abd El-Hack et al. (2016) reported that eggshell thickness decreased, Haugh unit increased, and egg shape index did not change by including 15% corn DDGS in the diets of Hy-Line Brown laying hens. Additionally, Trupia et al. (2016) demonstrated that Hy-Line W-36 White Leghorn hens consumed diets containing 10% corn DDGS significantly reduced eggshell quality, whereas Sun et al. (2012) recorded that adding 37% corn DDGS in White Leghorn hens diets had no effect on Haugh unit, yolk, albumen, and eggshell proportion, whereas yolk color significantly enhanced, but inclusion of 50% corn DDGS declined eggshell proportion and improved Haugh unit and yolk color. Corn DDGS is a concentrated source of xanthophylls and their substrates (lutein and zeaxanthin) (Salim et al., 2010; Shin et al., 2016a) so the yolk content of these are increased and color is improved when corn DDGS is included in the diets of laying hens (Sun et al., 2012; Shin et al., 2016b; Trupia et al., 2016).

Effect of Dietary Corn DDGS Substitution on Fatty Acid Composition of Yolk in Laying Ducks at Peak Laying Period

Dietary fatty acid composition is the most important factor influencing the fatty acid composition of broiler meat and hen eggs (Cortinas et al., 2004). Corn DDGS

contains approximately 10% fat, and is rich in PUFAs, particularly C18:2n-6. The proportions in yolk lipids of C18:1 and MUFAs decreased linearly with increased dietary inclusion of corn DDGS. While the contents of C18:2n-6 and C18:3n-3 linearly increased, which lead to an increase in PUFAs and n-6/n-3. There were no significant effects of up to 30% corn DDGS on fat, TG and TCH content of egg yolk. These results were consistent with previous reports (Cheon et al., 2008; Huang et al., 2006; Rew et al., 2009; Schilling et al., 2010; Jiang et al., 2013; Sun et al., 2013) though Schilling et al. (2010) and Jiang et al. (2013) found the proportions of palmitic acid, stearic acid, and SFAs to decrease in egg yolks with increased corn DDGS as was found here with ducks. Consequently, increasing dietary content of corn DDGS for the ducks here increased egg yolk concentrations of important fatty acids like C18:2n-6 and C18:3n-3.

Effect of Dietary Corn DDGS Substitution on Biochemical and Antioxidative Indices in Laying Ducks at Peak Laying Period

Very little is known of the effect of corn DDGS on the antioxidant status in laying ducks. It was found here that the addition of corn DDGS to the diets increased linearly GSH-Px activity and MDA content in liver while TC, HDL-C, LDL-C, T-AOC, T-SOD, or CAT activity were not significantly affected by corn DDGS. Also, the results in Table 7, showed that increasing corn DDGS concentration did not change hepatic expression of *SOD1* or *CAT* mRNA but did increase that of *GPX1*, *HO-1*, and *Nrf2*. These findings using ducks are consistent with studies in broilers (Min et al., 2012), where increasing levels of corn DDGS in the diets from 0 to 20% during fattening period (42 d) promoted lipid peroxidation, and so increased MDA. Therefore, they recommended including 15% corn DDGS in broiler's diets to optimize the activity of antioxidant enzymes including T-SOD and GSH-Px to maintain normal levels of lipid peroxidation (MDA). But Jiang et al. (2014) reported that inclusion of 15% corn DDGS in broiler diets during their fattening period (49 d) significantly elevated levels of MDA in plasma, liver, or breast muscles. Similarly, in diets of Chinese yellow broilers from 10% in starter phase, 20% in grower phase, and 30% in the finisher phase elevated hepatic MDA levels without significant effects on T-SOD or GSH-Px. Min et al. (2015) found that increasing corn DDGS level from 0 to 15% in broiler's diets from 1 to 21 d significantly reduced T-AOC and T-SOD and significantly increased MDA as a result of increasing lipid peroxidation. Moreover, inclusion of 15% corn DDGS in turkeys' diets during grower and finisher phases led to a significant decline in GSH-Px activity in plasma and red blood cells combined with an increase in MDA and a significant elevation in GSH-Px concentration in liver without significant effect on MDA in the liver compared with the controls receiving 0% corn DDGS) (Heincinger et al., 2011).

The transcription factor Nrf2, plays a crucial role in maintaining stability of the liver function through regulating the expression of antioxidant (*GPX*, *HO-1*, *CAT*, and *SOD*) and phase II detoxifying enzymes (Ishii et al., 2002; Li et al., 2009; Bai et al., 2016). The HO-1 is an essential enzyme in converting of heme into biliverdin, generating free iron and carbon monoxide. Biliverdin reductase is rapidly converts biliverdin to bilirubin, and both are involved in the antioxidant defense system (Maines, 1997; Ferrandiz and Devesa, 2008). GSH-Px production mainly occurs in the liver (Shi et al., 1996) and, in waterfowl, the activity of GSH-Px exceeds that of other antioxidant enzymes (Surai et al., 1998). Lipid peroxidation stimulates Nrf2 to induce antioxidant genes to protect the tissues from oxidative stress from the accumulation of end products of lipid peroxidation (Yan et al., 2014; Wang et al., 2016). This may explain why increasing corn DDGS content to more than 18% significantly elevated GSH-Px activity and *GPX1* and *HO-1* mRNA expression by stimulating *Nrf2* as a result of increasing MDA (the monitor of lipid peroxidation). Corn DDGS contains 10 to 13% oil, and most of the fatty acids are PUFAs (Świątkiewicz, and Koreleski, 2008). The high proportion of PUFAs in DDGS makes it susceptible to oxidation which increased the level of lipid peroxidation (MDA) when high levels of corn DDGS are consumed (Świątkiewicz, and Koreleski, 2008; Song and Shurson, 2013). The authors are unaware of any study on the effects of DDGS on expression of antioxidant related genes in poultry. As noted earlier, increasing corn DDGS beyond 18% significantly increased hepatic MDA concentration in the liver and in laying ducks, this would be associated with increased concentrations in the yolk (Ma et al., 2014) perhaps explaining why >18% corn DDGS significantly reduced Haugh unit. Increasing MDA level in the liver of laying hens could affect additional liver functions, including protein anabolism (Hiramoto et al., 1990; Yuan et al., 2016), which may explain why excessive corn DDGS significantly reduced albumen weight and its relative weight.

In conclusion, the current study shows that the use of corn DDGS is possible as a replacement for soybean meal in amounts up to 18% in the diets of laying ducks without affecting laying performance, egg quality and antioxidant capacity. Increasing amounts of corn DDGS linearly increased egg yolk concentrations of key fatty acids like C18:2n-6 and C18:3n-3. It appeared that antioxidant enzyme activity of GSH-Px was enhanced, through the Nrf2 pathway to offset any oxidative stress from the dietary PUFAs.

SUPPLEMENTARY DATA

Supplementary data are available at *PSCIEN* online.

ACKNOWLEDGMENTS

The authors sincerely thank W. Bruce Currie (Emeritus Professor, Cornell University, Ithaca, NY) for his help in presentation of this manuscript. This study was supported by the Earmarked Fund for Modern Agro-industry Technology Research System (CARS-42–13), Supporting Program for the Research of State Key Laboratory of Livestock and Poultry Breeding, Science & Technology Program of Guangdong Province (2013A061401020), Operating Funds for Guangdong Provincial Key Laboratory of Animal Breeding and Nutrition (2014B030301054), Supporting Program for Guangdong Agricultural Research & Development Center of Livestock and Poultry Healthy Breeding, and President Foundation of Guangdong Academy of Agricultural Sciences (201619).

REFERENCES

Abd EI-Hack, M. E., S. A. Mahgoub, M. Alagawany, and E. A. Ashour. 2016. Improving productive performance and mitigating harmful emissions from laying hen excreta via feeding on graded levels of corn DDGS with or without *bacillus subtilis* probiotic. J. Anim. Physiol. Anim. Nutr. doi: 10.1111/jpn.12522.

Adamski, M. P., A. M. Kowalczyk, E. T. Łukaszewicz, and M. Korzeniowska. 2011. Effects of sex and inclusion of dried distillers grains with solubles on slaughter yield and meat characteristics of Pekin ducks. Br. Poult. Sci. 52(6):742–749.

Alagón, G., O. Arce, P. Serrano, L. Ródenas, E. Martínez-Paredes, C. Cervera, J. J. Pascual, and M. Pascual. 2015. Effect of feeding diets containing barley, wheat and corn distillers dried grains with solubles on carcass traits and meat quality in growing rabbits. Meat. Sci. 101:56–62.

AOAC International. 2006. Official Methods of Analysis of AOAC, 18th edn. AOAC International, Washington, DC.

Awad, A. L., M. A. A. Hussein, A. I. I. Ghonim, and M. G. Kasim. 2011. Effect of dietary inclusion level of distillers dried grains with solubles on laying performance in domyati ducks. http://epsaegypt.com/pdf/2011_march/5-%201244%20(51-63)%20.pdf.

Bai, K., Q. Huang, J. Zhang, J. He, L. Zhang, and T. Wang. 2016. Supplemental effects of probiotic Bacillus subtilis fmbJ on growth performance, antioxidant capacity, and meat quality of broiler chickens. Poult. Sci. 96:74–82.

Cheon, Y. J., H. L. Lee, M. H. Shin, A. Jang, S. K. Lee, B. D. Lee, and C. K. Son. 2008. Effects of corn distiller's dried grains with solubles on production and egg quality in laying hens. Asian-Australas. J. Anim. Sci. 21:1318–1323.

Cherian, G., and J. S. Sim. 1991. Effect of feeding full fat flax and canola seeds to laying hens on the fatty acid composition of egg, embryos and newly hatched chicks. Poult. Sci. 70:917–922.

Cortinas, L., C. Villaverde, J. Galobart, M. D. Baucells, R. Codony, and A. C. Barroeta. 2004. Fatty acid content in chicken thigh and breast as affected by dietary polyunsaturation level. Poult. Sci. 83:1155–1164.

Corzo, A., M. W. Schilling, R. E. Loar, V. Jackson, S. Kin, and V. Radhakrisnan. 2009. The effect of feeding distillers dried grains with solubles on broiler meat quality. Poult. Sci. 88:432–439.

Creswell, D. C. 2006. DDGS: Benefits and limitations. Asian Poult. Mag. 22–24.

Deniz, G., H. Gencoglu, S. S. Gezen, I. I. Turkmen, A. Orman, and C. Kara. 2013. Effects of feeding corn distillers dried grains with solubles with and without enzyme cocktail supplementation to laying hens on performance, egg quality, selected manure parameters, and feed cost. Livest. Sci. 152:174–181.

El-Abd, N. M. 2013. Evaluation of using distillers dried grains with solubles (DDGS) in Japanese quail diets. World. Appl. Sci. J. 22:17–21.

Ferrandiz, M. L., and I. Devesa. 2008. Inducers of heme oxygenase-1. Curr. Pharm. Design. 14:473–486.

Folch, J., M. Lees, and G. H. Sloane-Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:497–509.

Hanson, A. R., L. Wang, and L. J. Johnston. 2015. Effect of feeding peroxidized dried distillers grains with solubles to sows progeny on growth performance and metabolic oxidative status of nursery pigs. J. Anim. Sci. 93:135–146.

Heincinger, M., K. Balogh, H. Fébel, M. Erdélyi, and M. Mézes. 2011. Effect of diets with different inclusion levels of distillers dried grain with solubles combined with lysine and methionine supplementation on the lipid peroxidation and glutathione status of chickens. Acta Vet. Hung. 59:195–204.

Hiramoto, K., T. Muramatsu, and J. Okumura. 1990. Effect of methionine and lysine deficiencies on protein synthesis in the liver and oviduct and in the whole body of laying hens. Poult. Sci. 69:84–89.

Huang, J. F., M. Y. Chen, H. F. Lee, S. H. Wang, Y. H. Hu, and Y. K. Chen. 2006. Effects of corn distiller's dried grains with soluble on the productive performance and egg quality of brown Tsaiya duck layers. http://biodieselfeeds.cfans.umn.edu/sites/biodieselfeeds.cfans.umn. edu/files/cfans_asset_414602.pdf.

Ishii, T., K. Itoh, and M. Yamamoto. 2002. Roles of Nrf2 in activation of antioxidant enzyme genes via antioxidant responsive elements. Methods Enzymol. 348:182–190.

Jiang, W., L. Zhang, and A. Shan. 2013. The effect of vitamin E on laying performance and egg quality in laying hens fed corn dried distillers grains with solubles. Poult. Sci. 92:2956–2964.

Jiang, W., S. Nie, Z. Qu, C. Bi, and A. Shan. 2014. The effects of conjugated linoleic acid on growth performance, carcass traits, meat quality, antioxidant capacity, and fatty acid composition of broilers fed corn dried distillers grains with solubles. Poult. Sci. 93:1202–1210.

Jie, Y. Z., J. Y. Zhang, L. H. Zhao, Q. G. Ma, and C. Ji. 2013. The correlationship between the metabolizable energy content, chemical composition and color score in different sources of corn DDGS. J. Anim. Sci. Biotech. 4:38.

Kowalczyk, A., E. Łukaszewicz, M. Adamski, and J. Kuźniacka. 2012. Carcass composition and meat characteristics of Pekin ducks in relation to age at slaughter and level of maize distiller's dried grains with solubles in diets. J. Anim. Feed Sci. 21:156–167.

Li, W., and A. N. Kong. 2009. Molecular mechanisms of Nrf2-mediated antioxidant response. Mol. Carcinog. 48:91–104.

Li, W., Q. F. Li, W. Powers, D. Karcher, R. Angel, and T. J. Applegate. 2014. Effects of distillers dried grains with solubles and mineral sources on gaseous emissions. J. Appl. Poult. Res. 23:41–50.

Li, X., Z. Wang, H. Yang, and X. Wang. 2012. Effects of feeding corn distillers dried grains with solubles on growth performance and meat quality in geese. Chin. J. Anim. Nutr. 24:897–904.

Livak, K. J., and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods. 25:402–408.

Loar II, R. E., M. W. Schilling, C. D. McDaniel, C. D. Coufal, S. F. Rogers, K. Karges, and A. Corzo. 2010. Effect of dietary inclusion level of distillers dried grains with solubles on layer performance, egg characteristics, and consumer acceptability. J. Appl. Poult. Res. 19:30–37.

Lumpkins, B., A. Batal, and N. Dale. 2005. Use of distillers dried grains plus solubles in laying hen diets. J. Appl. Poult. Res. 14:25–31.

Ma, X., Y. Lin, H. Zhang, W. Chen, S. Wang, D. Ruan, and Z. Jiang. 2014. Heat stress impairs the nutritional metabolism and reduces the productivity of egg-laying ducks. Anim. Reprod. Sci. 145:182–190.

Maines, M. D. 1997. The heme oxygenase system: A regulator of second messenger gases. Annu. Rev. Pharmacol. Toxicol. 37:517–554.

Masa'deh, M. K., S. E. Purdum, and K. J. Hanford. 2011. Drid distillers grains with solubles in laying hen diets. Poult. Sci. 90:1960–1966.

Min, Y. N., L. L. Li, S. K. Liu, J. Zhang, Y. P. Gao, and F. Z. Liu. 2015. Effects of dietary distillers dried grains with solubles

(DDGS) on growth performance, oxidative stress, and immune function in broiler chickens. J. Appl. Poult. Res. 24:23–29.
Min, Y. N., L. Li, P. W. Waldroup, Z. Y. Niu, Z. P. Wang, Y. P. Gao, and F. Z. Liu. 2012. Effects of dietary distillers dried grains with solubles concentrations on meat quality and antioxidant status and capacity of broiler chickens. J. Appl. Poult. Res. 21:603–611.
Pineda, L., S. Roberts, B. Kerr, R. Kwakkel, M. Verstegen, and K. Bregendahl. 2008. Maximum dietary content of dried distiller's grains with soluble in diets for laying hens: Effects on nitrogen balance, manure excretion, egg production and egg quality. Iowa State University Animal Industry Report 2008, Iowa State University. http://www.ans.iastate.edu/report/air/.
Rew, H. J., M. H. Shin, H. R. Lee, C. Jo, S. K. Lee, and B. D. Lee. 2009. Effects of corn distillers dried grains with solubles on production performance and economics in laying hens. Kor. J. Poult. Sci. 36:15–21.
Roberson, K. D., J. L. Kalbfleisch, W. Pan, and R. A. Charbeneau. 2005. Effect of corn distiller's dried grains with solubles at various levels on performance of laying hens and egg yolk color. Int. J. Poult. Sci. 4:44–51.
Ruan, D., S. Q. Jiang, Y. J. Hu, F. Y. Ding, Q. L. Fan, F. Chen, J. X. Lin, L. Li, and Y. Wang. 2016. Effects of corn distillers dried grains with solubles on performance, oxidative status, intestinal immunity and meat quality of Chinese Yellow broilers. J. Anim. Physiol. Anim. Nutr. doi: 10.1111/jpn.12637.
Salim, H. M., Z. A. Kruk, and B. D. Lee. 2010. Nutritive value of corn distillers dried grains with solubles as an ingredient of poultry diets: A review. World's Poult. Sci. J. 66:411–432.
SAS Institute Inc. 2004. SAS User's Guide: Statistics. Version 9.1. SAS Institute Inc., Gary, NC.
Schilling, M. W., V. Battula, R. E. Loar, V. Jackson, S Kin, and A. Corzo. 2010. Dietary inclusion level effects of distillers dried grains with solubles on broiler meat quality. Poult. Sci. 89:752–760.
Shi, Z. Z., B. Z. Carter, G. M. Habib, X. He, S. Sazer, R. M. Lebovitz, and M. W. A. Lieberman. 1996. Single mouse glutathione synthetase gene encodes six mRNAs with different 5' ends. Arch. Biochem. Biophys. 331:215–224.
Shin, H. S., J. W. Kim, D. G. Lee, S. Lee, and D. Y. Kil. 2016a. Bioavailability of lutein in corn distillers dried grains with solubles relative to lutein in corn gluten meal based on lutein retention in egg yolk. J. Sci. Food Agric. 96:3401–3406.
Shin, H. S., J. W. Kim, J. H. Kim, D. G. Lee, S. Lee, and D. Y. Kil. 2016b. 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. Poult. Sci. 95:2366–2371.
Song, R., and G. C. Shurson. 2013. Evaluation of lipid peroxidation level in corn dried distillers grains with solubles. J. Anim. Sci. 91:4383–4388.
Song, R., C. Chen, J. Johnston, B. J. Kerr, T. E. Weber, and G. C. Shurson. 2012. Effects of feeding diets containing highly oxidized dried distillers grains with solubles with increasing vitamin E levels to wean-finish pigs on growth performance, carcass characteristics, and pork fat composition. J. Anim. Sci. 90 (Suppl. 3):64 (Abstr).
Sun, H., E. J. Lee, H. Samaraweera, M. Persia, Dong, and U. Ahn. 2012. Effects of increasing concentrations of corn distillers dried grains with solubles on egg production and internal quality of eggs. Poult. Sci. 91:3236–3246.
Sun, H., E. J. Lee, H. Samaraweera, M. Persia, Dong, and U. Ahn. 2013. Effects of increasing concentrations of corn distillers dried grains with solubles on chemical composition and nutrient of egg. Poult. Sci. 92:233–242.
Surai, P. F., E. Blesbois, I. Grasseau, T. Chalah, J.-P. Brillard, G. J. Wishart, S. Cerolini, and N. H. C. Sparks. 1998. Fatty acid composition, glutathione peroxidase, and SOD activity and total antioxidant activity of avian semen. Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol. 120:527–533.
Świątkiewicz, S., and J. Koreleski. 2008. The use of distillers dried grains with solubles (DDGS) in poultry nutrition. World's Poult. Sci. J. 64:257–266.
Świątkiewicz, S., A. Arczewska-Włosek, J. Krawczyk, M. Puchała, and D. Józefiak. 2013. Effects of selected feed additives on the performance of laying hens given a diet rich in maize dried distiller's grains with solubles (DDGS). Br. Poult. Sci. 54:475–485.
Trupia, S., J. K. Winkler-Moser, A. C. Guney, R. Beckstead, and C-Y. O. Chen. 2016. Nutritional quality of eggs from hens fed distillers dried grains with solubles. Poult. Sci. 95:2592–2601.
Wang, J., H. Xu, R. Zuo, K. Mai, W. Xu, and Q. Ai. 2016. Effects of oxidised dietary fish oil and high-dose vitamin E supplementation on growth performance, feed utilisation and antioxidant defence enzyme activities of juvenile large yellow croaker (*Larmichthys crocea*). Br. J. Nutr. 115:1531–1538.
Wu-Haan, W., W. Powers, W. Angel, and T. J. Applegate. 2010. The use of distillers dried grains plus solubles as a feed ingredient on air emissions and performance from laying hens. Poult. Sci. 89:1355–1359.
Yan, X., S. Lee, B. G. Gugiu, L. Koroniak, M. E. Jung, J. Berliner, J. Cheng, and R. Li. 2014. Fatty acid epoxyisoprostane E2 stimulates an oxidative stress response in endothelial cells. Biochem. Biophys. Res. Commun. 444:69–74.
Yuan, C., X. C. Bu, H. X. Yan, J. J. Lu, and X. T. Zou. 2016. Dietary L-arginine levels affect the liver protein turnover and alter the expression of genes related to protein synthesis and proteolysis of laying hens. Poult. Sci. 95:261–267.