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High sulfur content in corn dried distillers grains with solubles protects against oxidized lipids by increasing sulfur-containing antioxidants in nursery pigs[1]

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ABSTRACT: Some sources of corn dried distillers grains with solubles (DDGS) contain relatively high amounts of oxidized lipids produced from PUFA peroxidation during the production process. These oxidized lipids may impair metabolic oxidation status of pigs. The objective of this study was to understand the effects of feeding corn–soybean meal diets (CON) or diets containing 30% highly oxidized DDGS with 1 of 3 levels of supplemental vitamin E (*dl*-α-tocopheryl acetate), none, the 1998 NRC level (11 IU/kg), and 10x the 1998 NRC level (110 IU/kg), on oxidative status of nursery pigs. The DDGS source used in this study contained the greatest thiobarbituric acid reactive substances (TBARS) value, peroxide value, and total S content (5.2 ng/mg oil, 84.1 mEq/kg oil, and 0.95%, respectively) relative to 30 other DDGS sources sampled (mean values = 1.8 ng/mg oil, 11.5 mEq/kg oil, and 0.50%, respectively). Barrows (n = 54) were housed in pens and fed the experimental diets for 8 wk after weaning and transferred to individual metabolism cages for collection of feces, urine, blood, and liver samples. Total S content was greater in DDGS diets than in CON (0.39 vs. 0.19%). Dietary inclusion of 30% DDGS improved apparent total tract digestibility of S (86.8 vs. 84.6%; $P < 0.001$) and S retained (2.94 vs. 2.07 g/d; $P < 0.01$) compared with CON. Although pigs were fed highly oxidized DDGS in this study, serum TBARS were similar between DDGS and CON treatments. There was an interaction between DDGS and dietary vitamin E level for serum concentrations of α-tocopherol. Serum α-tocopherol concentrations were greater ($P < 0.001$) in pigs fed DDGS diets than those fed CON when *dl*-α-tocopheryl acetate was not provided or provided at the NRC level but were similar when *dl*-α-tocopheryl acetate was supplemented at the 10x NRC level. Pigs fed DDGS diets had greater serum concentrations of S-containing AA, particularly Met ($P < 0.001$) and taurine ($P = 0.002$), compared with those fed CON. Liver glutathione concentration was greater in pigs fed DDGS diets than CON (56.3 vs. 41.8 nmol/g). Dietary inclusion of DDGS ($P < 0.001$) and vitamin E ($P = 0.03$) increased enzyme activity of glutathione peroxidase. The elevated concentrations of S-containing antioxidants (Met, taurine, and glutathione) in vivo may protect pigs against oxidative stress when feeding highly oxidized DDGS. Therefore, the increased S content in DDGS may be beneficial, and increasing concentrations of vitamin E in diets may not be necessary to protect pigs against metabolic oxidative stress when feeding high S and highly peroxidized DDGS.

Key words: corn dried distillers grains with solubles, lipid peroxidation, nursery pigs, sulfur-containing antioxidants, vitamin E

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INTRODUCTION

Oxidative damage of lipids in animal feed negatively affects pig health and growth performance (Miller et al., 1993; Pfalzgraf et al., 1995). Lipid peroxidation occurs during the production of corn dried

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distillers grains with solubles (**DDGS**). Corn oil, which is typically present at a concentration of approximately 10% in DDGS, contains high concentrations of PUFA, particularly linoleic acid, that are vulnerable to lipid peroxidation (NRC, 1998). Increased drying time and temperature used by ethanol plants also accelerates lipid peroxidation in DDGS. Furthermore, the total S content in corn DDGS can exceed 1% because of the addition of sulfuric acid during the ethanol production process, and S content in DDGS is highly variable (0.3 to 0.9%, as-fed basis; Kim et al., 2012). Sulfur is an essential component in many physiological functions and is incorporated into AA, proteins, enzymes, and micronutrients (Atmaca, 2004). However, very little is known about the impact of feeding DDGS containing greater concentrations of S on pig health and performance.

It appears that feeding DDGS containing oxidized lipids to pigs may require supplementation of greater concentrations of antioxidants (e.g., vitamin E) than currently being fed. For example, supplementation of additional antioxidants improved growth performance in pigs fed diets containing DDGS or oxidized corn oil (Harrell et al., 2010). However, results from other studies have shown that supplementation of antioxidants had no effect on growth performance in animals under a dietary oxidative stress challenge (Wang et al., 1997b; Anjum et al., 2002; Fernández-Dueñas, 2009). Therefore, the objective of this study was to evaluate the effects of feeding DDGS with a high content of oxidized lipids on pig growth performance and metabolic oxidation status and to determine if any of the negative effects could be overcome by increasing dietary concentration of vitamin E.

MATERIALS AND METHODS

All animal care and use procedures used in this experiment were approved by the University of Minnesota Institutional Animal Care and Use Committee.

Animals and Housing

Weanling terminal crossbred barrows (n = 54; initial BW = 7.0 ± 0.3 kg) of sows (Landrace × Yorkshire; Genetically Advanced Pigs, Winnipeg, MB, Canada) and Duroc boars (Comparts Boar Store, Nicollet, MN) were used in this experiment conducted at the University of Minnesota, Southern Research and Outreach Center (Waseca, MN). This experiment was conducted in 2 replicated groups with 24 pigs used in the first group and 30 pigs used in the second group. Pigs were blocked by initial BW, and pens within the blocks were assigned randomly to 1 of 6 dietary treatments in a 2 × 3 factorial arrangement (4 pens/treatment; 2 to 3 pigs/pen; 9 pigs/treatment). Pigs were fed corn–soybean meal (**CON**) or corn–soybean meal 30% DDGS diets containing 1 of 3 levels of supplemental vitamin E (*dl*-α-tocopheryl acetate): none (**No-E**), the NRC (1998) recommended concentration of vitamin E (**1X-E**), or 10x NRC (**10X-E**). Pigs were offered diets in a 3-phase feeding program with targeted BW of 7 to 11 kg, 11 to 25 kg, and 25 to 50 kg for Phases 1, 2, and 3, respectively. Pigs were group housed in pens (1.2 by 1.2 m) and fed their respective diets for 8 wk after weaning. All pigs were allowed ad libitum access to feed and water and were monitored for health daily. Individual pig BW was measured initially and pen feed disappearance were also measured when dietary phases were changed at the end of wk 2 and 6 as well as at the end of wk 8 to calculate ADG, ADFI, and G:F for this experimental period.

After housing pigs in groups for 8 wk, all pigs were transferred to individual metabolism cages for a 5-d adaptation period followed by a 3-d total collection of feces and urine following the procedure described by Liu et al. (2012) and 1-d collection of blood samples. After the collection of these samples, all pigs were sacrificed and liver samples from each pig were collected. Pigs were fed a daily amount of their respective Phase 3 diets equivalent to 4% of their BW determined on the first day in the metabolism crates (2% fed at 0700 h and 2% fed at 1900 h). The amount of feed provided to animals was recorded at each feeding time. All pigs consumed all of the feed provided at each meal throughout the entire adaptation and collection period. Feeders were located at the front of each metabolism cage, and a nipple waterer was located at the side of the cage to provide ad libitum access to water. Room temperature was maintained at 20 ± 1°C.

Diet Composition and Dried Distillers Grains with Solubles Source

Diet composition and nutrient concentrations of experimental diets for Phase 1 to 3 are presented in Tables 1 to 3. All diets were fed in meal form and were formulated on a standardized ileal digestible (**SID**) AA and available P basis with similar concentrations of ME and Ca. Crystalline Lys (L-Lys HCl) was added at 0.20 and 0.28% in Phase 2 and Phase 3 DDGS diets to provide comparable SID Lys concentration to CON. Nutrient concentrations of the diets met or exceeded NRC (1998) nutrient requirements for pigs with 350 g of fat-free lean gain/day, except for vitamin E concentration in the No-E treatments. Vitamin E was supplemented in the form of *dl*-α-tocopheroyl acetate for 1X-E and 10X-E treatments. The NRC (1998) requirements of vitamin E for BW of 7 to 11 kg (Phase 1), 11 to 25 kg (Phase 2), and 25 to 50 kg (Phase 3) are 13.2, 11.0, and 11.0 IU/kg, respectively. Therefore, these levels and 10x these levels were used as target concentrations of dietary vitamin E when formu-

Table 1. Composition and nutrient analysis of Phase 1 diets (7 to 11 kg BW, as-fed basis)

	CON[1]			DDGS[2]		
Item	No-E[3]	1X-E[3]	10X-E[3]	No-E	1X-E	10X-E
Ingredient, %						
Corn	48.67	48.64	48.37	22.92	22.89	22.62
Soybean meal (46.5% CP)	18.40	18.40	18.40	14.50	14.50	14.50
DDGS	–	–	–	30.00	30.00	30.00
Fish meal, menhaden	10.00	10.00	10.00	10.00	10.00	10.00
Whey powder	20.00	20.00	20.00	20.00	20.00	20.00
Limestone	0.78	0.78	0.78	1.28	1.28	1.28
Dicalcium phosphate	0.83	0.83	0.83	–	–	–
Salt	0.25	0.25	0.25	0.25	0.25	0.25
DL-Met	0.02	0.02	0.02	–	–	–
Vitamin/trace mineral premix[4]	0.50	0.50	0.50	0.50	0.50	0.50
Antibiotic (Mecadox)	0.50	0.50	0.50	0.50	0.50	0.50
Zinc oxide	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin E[5]	–	0.03	0.30	–	0.03	0.30
Calculated nutrient composition						
ME,[6] kcal/kg	3,283	3,282	3,273	3,297	3,295	3,286
NE,[7] kcal/kg	2,214	2,213	2,207	2,138	2,137	2,131
CP, %	21.3	21.3	21.2	25.3	25.3	25.3
Lys, %	1.34	1.34	1.34	1.41	1.41	1.41
Met + Cys, %	0.77	0.77	0.77	0.91	0.91	0.91
Thr, %	0.89	0.89	0.89	1.02	1.02	1.02
Trp, %	0.25	0.25	0.25	0.26	0.26	0.26
SID Lys,[8] %	1.20	1.20	1.20	1.20	1.20	1.20
SID Met + Cys, %	0.68	0.68	0.68	0.77	0.77	0.77
SID Thr, %	0.75	0.75	0.75	0.82	0.82	0.82
SID Trp, %	0.22	0.22	0.22	0.22	0.22	0.22
Ca, %	1.22	1.22	1.22	1.22	1.22	1.22
P, %	0.86	0.86	0.86	0.90	0.90	0.90
Available P, %	0.62	0.62	0.62	0.62	0.62	0.62
α-Tocopherol, IU/kg	0.00	13.2	132	0.00	13.2	132
Analyzed nutrient composition						
CP, %	20.6	20.1	21.9	24.7	25.0	24.6
Crude fat,[9] %	2.54	–	–	3.91	–	–
Crude fiber,[9] %	1.5	–	–	3.0	–	–
Lys, %	1.31	1.33	1.43	1.34	1.40	1.36
Met, %	0.38	0.37	0.41	0.44	0.48	0.47
Thr, %	0.83	0.82	0.88	0.96	1.00	0.99
Trp, %	0.22	0.22	0.25	0.24	0.25	0.25
Ca,[9] %	1.72	–	–	1.40	–	–
P,[9] %	0.99	–	–	0.89	–	–
α-Tocopherol, IU/kg	<10	13	151	<10	14	127

[1]CON = corn–soybean meal based control diet.

[2]DDGS = dried distillers grains with solubles; 30% inclusion of DDGS.

[3]No-E = no supplemental vitamin E; 1X-E = NRC (1998) level of vitamin E supplied as *dl*-α-tocopheryl acetate, which is 13.2 IU/kg for BW of 9 kg; 10X-E = 10x NRC (1998) level of vitamin E supplied as *dl*-α-tocopheryl acetate, which is 132 IU/kg for BW of 9 kg.

[4]Vitamin–trace mineral premix (without vitamin E) provided these nutrients per kilogram of diet: 11,023 IU of vitamin A as retinyl acetate, 2,756 IU of vitamin D_3 as cholecalciferol, 4.41 mg of vitamin K as menadione dimethylpyrimidinol bisulfite, 9.92 mg of riboflavin, 55.11 mg of niacin, 33.07 mg of pantothenic acid as D-calcium pantothenate, 496.03 mg of choline as choline chloride, 0.06 mg of vitamin B_{12}, 2.20 mg of pyridoxine, 1.65 mg of folic acid, 1.10 mg of thiamine, 0.22 mg of biotin, 2.20 mg of I as ethylenediamine dihydroiodide, 0.30 mg of Se as sodium selenite, 90.39 mg of Zn as zinc oxide (SQM; Actifeed, Saint Malo, France), 55.11 mg of Fe as ferrous sulfate (SQM; Actifeed), 5.51 mg of Cu as copper sulfate (SQM; Actifeed), and 17.64 mg of Mn as manganese oxide (SQM; Actifeed).

[5]Vitamin E was supplied as *dl*-α-tocopheryl acetate with concentration of 44,092 IU/kg.

[6]ME values were calculated using NRC (1998) values for corn and soybean meal and 3,416 kcal/kg for DDGS, which was the average value of 10 DDGS samples analyzed by Pedersen et al. (2007).

[7]NE values were calculated using NRC (1998) values for corn, soybean meal, and DDGS.

[8]SID = standardized ileal digestible.

[9]Nutrient concentrations were only analyzed in No-E/CON and No-E/DDGS treatments because values were expected to be similar to those in corresponding 1X-E and 10X-E treatments.

Table 2. Composition and nutrient analysis of Phase 2 diets (11 to 25 kg BW, as-fed basis)

	CON[1]			DDGS[2]		
Item	No-E[3]	1X-E[3]	10X-E[3]	No-E	1X-E	10X-E
Ingredient, %						
Corn	65.97	65.94	65.72	44.13	44.10	43.88
Soybean meal (46.5% CP)	31.00	31.00	31.00	23.00	23.00	23.00
DDGS	–	–	–	30.00	30.00	30.00
Limestone	0.78	0.78	0.78	1.30	1.30	1.30
Dicalcium phosphate	1.33	1.33	1.33	0.52	0.52	0.52
Salt	0.35	0.35	0.35	0.35	0.35	0.35
L-Lys HCl	0.08	0.08	0.08	0.20	0.20	0.20
Vitamin/trace mineral premix[4]	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin E[5]	–	0.025	0.25	–	0.025	0.25
Calculated nutrient composition						
ME,[6] kcal/kg	3,293	3,292	3,284	3,303	3,302	3,294
NE,[7] kcal/kg	2,206	2,205	2,200	2,141	2,140	2,135
CP, %	19.9	19.9	19.9	22.5	22.5	22.5
Lys, %	1.17	1.17	1.17	1.22	1.22	1.22
Met + Cys, %	0.67	0.67	0.67	0.79	0.79	0.79
Thr, %	0.76	0.76	0.76	0.84	0.84	0.83
Trp, %	0.24	0.24	0.24	0.23	0.23	0.23
SID Lys,[8] %	1.01	1.01	1.01	1.01	1.01	1.01
SID Met + Cys, %	0.58	0.58	0.58	0.65	0.65	0.65
SID Thr, %	0.64	0.64	0.64	0.66	0.66	0.66
SID Trp, %	0.21	0.21	0.21	0.19	0.19	0.19
Ca, %	0.70	0.70	0.70	0.70	0.70	0.70
P, %	0.64	0.64	0.64	0.66	0.66	0.66
Available P, %	0.32	0.32	0.32	0.32	0.32	0.32
α-Tocopherol, IU/kg	0.00	11.0	110	0.00	11.0	110
Analyzed nutrient composition						
CP, %	19.3	19.0	18.6	22.2	21.5	20.7
Crude fat,[9] %	2.68	–	–	3.70	–	–
Crude fiber,[9] %	2.3	–	–	3.6	–	–
Lys, %	1.21	1.25	1.07	1.23	1.16	1.20
Met, %	0.27	0.27	0.27	0.37	0.34	0.35
Thr, %	0.75	0.76	0.67	0.87	0.83	0.82
Trp, %	0.24	0.22	0.20	0.22	0.22	0.21
Ca,[9] %	0.77	–	–	0.98	–	–
P,[9] %	0.64	–	–	0.66	–	–
α-Tocopherol, IU/kg	<10	14	124	<10	13	118

[1]CON = corn–soybean meal based control diet.

[2]DDGS = dried distillers grains with solubles; 30% inclusion of DDGS.

[3]No-E = no supplemental vitamin E; 1X-E = NRC (1998) level of vitamin E supplied as *dl*-α-tocopheryl acetate, which is 11.0 IU/kg for BW of 18 kg; 10X-E = 10x NRC (1998) level of vitamin E supplied as *dl*-α-tocopheryl acetate, which is 110 IU/kg for BW of 18 kg.

[4]Vitamin–trace mineral premix (without vitamin E) provided these nutrients per kilogram of diet: 11,023 IU of vitamin A as retinyl acetate, 2756 IU of vitamin D_3 as cholecalciferol, 4.41 mg of vitamin K as menadione dimethylpyrimidinol bisulfite, 9.92 mg of riboflavin, 55.11 mg of niacin, 33.07 mg of pantothenic acid as D-calcium pantothenate, 496.03 mg of choline as choline chloride, 0.06 mg of vitamin B_{12}, 2.20 mg of pyridoxine, 1.65 mg of folic acid, 1.10 mg of thiamine, 0.22 mg of biotin, 2.20 mg of I as ethylenediamine dihydroiodide, 0.30 mg of Se as sodium selenite, 90.39 mg of Zn as zinc oxide (SQM; Actifeed, Saint Malo, France), 55.11 mg of Fe as ferrous sulfate (SQM; Actifeed), 5.51 mg of Cu as copper sulfate (SQM; Actifeed), and 17.64 mg of Mn as manganese oxide (SQM; Actifeed).

[5]Vitamin E was supplied as *dl*-α-tocopheryl acetate with concentration of 44,092 IU/kg.

[6]ME values were calculated using NRC (1998) values for corn and soybean meal and 3,416 kcal/kg for DDGS, which was the average value of 10 DDGS samples analyzed by Pedersen et al. (2007).

[7]NE values were calculated using NRC (1998) values for corn, soybean meal, and DDGS.

[8]SID = standardized ileal digestible.

[9]Nutrient concentrations were only analyzed in No-E/CON and No-E/DDGS treatments because values were expected to be similar to those in corresponding 1X-E and 10X-E treatments.

Table 3. Composition and nutrient analysis of Phase 3 diets (25 to 50 kg BW, as-fed basis)

	CON[1]			DDGS[2]		
Item	No-E[3]	1X-E[3]	10X-E[3]	No-E	1X-E	10X-E
Ingredient, %						
Corn	71.43	71.40	71.18	51.74	51.71	51.49
Soybean meal (46.5% CP)	26.00	26.00	26.00	15.75	15.75	15.75
DDGS	–	–	–	30.00	30.00	30.00
Limestone	0.76	0.76	0.76	1.28	1.28	1.28
Dicalcium phosphate	0.88	0.88	0.88	0.08	0.08	0.08
Salt	0.35	0.35	0.35	0.35	0.35	0.35
L-Lys HCl	0.08	0.08	0.08	0.28	0.28	0.28
L-Trp	–	–	–	0.02	0.02	0.02
Vitamin/trace mineral premix[4]	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin E[5]	–	0.025	0.25	–	0.025	0.250
Calculated nutrient composition						
ME,[6] kcal/kg	3,312	3,312	3,304	3,321	3,320	3,312
NE,[7] kcal/kg	2,236	2,235	2,230	2,177	2,176	2,171
CP, %	18.1	18.1	18.1	19.8	19.8	19.8
Lys, %	1.03	1.03	1.03	1.08	1.08	1.08
Met + Cys, %	0.62	0.62	0.62	0.71	0.71	0.71
Thr, %	0.69	0.69	0.69	0.72	0.72	0.72
Trp, %	0.21	0.21	0.21	0.20	0.20	0.20
SID Lys,[8] %	0.89	0.89	0.89	0.89	0.89	0.89
SID Met + Cys, %	0.54	0.54	0.54	0.59	0.59	0.59
SID Thr, %	0.57	0.57	0.57	0.57	0.57	0.57
SID Trp, %	0.18	0.18	0.18	0.17	0.17	0.17
Ca, %	0.58	0.58	0.58	0.58	0.58	0.58
P, %	0.54	0.54	0.54	0.56	0.56	0.55
Available P, %	0.23	0.23	0.23	0.23	0.23	0.23
α-Tocopherol, IU/kg	0.00	11.0	110	0.00	11.0	110
Analyzed nutrient composition						
CP, %	17.8	17.2	17.0	18.3	18.3	17.8
Crude fat,[9] %	2.23	–	–	3.71	–	–
Crude fiber,[9] %	2.6	–	–	3.6	–	–
Lys, %	1.12	1.10	1.04	1.07	0.99	1.03
Met, %	0.26	0.25	0.24	0.32	0.31	0.30
Thr, %	0.70	0.69	0.67	0.73	0.68	0.67
Trp, %	0.20	0.23	0.23	0.21	0.20	0.20
Ca,[9] %	0.76	–	–	0.76	–	–
P,[9] %	0.62	–	–	0.56	–	–
α-Tocopherol, IU/kg	<10	13	117	<10	14	105

[1]CON = corn–soybean meal based control diet.

[2]DDGS = dried distillers grains with solubles; 30% inclusion of DDGS.

[3]No-E = no supplemental vitamin E; 1X-E = NRC (1998) level of vitamin E supplied as *dl*-α-tocopheryl acetate, which is 11.0 IU/kg for BW of 37 kg; 10X-E = 10x NRC (1998) level of vitamin E supplied as *dl*-α-tocopheryl acetate, which is 110 IU/kg for BW of 37 kg.

[4]Vitamin–trace mineral premix (without vitamin E) provided these nutrients per kilogram of diet: 11,023 IU of vitamin A as retinyl acetate, 2,756 IU of vitamin D_3 as cholecalciferol, 4.41 mg of vitamin K as menadione dimethylpyrimidinol bisulfite, 9.92 mg of riboflavin, 55.11 mg of niacin, 33.07 mg of pantothenic acid as D-calcium pantothenate, 496.03 mg of choline as choline chloride, 0.06 mg of vitamin B_{12}, 2.20 mg of pyridoxine, 1.65 mg of folic acid, 1.10 mg of thiamine, 0.22 mg of biotin, 2.20 mg of I as ethylenediamine dihydroiodide, 0.30 mg of Se as sodium selenite, 90.39 mg of Zn as zinc oxide (SQM; Actifeed, Saint Malo, France), 55.11 mg of Fe as ferrous sulfate (SQM; Actifeed), 5.51 mg of Cu as copper sulfate (SQM; Actifeed), and 17.64 mg of Mn as manganese oxide (SQM; Actifeed).

[5]Vitamin E was supplied as *dl*-α-tocopheryl acetate with concentration of 44,092 IU/kg.

[6]ME values were calculated using NRC (1998) values for corn and soybean meal and 3,416 kcal/kg for DDGS, which was the average value of 10 DDGS samples analyzed by Pedersen et al. (2007).

[7]NE values were calculated using NRC (1998) values for corn, soybean meal, and DDGS.

[8]SID = standardized ileal digestible.

[9]Nutrient concentrations were only analyzed in No-E/CON and No-E/DDGS treatments because values were expected to be similar to those in corresponding 1X-E and 10X-E treatments.

Table 4. Analyzed nutrient composition of dried distillers grains with solubles source used (as-fed basis)

Item	%
DM	91.3
CP	26.6
Crude fat	9.7
Crude fiber	7.7
ADF	12.5
NDF	25.0
Ash	5.3
P	0.97
Ca	0.03
S	0.95
Indispensable AA	
Arg	1.19
His	0.69
Ile	1.00
Leu	2.86
Lys	0.85
Met	0.50
Phe	1.08
Thr	0.94
Trp	0.17
Val	1.34
Dispensable AA	
Ala	1.68
Asp	1.57
Cys	0.51
Glu	3.17
Gly	1.01
Pro	1.77
Ser	1.00
Tyr	0.93

lating the 1X-E and 10X-E diets, respectively. The actual concentration of α-tocopherol in each experimental diet was analyzed by Michigan State University Diagnostic Center for Population & Animal Health (Lansing, MI) using ethanol and hexane extraction followed by quantification via HPLC (Separation Module 2690, Waters, Milford, MA). The highly oxidized DDGS source used in this study was selected out of 31 corn DDGS sources produced by U.S. ethanol plants (Song et al., 2011; Table 4). This DDGS source contained the greatest thiobarbituric acid reactive substances (**TBARS**) value, peroxide value (**PV**), and total S content (5.2 ng/mg oil, 84.1 mEq/kg oil, and 0.95%, respectively) relative to the other 30 DDGS sources sampled (mean values = 1.8 ng/mg oil, 11.5 mEq/kg oil, and 0.50%, respectively).

Sample Collection

Total feces and urine from each pig were collected twice (0700 and 1900 h) daily and stored at –20°C. Fecal samples from each pig were pooled, weighed, and dried in a forced-draft oven at 55 to 60°C, and subsamples were obtained for further analysis of S content. At the same time as the fecal collection, total urine output was collected from each pig using plastic containers located under funnels beneath the metabolism cages. Thirty milliliters of 6 *N* HCl were added to the collection containers to limit microbial growth and to reduce loss of ammonia. Total urine volume was recorded and a subsample of approximately 20% of the urine excreted from each pig was collected and stored at –20°C until analysis of S content was conducted.

Blood samples (approximately 8 mL) were collected 1 h after feeding at 0700 h from all pigs in the metabolism crates using serum separation tubes coated with silicone and micronized silica particles (BD SST* brand, Franklin Lakes, NJ). Blood samples were stored at 4°C overnight before centrifugation at 2,000 × *g* for 20 min at room temperature. Serum was then removed and stored at –20°C until analyses of TBARS, α-tocopherol, AA profile, and glutathione peroxidase (**GPx**) were performed.

Liver samples (approximately 50 g) were collected after pigs were euthanized using a captive bolt pistol on the last day of the study. Liver samples were frozen immediately on dry ice and stored at –80°C for glutathione (**GSH**) analysis.

Thiobarbituric Acid Reactive Substance Assay in Serum

To evaluate the metabolic status in vivo, a serum TBARS assay was performed (version 1; AMDCC, 2005). Generally, 100 μL serum samples and standards of malonaldehyde (catalog number AC14861-1000; Fisher Scientific, Pittsburgh, PA) were mixed with 200 μL ice-cold 10% trichloroacetic acid (Sigma-Aldrich, St. Louis, MO) and centrifuged at 2,200 × *g* for 15 min at 4°C. Two hundred microliters of supernatant were removed and incubated with an equal volume of 0.67% (wt/vol) thiobarbituric acid (Sigma-Aldrich) for 10 min in a boiling water bath. The mixture was cooled to room temperature and the absorbance was read at 532 nm using a spectrometer (SpectraMax 250; Molecular Device, Sunnyvale, CA). This assay was conducted in 4 batches with duplicate samples and a standard. The intra-assay CV was 6.7% and the interassay CV was 5.2%.

Analysis of α-Tocopherol Concentration in Serum

Analysis of α-tocopherol concentration in serum was conducted by Michigan State University Diagnostic Center for Population & Animal Health. Briefly, serum samples were mixed with equal volumes of ethanol and hexane. Mixtures were centrifuged and a known aliquot of hexane was removed and then dried under vacuum. The samples were dissolved in chromatographic mobile

phase and analyzed by HPLC (Separation Module 2690; Waters).

Analysis of Sulfur-Containing Compounds

Hepatic Glutathione. Glutathione concentration in liver was analyzed using a commercial GSH assay kit (catalog number CS0260; Sigma-Aldrich). Fifty milligrams of each liver sample were extracted by homogenizing in 500 μL of 5% 5-sulfosalicylic acid followed by centrifugation at 10,000 × *g* for 10 min at 4°C. Ten microliters of supernatant from each sample were used for GSH measurement following the manufacturer's instructions. Each sample and standard were analyzed in duplicate. This assay was conducted in 1 batch, with the intra-assay CV of 3.2%.

Serum Glutathione Peroxidase Activity. Enzyme activity of GPx in the serum was determined using a commercial GPx Assay kit (catalog number 703102; Cayman Chemical, Ann Arbor, MI). Briefly, indirect GPx activity was measured by a coupled reaction with GSH reductase. The reaction was initiated after cumene hydroperoxide addition. Oxidation of reduced nicotinamide adenine dinucleotide phosphate (**NADPH**) to nicotinamide adenine dinucleotide phosphate (**NADP**) was measured colorimetrically using a spectrometer (SpectraMax 250; Molecular Device) at 340 nm for at least 5 min. Glutathione peroxidase activity was expressed as micromoles NADPH oxidized per minute per milliliter of serum of protein and compared with a bovine erythrocyte GPx standard curve over time. Each sample and standard were analyzed in duplicate. This assay was conducted in 1 batch with the intra-assay CV of 5.9%.

Serum Sulfur-Containing AA. Serum concentrations of Met, Cys, and taurine were determined by liquid chromatography–mass spectrometry (**LC-MS**) using a modified method based on Márquez et al. (1986). Generally, each serum sample and standard was prepared with 100 μ*M* p-chlorol-L-phenylalanine as the internal standard. Five microliters of each sample and standard were mixed with 40 μL Na_2CO_3 (10 m*M* and pH 11) and 100 μL dansyl chloride (3 mg/mL in acetone). The mixture was incubated in a water bath at 60°C for 10 min followed by centrifugation at 10,000 × *g* for 10 min at 4°C. The top supernatant was transferred to a high recovery vial and 5 μL was injected into the LC-MS system for analysis.

Analysis of S Content in Feed, Feces, and Urine

Feed samples (50 g) of each diet in Phase 3 were sent to the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO) for analysis of total S content and sulfur-containing AA (**SAA**), including Met, Cys, and taurine, to determine the organic and inorganic S content. The organic S content in the feed was calculated as sum of the S content from Met, Cys, and taurine using this equation:

$$\begin{aligned}\text{organic S in feed (\%)} = {} & \text{Met in feed (\%)} \\ & \times \text{S in Met (\%)} \\ & + \text{Cys in feed (\%)} \\ & \times \text{S in Cys (\%)} \\ & + \text{taurine in feed (\%)} \\ & \times \text{S in taurine (\%)},\end{aligned}$$

in which S in Met (%) = [32 (atomic weight of S)/149 (molecular weight of Met)] × 100 = 21%, S in Cys (%) = [32 (atomic weight of S)/121 (molecular weight of Cys)] × 100 = 26%, and S in taurine (%) =[32 (atomic weight of S/125 (molecular weight of taurine)] × 100 = 26%.

The inorganic S content in the feed was then estimated by subtracting organic S content from the total S content in each diet. Total S concentration in feces and urine was determined using a combustion method described by Greweling et al. (1972) to calculate S daily balance, and apparent total tract digestibility (**ATTD**) of S in each diet was calculated according to this equation:

$$\text{ATTD (\%)} = [(\text{St} - \text{Sf})/\text{St}] \times 100\%,$$

in which **St** = the total consumption of S (g) in 3 consecutive days and **Sf** = the total fecal excretion of S (g) in 3 consecutive days.

Statistical Analysis

All data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) to evaluate the main effects of DDGS, 3 dietary concentrations of vitamin E, and any 2-way interactions. Analysis of variance was conducted as a 2 × 3 factorial arrangement. The pen was used as the experimental unit for growth performance responses, and the individual pig served as the experimental unit for all other responses. The statistical model included the fixed effects of DDGS, vitamin E concentration, and DDGS × vitamin E interactions as well as random effects of block and group. All results are reported as least squares means. Multiple comparisons among treatments were performed using the Tukey adjustment option of SAS. The significance level chosen was $\alpha = 0.05$. Treatment effects were considered significant if $P < 0.05$ whereas values between $0.05 \leq P \leq 0.10$ were considered statistical trends.

Table 5. Growth performance of pigs fed corn dried distillers grains with solubles (DDGS) and increasing levels of vitamin E (Vit E)[1]

	CON[2]			DDGS[3]				*P*-value[4]	
Item	No-E[5]	1X-E[5]	10X-E[5]	No-E	1X-E	10X-E	SE	Vit E	DDGS
Phase 1									
ADG, kg	0.18	0.21	0.25	0.18	0.17	0.19	0.02	0.40	0.14
ADFI, kg	0.27	0.30	0.33	0.28	0.24	0.29	0.03	0.36	0.17
G:F, kg/kg	0.66	0.71	0.74	0.64	0.72	0.65	0.06	0.52	0.51
Phase 2									
ADG, kg	0.59	0.62	0.63	0.58	0.58	0.58	0.03	0.83	0.28
ADFI, kg	1.02	1.13	1.19	1.13	1.08	1.12	0.05	0.57	0.93
G:F, kg/kg	0.58	0.55	0.53	0.52	0.55	0.52	0.03	0.68	0.40
Phase 3									
ADG, kg	0.88	0.83	0.86	0.81	0.83	0.80	0.07	0.98	0.44
ADFI, kg	1.80	1.80	1.94	1.74	1.82	1.91	0.08	0.49	0.82
G:F, kg/kg	0.49	0.45	0.44	0.46	0.45	0.42	0.02	0.07	0.37
Overall[6]									
Initial BW, kg	6.9	6.9	6.9	7.0	6.9	7.0	0.4	0.69	1.00
Final BW, kg	42.0	41.3	43.8	39.9	39.9	40.0	1.5	0.67	0.05
ADG, kg	0.56	0.57	0.59	0.54	0.54	0.54	0.03	0.83	0.12
ADFI, kg	0.99	1.10	1.16	1.07	1.05	1.11	0.05	0.16	0.89
G:F, kg/kg	0.57	0.52	0.51	0.50	0.51	0.49	0.02	0.26	0.14

[1]Values are least square means of 4 replicate pens per dietary treatment.

[2]CON = corn–soybean based control diet.

[3]30% inclusion of DDGS.

[4]No interactions between DDGS and vitamin E were observed for any of the growth performance responses in this table ($P > 0.34$).

[5]No-E = no supplemental of vitamin E; 1X-E = NRC (1998) level of vitamin E; 10X-E = 10x NRC (1998) level of vitamin E. Vitamin E was supplied as *dl*-α-tocopheryl acetate.

[6]Initial BW = at weaning; final BW = 8 wk after weaning.

RESULTS

Growth Performance

No interactions between DDGS and vitamin E were observed for any of the growth performance responses during each phase or the overall feeding period (Table 5). Vitamin E did not affect final BW, overall ADG, ADFI, or G:F. Pigs fed DDGS had a lower final BW at 8 wk post-weaning than pigs fed CON (42.4 vs. 40.0 kg, respectively; $P = 0.05$), but overall ADG, ADFI, and G:F were not affected in pigs fed DDGS compared with those fed CON.

Metabolic Oxidation Status

There were no effects of DDGS, vitamin E supplementation level, or DDGS × vitamin E concentration on TBARS values in serum (Table 6). An effect of DDGS × vitamin E concentration was detected ($P < 0.001$) for serum α-tocopherol concentration. Specifically, pigs fed DDGS with No-E and 1X-E had a greater concentration ($P < 0.001$) of serum α-tocopherol concentration compared with those fed CON with No-E and 1X-E (1.61 vs. 0.69 μg/mL). However, when vitamin E supplementation was increased to 10x NRC level, serum α-tocopherol concentration was similar for pigs fed DDGS and CON diets. Serum α-tocopherol concentration was greater in pigs fed CON/10X-E than those fed CON/1X-E, which was greater than those fed CON/No-E (3.32 vs. 0.95 vs. 0.42 μg/mL, respectively; $P < 0.001$). However, in pigs fed DDGS, serum α-tocopherol concentration was greater ($P < 0.001$) in pigs fed 10X-E than those fed 1X-E and No-E (3.54 vs. 1.61 and 1.60 μg/mL) but similar for pigs fed 1X-E and No-E diets. These responses also reflect the DDGS × dietary vitamin E concentration interaction observed.

Sulfur-Containing Antioxidants

Sulfur-containing antioxidants, including Met, Cys, taurine, GSH, and GPx activity, were evaluated in vivo in this study (Table 7). The Cys concentration in the serum was not detectable due to the low concentration or poor derivation by dansyl chloride reagents that were used in this study. No effects of DDGS × vitamin E concentration were detected for any of the S-containing antioxidants measured. Pigs fed DDGS had greater concentrations of Met and taurine in serum compared with those fed CON (70.8 vs. 45.8 and 197.3 vs. 143.7 μ*M*, respectively). No effect of vitamin E supplementation level was observed for serum SAA concentrations. Liver GSH concentration

Table 6. Influence of corn dried distillers grains with solubles (DDGS) and vitamin E (Vit E) supplementation on thiobarbituric acid reactive substance (TBARS) and α-tocopherol concentration in serum[1]

	CON[2]			DDGS[3]				*P*-value		
Item	No-E[4]	1X-E[4]	10X-E[4]	No-E	1X-E	10X-E	SE	Vit E	DDGS	Vit E × DDGS
TBARS, μ*M*	3.69	3.54	3.68	3.72	3.63	3.56	0.08	0.23	0.95	0.27
α-Tocopherol, μg/mL	0.42	0.95	3.32	1.60	1.61	3.54	0.11	<0.001	<0.001	<0.001

[1]Values are least square means of 9 replicate pigs per dietary treatment.

[2]CON = corn–soybean based control diet.

[3]30% inclusion of DDGS.

[4]No-E = no supplemental of vitamin E; 1X-E = NRC (1998) level of vitamin E; 10X-E = 10x NRC (1998) level of vitamin E. Vitamin E was supplied as *dl*-α-tocopheryl acetate.

was greater in pigs fed DDGS than CON (56.3 vs. 41.8 nmol/g; $P < 0.001$). Dietary supplementation of vitamin E increased ($P = 0.01$) liver GSH concentration in both DDGS and CON treated pigs. Similar to GSH, pigs fed DDGS had a greater serum GPx activity compared with those fed CON (1.25 vs. 1.00 units/mL; $P < 0.001$). Serum GPx activity increased when supplemental vitamin E levels increased ($P = 0.03$).

Sulfur Content and Digestibility

Total S content in DDGS containing diets was 2 times greater than that in CON (0.39 vs. 0.19%; Table 8), which was largely contributed by greater inorganic S content in DDGS diets than CON (0.23 vs. 0.16%), whereas the organic S content was similar between DDGS and CON diets (0.15 vs. 0.13%). As a result of greater total S content in DDGS diets, with similar feed intake, daily S intake was almost 2 times greater in pigs fed DDGS than those fed CON (5.7 vs. 3.0 g/d; $P < 0.001$; Table 8). Daily S excretion in feces and urine was greater, and more S was absorbed and retained in pigs fed DDGS compared with CON ($P < 0.001$). The ATTD of S was improved when DDGS was included in the diets compared with the CON (86.8 vs. 84.6%; $P < 0.001$), which is presumably due to a greater amount of inorganic S in DDGS. However, there was no effect of vitamin E concentration or interaction between DDGS and vitamin E supplementation level on daily S balance or ATTD of S. It should be noted that fecal samples from 1 pig from the No-E/CON treatment, 2 pigs from the 10X-E/CON treatment, and 1 pig from the No-E/DDGS treatment were lost during storage. As a result, all S balance data from these pigs were discarded and only pigs with complete feces and urine data were included in the results presented in Table 8.

DISCUSSION

The use of corn co-products, such as DDGS, in swine feeds has increased dramatically in recent years because of increased availability and cost competitiveness compared with corn and soybean meal. With DDGS, limits on dietary inclusion rates often occur because pig performance and pork quality decline when high dietary levels of DDGS (30 to 40%) are fed to growing–finishing pigs (Xu et al., 2010). The reduction in growth performance sometimes observed when feeding high dietary levels of DDGS could be potentially caused by antinutritional factors, toxins, low NE concentration, poor AA digestibility,

Table 7. Sulfur-containing antioxidants in pigs fed corn dried distillers grains with solubles (DDGS) and increasing levels of vitamin E (Vit E)[1]

	CON[2]			DDGS[3]				*P*-value		
Item	No-E[4]	1X-E[4]	10X-E[4]	No-E	1X-E	10X-E	SE	Vit E	DDGS	Vit E × DDGS
Serum sulfur AA, μ*M*										
Met	53.7	41.1	42.4	73.6	77.1	61.7	4.8	0.12	<0.001	0.16
Taurine	138.4	135.9	156.3	194.3	206.8	190.2	19.4	0.93	0.002	0.63
Liver glutathione, nmol/g	35.3	44.6	45.7	50.2	56.4	62.3	4.9	0.01	<0.001	0.78
Serum GPx activity,[5] units/mL	0.95	0.92	1.13	1.15	1.30	1.30	0.06	0.03	<0.001	0.17

[1]Values are least square means of nine replicate pigs per dietary treatment.

[2]CON = corn–soybean meal based control diet.

[3]30% inclusion of DDGS.

[4]No-E = no supplemental of vitamin E; 1X-E = NRC (1998) level of vitamin E; 10X-E = 10x NRC (1998) level of vitamin E. Vitamin E was supplied as *dl*-α-tocopheryl acetate.

[5]GPx = glutathione peroxidase, and 1 unit of activity equals 1 μmol reduced nicotinamide adenine dinucleotide phosphate oxidized per min/mL serum.

Table 8. Sulfur content, daily S balance, and apparent total tract digestibility (ATTD) in experimental diets (as-fed basis)

	CON[1]			DDGS[2]				*P*-value		
Item	No-E[3]	1X-E[3]	10X-E[3]	No-E	1X-E	10X-E	SE	Vit E[4]	DDGS	Vit E × DDGS
Dietary S content[5]										
Organic S, %	0.13	0.13	0.12	0.15	0.15	0.14	–	–	–	–
Inorganic S, %	0.06	0.06	0.07	0.22	0.23	0.26	–	–	–	–
Total S, %	0.19	0.19	0.19	0.37	0.38	0.40	–	–	–	–
Daily S balance[6]										
No. of pigs	8	9	7	8	9	9				
S intake, g	3.13	2.90	3.19	5.57	5.64	5.95	0.21	0.32	<0.001	0.69
S in feces, g	0.50	0.46	0.48	0.70	0.79	0.76	0.06	0.79	<0.001	0.31
S in urine, g	0.65	0.40	0.09	1.97	1.95	1.88	0.26	0.14	<0.001	0.21
S absorbed, g	2.63	2.44	2.70	4.86	4.84	5.19	0.18	0.23	<0.001	0.78
S retained, g	1.90	1.89	2.42	2.78	2.84	3.20	0.28	0.20	0.001	0.95
ATTD S, %	84.4	84.0	85.5	87.3	85.9	87.3	0.85	0.12	<0.001	0.66

[1]CON = corn–soybean meal based control diet.

[2]DDGS = dried distillers grains with solubles; 30% inclusion of DDGS.

[3]No-E = no supplemental of vitamin E; 1X-E = NRC (1998) level of vitamin E; 10X-E = 10x NRC (1998) level of vitamin E. Vitamin E was supplied as *dl*-α-tocopheryl acetate.

[4]Vit E = vitamin E.

[5]Values are from Phase 3 experimental diets.

[6]Values are least square means.

or peroxidized lipid or all of these (Stein and Shurson, 2009). Corn DDGS contains approximately 10% corn oil. Corn oil contains high concentrations of PUFA (particularly linoleic acid; NRC, 1998) that are vulnerable to lipid peroxidation, which is a free-radical chain reaction that produces oxidized lipids and a series of toxic aldehydes (Blokhina et al., 2003). In addition, drying temperatures used by ethanol plants vary substantially, and increased drying time and temperature during the production process of DDGS may accelerate lipid peroxidation by oxidizing unsaturated lipids in DDGS. In the present study, a DDGS source with high level of lipid peroxidation was selected according to a recent study conducted in our laboratory (Song et al., 2011). In this study, 2 commonly used indicators of lipid peroxidation, TBARS and PV, were measured to evaluate the lipid peroxidation level in DDGS samples obtained from 31 ethanol plants in the United States. The TBARS values for DDGS samples ranged from 1.0 to 5.2 ng MDA/mg oil, and PV ranged from 4.2 to 84.1 mEq/kg oil, which indicates that lipid peroxidation varies among DDGS sources. The DDGS source with the greatest TBARS and PV values was 25 and 27 times greater, respectively, than the concentrations in a corn reference sample (0.2 ng MDA/mg oil and 3.1 mEq/kg oil, respectively). The DDGS source with the greatest amount of oxidized lipids was used in the current study to evaluate effects of oxidized lipids in DDGS on pig growth performance and metabolic oxidation status. It should be noted that we did not compare this highly oxidized DDGS source to a low oxidized DDGS source in the present study. However, the CON used in the current study was used as a standard reference point for typical U.S. swine diets, and therefore, all the comparisons are relative to responses when feeding a corn–soybean meal diet.

In the present study, pigs fed diets containing highly oxidized DDGS had reduced final BW compared with those fed CON, regardless of dietary concentrations of vitamin E. However, without the comparison with a low oxidized DDGS source, it is difficult to determine if the depressed growth performance was due to oxidized lipids or other factors, such as low NE content or overestimation of Lys digestibility in DDGS. Growth suppression from oxidized lipids has been well documented in several different animal species (Dibner et al., 1996; DeRouchey et al., 2004; Harrell et al., 2010). The presence of greater amounts of oxidized lipids in the diet raises the concentrations of free radicals, aldehydes, and other oxidized metabolites that are toxic to animals. These secondary lipid peroxidation products are highly reactive and potentially cause damage to lipids, proteins, and nucleic acids and, thus, impair animal health and growth performance (Logani and Davies, 1980; Comporti, 1993). Reduced BW has been reported in pigs fed oxidized corn oil (Fernández-Dueñas, 2009; Harrell et al., 2010) and in chickens fed heated sunflower oil (Sheehy et al., 1994), oxidized rapeseed–soybean oil (Engberg et al., 1996), and oxidized poultry fat (Dibner et al., 1996). However, some other studies reported no differences in growth rate and feed intake when diets contained oxidized lipids for poultry and swine (Sheehy et al., 1994; Mitchaothai et al., 2007; Fernández-Dueñas et al., 2008). The lack of

negative effects on animal performance reported in these studies may be due to insufficient dietary oxidative challenge as measured by PV in oil or fat or final diet. There seems to be a threshold for rancidity above which growth performance is decreased. DeRouchey et al. (2004) suggested that a PV of oxidized lipids (6% dietary inclusion rate) less than 40 mEq/kg, which is approximately equal to a PV of the diet less than 2.4 mEq/kg (2.4 mEq/kg = 40 mEq/kg × 6%), might not result in decreased growth performance in nursery pigs. The highly oxidized DDGS source used in the current study contained 9.66% crude fat and a PV of 84.1 mEq/kg oil. Therefore, by including 30% DDGS in the diet, the PV of the diet could be calculated using this equation: PV of the diet (mEq/kg) = 84.1 mEq/kg oil × 9.66% crude fat × 30% inclusion rate = 2.4 mEq/kg, which is at the threshold concentration suggested by DeRouchey et al. (2004). However, it should be pointed out that using PV as the only indicator of lipid peroxidation may not be accurate or sufficient. Peroxide value is a measurement of hydroperoxides. However, the hydroperoxides generated by lipid peroxidation begin to decompose as soon as they are formed, and the breakdown of hydroperoxide by cleavage yields a variety of smaller molecular weight compounds, such as aldehydes, ketones, alcohols, esters, hydrocarbons, and aromatic compounds (Gray, 1978). Therefore, a low PV could be due to either minimal oxidation or decomposition of hydroperoxides that has already begun.

One objective of the present study was to investigate if any of the negative effects of feeding DDGS containing oxidized lipids could be overcome or alleviated by increasing the concentration of dietary vitamin E. However, we did not observe a beneficial effect of vitamin E. This result agrees with results reported by Fernández-Dueñas (2009) that showed supplementation of a synthetic antioxidant did not increase ADG, ADFI, and G:F of finishing pigs fed 5% oxidized corn oil. Similarly in previous poultry studies, dietary ethoxyquin failed to improve growth rate and feed consumption of broilers fed oxidized oil (Wang et al., 1997b; Anjum et al., 2002). The lack of response to vitamin E supplementation in the present study may be due to limited dietary oxidative challenge or the protective effects from other antioxidants in animals fed DDGS. Therefore, it appears that the concentration of natural vitamin E present in our diets, without additional vitamin E supplementation, was sufficient to protect pigs against the negative effects of oxidized lipids from DDGS.

Serum concentrations of α-tocopherol and TBARS were determined in this study to evaluate the metabolic oxidation status of pigs. Interestingly, pigs fed DDGS exhibited a greater concentration of serum α-tocopherol than those fed CON whereas the TBARS value was not different in pigs fed DDGS or CON diets. Liver concentrations of cholesterol and triglycerides were also analyzed in the current study, but they were similar among dietary treatments (data not shown). These results are in contrast with previous poultry and swine studies, in which plasma TBARS increased and plasma vitamin E decreased when diets contained oxidized oil or fat (Sheehy et al., 1994; Engberg et al., 1996; Fernández-Dueñas, 2009). The divergent findings regarding serum α-tocopherol and TBARS in response to lipid peroxidation indicate that either feeding DDGS may not induce an oxidative challenge as strong as feeding oxidized fat or oil directly and/or feeding DDGS may cause a vitamin E-sparing effect by increasing other antioxidants and thus alleviating the oxidative stress induced by oxidized lipids in DDGS.

Biological S-containing compounds, including Met, Cys, taurine, and GSH, have been studied extensively for their antioxidant properties via mechanisms of radical scavenging, GPx activity, and metal-binding interactions (Parcell, 2002; Atmaca, 2004; Battin and Brumaghim, 2009). For example, Met can have a free radical scavenging effect by being oxidized to Met sulfoxide in many animal species (Levine et al., 2000; Atmaca, 2004). Taurine is the most abundant free AA in the body, and it has potent antioxidant properties (Atmaca, 2004). Taurine can scavenge reactive oxygen species and prevent changes in cell membrane permeability, which can reduce lipid peroxidation (Alvarez and Storey, 1983; Hwang et al., 1998; Atmaca, 2004). Hwang et al. (2000) fed 5% taurine to rats and observed increased BW and decreased liver TBARS caused by feeding diets containing 3% oxidized fish oil, which indicates that taurine may protect against lipid peroxidation. Glutathione is the major cellular antioxidant in pigs, and it is a co-factor of the antioxidant enzyme GPx (Battin and Brumaghim, 2009).

In the current study, compared with pigs fed CON, feeding 30% DDGS increased Met and taurine concentrations in the serum, GSH concentration in the liver, and serum activity of GPx of pigs by 55, 37, 35, and 24%, respectively, which indicates an improved antioxidant status and oxidation defense system. The increase in these S-containing antioxidants could be due to the combined effects of increased dietary concentrations of inorganic S and Met and improved S digestibility (Table 8) in DDGS diets compared with CON.

Corn DDGS contains a relatively high S content (0.33 to 1.04% on a DM basis; Kim et al., 2012) compared with other feed ingredients because of the addition of sulfuric acid for pH adjustment and cleaning of fermenters used in the dry-grind ethanol production process. In addition, organic S, mainly in the form of SAA, is present in DDGS intrinsically because the corn kernel contains approximately 0.1% S (Kerr et al., 2008), and this concentration is expected to be increased by a fac-

tor of 3 in DDGS because of the removal of most of the starch during ethanol production. Effects of feeding DDGS with high S concentration on animal health and performance have been evaluated extensively in cattle (Sarturi et al., 2011; Uwituze et al., 2011a,b). The maximum tolerable concentration of dietary S in diets fed to cattle is 0.3% of DM in grain-based diets and 0.5% of DM in forage-based diets (NRC, 2005), but the tolerance for S in diets fed to pigs has not been established. Kim et al. (2012) concluded that high S content in DDGS-containing diets did not influence growth performance of weanling or growing–finishing pigs and suggested that a high S content in DDGS may not be the cause for reduced growth performance of pigs observed in some previous experiments (Whitney et al., 2006; Barbosa et al., 2008; Linneen et al., 2008).

In the 31 DDGS sources that were considered for this experiment, total S content varied from 0.27 to 0.95%, which was in agreement with data published by others (Spiehs et al., 2002; Kerr et al., 2008; Kim et al., 2012). The source of DDGS used in the present study contained 0.95% total S. Including 30% of this high S source of DDGS in the diet, total S content was 2 times greater than CON (0.39 vs. 0.19%). This increase in total S content was largely contributed by the 2.7 times greater concentration of inorganic S content in DDGS diets than CON whereas the organic S content was similar between DDGS and CON diets. In a study conducted by Anderson et al. (1975), dietary inclusion of 0.1% sulfate decreased the SAA requirements in chickens through the sparing effect of SAA. Additionally, Machlin et al. (1953) reported that when diets were low in SAA, hens appeared to synthesize Met and Cys from orally administered inorganic sulfate. Ruminants are able to synthesize SAA from inorganic sulfate in the diet and these mechanisms have been well documented (Block et al., 1951; Emery et al., 1957); however, the capability of nonruminant animals to utilize inorganic S to synthesize organic S is still unclear.

In addition to high S content in DDGS and DDGS containing diets, greater dietary Met concentrations may be another reason for increased S-containing compounds observed in pigs in the present study. Charkey et al. (1953) and Denton et al. (1953) presented evidence that the concentration of any AA in the blood is usually in agreement with the relative concentration of that AA in the diet, and that dietary supplementation of AA increases blood-borne concentrations of corresponding AA. This statement was further confirmed by the study from Puchal et al. (1962), who found that the plasma concentration of indispensable AA, including Met, in young pigs were related to the AA composition in the diet. Results from the current study are in agreement with those reported from previous studies, where pigs fed DDGS diets containing greater concentrations of Met showed greater concentrations of Met in the serum compared with those fed CON. Furthermore, increased serum taurine and liver GSH concentrations were also observed in pigs fed DDGS diets. The increase in taurine and GSH concentrations were likely due to the increase in Met because Met can be converted rapidly to Cys via the transsulfurylation pathway, and in turn, Cys serves as a precursor for the synthesis of taurine and GSH (Atmaca, 2004; Bauchart-Thevret et al., 2009). In fact, dietary Met concentration (mean of analyzed values in Phase 1, 2, and 3) in the present study was positively correlated ($P = 0.05$) with liver GSH concentration with Pearson's correlation coefficient of 0.81 (data not shown). This observation is consistent with previous findings in rats where reducing dietary Met concentration decreased taurine concentration in serum and GSH content in liver (Glazenburg et al., 1983). Additionally, Wang et al. (1997a) observed that increased concentrations of Met in cultured rat hepatocytes increased intracellular GSH concentration and GPx activity. Similarly, rats fed additional Met (Hunter and Grimble, 1997) and mice fed additional taurine (Ebrahim and Sakthisekaran, 1997) expressed increased GPx activity in blood. The supplementation of SAA appears to be an effective method of restoring GSH status because SAA play a role in determining the flux of Cys between Cys catabolism and GSH synthesis (Atmaca, 2004). However, the detailed mechanism of this response has not been determined. Regardless of the mechanism, greater hepatic GSH concentrations in pigs fed DDGS diets would be beneficial for increasing the ability of GSH to conjugate toxins or combat metabolic oxidative challenges encountered by the animal.

Increased S-containing antioxidants together with an increased activity of GPx in pigs fed DDGS indicate an improved antioxidant status and oxidation defense system, which appear to protect the animal against the possible oxidative challenge by feeding DDGS containing peroxidized lipids. However, it remains unclear which source or sources of S in DDGS (inorganic S, organic S, or both) result in this protective mechanism. It appears that greater Met concentration in DDGS diets may be an important factor, and therefore, diets formulated with more crystalline Lys and consequently a reduced Met concentration may have less S-containing antioxidants in vivo than observed in this study. Even though supplementation of vitamin E did increase the liver GSH concentration and activity of GPx in the serum, which was in agreement with the findings reported by Ebrahim and Sakthisekaran (1997) and Wang et al. (1997b), it may not be necessary to increase the concentrations of vitamin E greater than those recommended by NRC (1998)

to protect pigs against oxidative stress when feeding high S and highly oxidized DDGS.

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