RESEARCHREPORT

ANIMAL SCIENCE — Nutritional Efficiency Consortium

Title: Evaluation of lipid oxidation levels in DDGS sources and impact of feeding (with or without antioxidants) on swine health, performance, and metabolic oxidation **– NPB #10-002**

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Industry Summary

Background: Oxidative damage in feedstuffs represents a significant economic loss because it negatively affects pig health and growth performance. Lipid peroxidation occurs during the production of corn dried distillers grains with solubles (DDGS). Corn oil, which is typically present at a concentration of 10% in DDGS, contains high levels of polyunsaturated fatty acids, particularly linoleic acid, which are vulnerable to lipid peroxidation. Increased drying time and temperature used by ethanol plants accelerates lipid peroxidation in DDGS. It appears that that feeding diets containing DDGS with oxidized fat to pigs may require supplementation of higher levels of antioxidants (e.g. vitamin E) than currently being fed. Improved growth performance was reported in one study when pigs were fed diets containing DDGS or oxidized corn oil supplemented with antioxidants, but results from other studies have shown that supplementation of antioxidants had no effect on growth performance in animals under a dietary oxidative stress challenge. Therefore, one of the objectives of this study was to evaluate the effects of feeding a diet containing DDGS with a high content of oxidized lipids and sulfur on pig growth performance and metabolic oxidation status, and to determine if any of the negative effects could be overcome by increasing dietary level of vitamin E. Sulfur-containing compounds, including methionine, cysteine, taurine, and glutathione, have been shown to have potent antioxidant properties, and DDGS often containa high concentrations of sulfur due to the large amount of sulfuric acid added during the ethanol production process. There is no published information available regarding sulfur-containing antioxidants in response to feeding DDGS with high sulfur content to pigs, nor if sulfur-containing compounds produced by pigs could protect them against lipid peroxidation induced by feeding DDGS. Therefore, we were also interested in determining whether the high sulfur content in DDGS could protect against lipid peroxidation in pigs fed DDGS diets.

Objectives: To determine the nutrient content and lipid oxidation levels in DDGS samples from different sources and investigate the effects of feeding diets containing oxidized DDGS, with and without an antioxidant, on growth performance, health and immune status, nutrient digestibility, and metabolic oxidation balance in nursery pigs.

These research results were submitted in fulfillment of the Nutritional Efficiency Consortium research projects.
Contributing organizations for 2010 include: AgriSolutions, Inc., DPI Global, Iowa Pork Producers Association, Illinois Corn Marketing Board, Illinois Pork Producers Association, Kansas Pork Association, Missouri Pork Producers Association, Mississippi Pork Producers Association, National Pork Board, Nebraska Corn Board and the Utah Pork Producers Association.

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Procedures: The DDGS source used in this study was selected out of 31 DDGS sources produced by the ethanol plants in the U.S., and contained the highest thiobarbituric acid reactive substances (TBARS) value, peroxide value, and total sulfur content (5.2 ng/mg oil, 84.1 meq/kg oil, and 0.95%, respectively) among the other 30 DDGS sources sampled (mean values = 1.8 ng/mg oil, 11.5 meq/kg oil, and 0.50%, respectively). Fifty-four barrows were fed corn-soybean meal (CON) or 30% DDGS diets containing one of 3 levels of vitamin E (α-tocopheryl acetate): none supplemented (No-E), NRC (1X-E), or 10X NRC (10X-E) using a 3-phase nursery feeding program with targeted body weight of 7-11 kg, 11-25 kg, and 25-50 kg for Phase 1, 2, and 3, respectively. Barrows 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.
Findings: Total sulfur content was higher in DDGS diets than CON (0.39 vs. 0.19%). Dietary inclusion of 30% DDGS improved apparent total tract digestibility of sulfur (86.8 vs. 84.6%), as well as sulfur absorbed and retained compared to CON. Although pigs were fed highly oxidized DDGS in this study, serum TBARS were similar between DDGS and CON treatments. There was no interaction between dietary DDGS and α-tocopherol concentration in serum TBARS. Serum α-tocopherol (vitamin E) increased by feeding DDGS diets compared to CON (2.25 vs. 1.56 µg/mL). In addition, pigs fed DDGS diets had higher concentrations of sulfur-containing amino acids, particularly methionine and taurine in serum of fed pigs, and a higher concentration of taurine in serum of fasted pigs compared with those fed CON. Liver glutathione concentration was higher in pigs fed DDGS diets than CON (56.3 vs. 41.8 nmol/g). Dietary inclusion of DDGS and α-tocopherol increased serum enzyme activity of glutathione peroxidase.
Conclusions: The elevated concentrations of sulfur-containing antioxidants (methionine, taurine, glutathione) may protect pigs against oxidative stress when feeding highly oxidized DDGS. Therefore, increasing levels of α-tocopherol (vitamin E) in diets containing DDGS with high oxidized lipid content may not be necessary to protect pigs from metabolic oxidation stress.

Key Words: DDGS, glutathione, lipid oxidation, nursery pigs, sulfur-containing antioxidants, vitamin E

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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 negatively affect growth performance and 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% DDGS containing one of 3 levels of vitamin E (α-tocopheryl acetate): none supplemented, NRC level (11 IU/kg), and 10X NRC level (110 IU/kg) on oxidative status of nursery pigs. The DDGS source used in this study contained the highest 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) among 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 higher in DDGS diets than CON (0.39 vs. 0.19%). Dietary inclusion of 30% DDGS improved apparent total tract digestibility of S (86.8 vs. 84.6%, $P < 0.001$), S absorbed and retained ($P < 0.01$) compared to CON. Although pigs were fed highly oxidized DDGS in this study, serum TBARS were similar between DDGS and CON treatments. There was no interaction between dietary DDGS and α-tocopherol concentration in serum TBARS. Serum α-tocopherol increased by feeding DDGS diets compared to CON (2.25 vs. 1.56 μg/mL; $P < 0.001$). Pigs fed DDGS diets had higher concentrations of S-containing AA, particularly methionine ($P < 0.001$) and taurine ($P = 0.002$) in serum of fed pigs, and a higher concentration of taurine in serum of fasted pigs ($P = 0.006$) compared with those fed CON. Liver glutathione concentration was higher in pigs fed DDGS diets than CON (56.3 vs. 41.8 nmol/g). Dietary inclusion of DDGS ($P < 0.001$) and α-tocopherol ($P = 0.03$) increased serum enzyme activity of glutathione peroxidase. The elevated concentrations of S-containing antioxidants (methionine, taurine, glutathione) *in vivo* may protect pigs against oxidative stress when feeding highly oxidized DDGS. Therefore, increasing levels of α-tocopherol in diets containing DDGS with high oxidized lipid content may not be necessary to protect pigs from metabolic oxidation stress.

Introduction

Oxidative damage in feedstuffs represents a significant economic loss. Oxidized lipids in animal feed negatively affects pig health and growth performance (Miller and Brzezlnska-Slebodzlnska, 1993; Pfalzgraf et al., 1995). Lipid peroxidation occurs during the production of corn dried distillers grains with solubles (DDGS). Corn oil, which is typically present at a concentration of 10% in DDGS, contains high levels of PUFA, particularly linoleic acid, that are vulnerable to lipid peroxidation (NRC, 1998). Increased drying time and temperature used by ethanol plants accelerates lipid peroxidation in DDGS.

It appears that that feeding diets containing DDGS with oxidized fat to pigs may require supplementation of higher levels of antioxidants (e.g. vitamin E) than currently being fed. Improved growth performance was reported when pigs were fed diets containing DDGS or oxidized corn oil supplemented with antioxidants (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., 1997; Anjum et al., 2002; Fernández-Dueñas, 2009). Therefore, one of the objectives of this study was to evaluate the effects of feeding a diet containing DDGS with a high content of oxidized lipids and S on pig growth performance and metabolic oxidation status, and to determine if any of the negative effects could be overcome by increasing dietary level of vitamin E.

Sulfur is an essential component in many physiological functions of animal and is incorporated into amino acids, proteins, enzymes and micronutrients (Atmaca, 2004). Sulfur-containing compounds, including methionine (Met), cysteine (Cys), taurine, and glutathione (GSH), have been shown to have potent antioxidant properties (Battin and Brumaghim, 2009). Dried distiller's grains with solubles (DDGS) may contains high concentrations of S mainly due to the large amount of sulfuric acid added during the ethanol production process

(Kim et al., 2012). There is no published information available regarding S-containing antioxidants in response to feeding DDGS with high S content to pigs, nor if the S-containing compounds *in vivo* could protect the pigs against lipid peroxidation induced by feeding DDGS. Therefore, the second objective of this study was to determine if high S content in DDGS could protect against lipid peroxidation in pigs fed DDGS diets.

Objectives

1. Determine the nutrient content and lipid oxidation levels in DDGS samples from different sources, and develop equations to predict the oxidation level in DDGS using chemical and physical characteristics.

2. Determine the effects of feeding diets containing oxidized DDGS, with and without an antioxidant, on growth performance, health and immune status, nutrient digestibility, and metabolic oxidation balance in nursery pigs.

Materials and Methods

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

Animals and Housing

A total of 54 weanling terminal cross barrows (Duroc x Landrace x Yorkshire, initial BW = 7.0±0.3 kg) were used in this experiment, which was conducted at University of Minnesota, Southern Research and Outreach Center (SROC, Waseca, MN). Pigs were blocked by initial BW, and pens within the blocks were randomly assigned to one of 6 dietary treatments in a 2 x 3 factorial arrangement, resulting in 9 pigs and 4 pens / treatment. Pigs were fed corn-soybean meal (CON) or 30% DDGS diets containing one of 3 levels of vitamin E (α-tocopheryl acetate): none supplemented (No-E), NRC (1X-E), or 10X NRC (10X-E). Pigs were fed in a 3-phase nursery feeding program with targeted BW of 7-11 kg, 11-25 kg, and 25-50 kg for Phase 1, 2, and 3, respectively. Pigs were group-housed in pens (1.2 m x 1.2 m) and fed their respective diets for 8 wk after weaning. All pigs were allowed *ad libitum* access to feed and water throughout this time period and were monitored for health on a daily basis. Individual pig BW and pen feed disappearance were measured initially and 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 located at SROC for a 5-d (d 1 - 5) adaptation period followed by a 3-d (d 6 - 8) total collection of feces and urine, and 2-d (d 9-10) collection of blood samples. On d 11, 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 measured on d 1 (2% fed at 0700 h and 2% fed at 1900 h). The amount of feed provided to animals was recorded at each feeding time. If there was any feed remaining from the previous feeding, it was removed, weighed, and subtracted from the amount offered to determine the average daily feed disappearance. 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 to meet the comfort needs of the pig.

Diet Composition and DDGS Source

Diet composition and nutrient concentrations of experimental diets for Phase 1-3 are presented in **Table 1-3**. All diets were fed in meal form and were formulated on a standardized ileal digestible (SID) AA and available P basis, and nutrient amounts of the diets met or exceeded NRC (1988) nutrient requirements for pigs with 350 g of fat-free lean gain/d, except for vitamin E concentration in the No-E treatments. Vitamin E was supplemented in the form of *dl-α*-tocopheroyl acetate in 1X-E and 10X-E treatments. The DDGS source used in this study was selected out of 31 DDGS sources produced by the ethanol plants in the U.S. As previously

analyzed by our lab (Song et al., 2011), this source of DDGS contained the highest 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) among 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 h and 1900 h) daily from d 6 to8 and stored at -20° C. Fecal samples from each pig were pooled, weighed, and dried in a forced-draft oven at 55to60° 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 the funnels of the metabolism cages. Thirty mL of 6N 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 in fed pigs were collected 1 h after feeding at 0700 h on d 7. Approximately 8-mL blood samples were collected using BD SST* brand serum separation tubes coated with silicone and micronized silica particles (Franklin Lakes, NJ) from all pigs in the metabolism crates. Blood samples in fasted pigs were collected on d 10 after pigs were fasted for 24 h on d-9, following the same procedure used for collection of blood samples in fed pigs. All 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 GPX were performed.

Approximately 50 g liver samples from each pig were collected on d-11 after pigs were euthanized using captive bolt. Liver samples were frozen immediately on dry ice and stored at -80°C for GSH analysis.

TBARS Assay

To evaluate the metabolic status *in vivo*, TBARS assay was performed using serum of fed pigs following the method described by Animal Models of Diabetic Complications Consortium (AMDCC, Version 1). 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 4º C. Two hundred µL supernatant was then removed and incubated with an equal volume of 0.67% (w/v) thiobarbituric acid (Sigma-Aldrich, St. Louis, MO) for 10 min in a boiling water bath. The mixture was then cooled to room temperature and read at 532 nm using spectrometer (SpectraMax 250, Molecular Device, Sunnyvale, CA). This assay was conducted in 4 batches with duplicatesamples and a standard. The intra-assay CV was 6.7% and the inter-assay CV was 5.2%.

Analysis of α-Tocopherol Concentration in Serum

Analysis of α-tocopherol concentration in serum of fed pigs was conducted by Michigan State University Diagnostic Center for Population & Animal Health (DCPAH, Lansing, MI). 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 high-performance liquid chromatography (HPLC, Separation Module 2690, Waters, Milford, MA).

Analysis of Sulfur-Containing Compounds

Serum sulfur-containing amino acids. Serum levels 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 µM p-chlorol-L-phenylalanine as the internal standard. Five µL of each sample and standard was mixed with 40 µL Na_2CO_3 (10 mM, pH:11) and 100 µL Dansyl chloride (3 mg/ml in acetone). The mixture was then incubated in a water bath at 60º C for 10 min, followed by centrifugation at 14.8×10^3 rpm for 10 min. The top supernatant was transferred to a high recovery vial and 5 µL was injected into the LC-MS system for analysis.

Hepatic glutathione. Glutathione concentration in liver was analyzed using a commercial GSH Assay kit from Sigma-Aldrich (Catalog number: CS0260, St. Louis, MO). Fifty milligrams of each liver sample was extracted by homogenizing in 500 µL of 5% 5-sulfosalcyclic acid followed by centrifugation at 10,000 × *g* for 10 min at 4º C. Ten µL supernatant of each sample was then used for GSH measurement following the manufacturer's instructions. Each sample and standard were analyzed in duplicate. This assay was conducted in one batch, with the inter-assay CV of 3.2%.

Serum glutathione peroxidase activity. Enzyme activity of GPX in the serum of fed pigs was determined using a commercial GPX Assay kit (Cayman Chemical, Catalog number: 703102, Ann Arbor, MI). Briefly, indirect GPX activity was measured by a coupled reaction with glutathione reductase (GR). The reaction was initiated after cumene hydroperoxide addition. Oxidation of NADPH to NADP was measured colorimetrically at 340 nm for at least 5 min; GPX activity was expressed as µmol/min/mg of protein and compared to a bovine erythrocyte GPX standard curve over time. Each sample and standard was analyzed in duplicate. This assay was conducted in one batch with the inter-assay CV of 5.9%.

Analysis of S Content in Feed, Feces and Urine

Fifty gram feed samples of each diet in Phase 3 were sent to the University of Missouri Agricultural Experiment Station Chemical Laboratories (ESCL) for analysis of total S content, and S-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 the following equation: Organic S in feed, % = Met in feed, % × 21% + Cys in feed, % × 26% + taurine in feed, % × 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 by the magnesium nitrate procedure of AOAC (1995) to calculate S daily balance and apparent total tract digestibility (ATTD) of S in each diet, using the following equation: ATTD, % = [(St − Sf)/Si] × 100%, where St = the total consumption of S (g) from d 6 to 8, and Sf = the total fecal excretion of S (g) originating from the feed fed from d 6 to 8.

Statistical Analysis

All data were analyzed using the MIXED procedure of 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 for this complete 2 x 3 factorial arrangement. Pen was used as the experimental unit for growth performance responses. 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 x vitamin E interactions. 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.

Results

Growth Performance

No interactions ($P > 0.39$) of DDGS and vitamin E were observed for any of the growth performance

responses (**Table 4**). Vitamin E did not affect BW, ADG, ADFI, or gain:feed. Pigs fed DDGS had a lower final BW at 8 wk than pigs fed CON (42.4 vs. 40.0 kg, respectively, $P = 0.05$). Average daily gain, ADFI, and G:F were not affected in pigs fed DDGS compared with those fed CON. It should be noted in the present study that, all the growth performance measurements were obtained from four replications per treatment. Differences among DDGS and CON treatments would be likely be significantly different if more replications were used.

Metabolic Oxidation Status

There were no effects of DDGS, vitamin E supplementation level, or their interaction on TBARS values in serum of fed pigs (**Table 5**). An interaction of DDGS × vitamin E concentration was detected ($P < 0.001$) for α-tocopherol concentration in serum of fed pigs (**Table 5**). Specifically, pigs fed DDGS with No-E and 1X-E had a higher concentration ($P < 0.001$) of serum α-tocopherol 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 in DDGS and CON treatments. As expected, serum α-tocopherol concentration was higher in pigs fed CON/10X-E than those fed CON/1X-E, which was higher 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 higher ($P < 0.001$) in pigs fed 10X-E than those fed 1X-E and No-E (3.54 vs. 1.61 and 1.60 μg/mL), but pigs fed 1X-E was similar to those fed No-E.

Sulfur-containing Antioxidants

Sulfur-containing antioxidants, including Met, Cys, taurine, GSH, and GPX activity, were evaluated *in vivo* in this study (**Table 6**). However, the Cys concentration in the serum was below detection limit. Therefore, we were not able to report Cys concentrations in the present study. No DDGS and vitamin E interactions were detected for any of these measurements of S-containing antioxidants. Pigs fed DDGS had higher concentrations of Met (70.8 μM, $P < 0.001$) and taurine (197.3 μM, $P = 0.002$) in serum of fed pigs, and a higher level of taurine in serum of fasted pigs (183.3 μM, $P = 0.006$) compared with those fed CON (45.8, 143.7 and 141.5 μM, respectively). Comparing fasted versus fed status, pigs fed DDGS had lower fasted serum Met and taurine concentrations than in fed serum (70.8 vs. 41.8 and 197.3 vs. 183.3 μM, respectively). However, pigs fed CON had a similar serum level of Met and taurine in fasted and fed states (45.8 vs. 44.2 and 143.7 vs. 141.5 μM, respectively). No effect of vitamin E supplementation level or any interactions were observed for serum SAA concentrations.

Liver GSH concentration was higher 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. No interaction between DDGS and vitamin E supplementation level was detected for liver GSH concentration.

Similar to GSH, serum GPX activity increased when supplemental vitamin E levels increased ($P = 0.03$). Pigs fed DDGS had a higher serum GPX activity compared with those fed CON (1.25 vs. 1.00 units/ml, $P < 0.001$). There was no interaction between DDGS and vitamin E concentration on serum GPX activity.

Sulfur Content and Digestibility in Experimental Diets

Total S content in DDGS containing diets was 2 times higher than that in CON (0.39 vs. 0.19%, **Table 7**). As a result, with similar feed intake, daily S intake was almost 2 times higher in pigs fed DDGS than those fed CON (5.7 vs. 3.0 g/d, $P < 0.001$). Daily S excretion in feces and urine was higher, 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 to the CON (86.8 vs. 84.6 %, $P < 0.001$). 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.

Discussion

The utilization of corn co-products in livestock feeds, such as DDGS, has increased dramatically in recent years due to increased availability and cost competitiveness compared with corn and soybean meal. Often with DDGS, limits on dietary inclusion rates occur because pig performance and pork quality decline when high dietary levels of DDGS (30-40%) are fed to growing-finishing pigs. The reduction in growth performance sometimes observed when feeding high dietary levels of DDGS could be potentially caused by antinutritional factors, toxins, low net energy level, poor amino acid digestibility, and oxidized fat (Stein and Shurson, 2008). Corn DDGS contains approximately 10% corn oil. Corn oil contains high levels of PUFA (particularly linoleic acid; NRC, 1998) that are vulnerable to lipid peroxidation, which is a free-radical chain reaction producing oxidized lipids and a series of toxic aldehydes (Blokhina et al., 2003). In addition, drying temperatures used by ethanol plants vary substantially (185 to 1100° F), and increased drying time and temperature during the production process of DDGS accelerates lipid peroxidation. In the present study, a DDGS source with high level of lipid peroxidation was selected according to a recent study conducted by our laboratory (Song et al., 2011), where TBARS and PV were measured to evaluate the lipid peroxidation level in DDGS samples obtained from 31 ethanol plants in the U.S. 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, suggesting that lipid peroxidation varies among DDGS sources. The DDGS source with the highest TBARS and PV values was 25 and 27 times greater, respectively, than the level in a reference corn sample (0.2 ng MDA/mg oil and 3.1 meq/kg oil, respectively), and this source of DDGS was then used in the current study to evaluate the effect of oxidized lipids in DDGS on pig growth performance and metabolic oxidation status.

Growth suppression from oxidized lipids has been well documented in several different animal species (Reddy and Tappel, 1974; Dibner et al., 1996; Wang et al., 1997; Derouchey et al., 2004; Harrell et al., 2010). The presence of high amounts of oxidized fat in the diet raises the levels 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, 1979; Comporti, 1993). In the present study, pigs fed diets with highly oxidized DDGS had reduced final BW regardless of dietary levels of vitamin E. This observation is in agreement with previous studies where reduced BW was reported in pigs fed oxidized corn oil (Harrell et al., 2010; Fernández-Dueñas, 2009) 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 might be due to insufficient dietary oxidative challenge as measured by PV in oil/fat or in the final diet, and there seems to be a threshold for rancidity above which growth performance is decreased. Derouchey et al. (2004) suggested that a peroxide value of oxidized lipids less than 40 meq/kg might not result in decreased growth performance in nursery pigs if hydroperoxides have not already begun the degradation. However, it should be pointed out that using PV as the only indicator of lipid peroxidation may not be sufficient since a low PV could be due to minimal oxidation or decomposition of hydroperoxides that have 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 level of dietary vitamin E. However, we did not observe a beneficial effect of vitamin E in this study. This result was in agreement with results reported from a recent study (Fernández-Dueñas, 2009), where the author showed that supplementation

of a synthetic antioxidant did not increase ADG, ADFI and G:F in finishing pigs fed 5% oxidized corn oil. Similarly, in previous poultry studies, providing ethoxyquin in the diet failed to improve growth rate and feed consumption in broilers fed oxidized oil (Wang et al., 1997; 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 natural vitamin E present in our diets, without additional vitamin E supplementation, was sufficient to protect the 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 in pigs. It should be noted that although the vitamin E was supplemented in the form of α-tocophoeryl acetate in the diet, only free α-tocopherol was identified in the blood serum based on the fact that tocopheryl acetate is converted to tocopherol in the intestine before or during absorption (Ogihara, et a1. 1985). Interestingly, pigs fed DDGS exhibited a higher level of serum α-tocopherol than those fed CON, and the TBARS value was not different in DDGS vs. CON treatments. These results were in contrast with some poultry and swine studies where increased plasma TBARS and decreased plasma vitamin E were observed when diets were formulated with oxidized oil/fat (Sallmann, et al., 1988; Sheehy et al., 1994; Engberg et al. 1996; Fernández-Dueñas, 2009). The divergent findings regarding the serum α-tocopherol and TBARS in response to lipid peroxidation suggest that either feeding DDGS may not induce an oxidative challenge as strong as feeding oxidized fat/oil directly, or feeding DDGS may cause a vitamin E-sparing effect by increasing other antioxidants and thus, alleviate the oxidative stress induced by oxidized fat in DDGS, or both.

The above assumptions were then verified by measuring the S content in feed and S-containing antioxidants *in vivo*. The reason that we chose to focus on S and S-containing antioxidants rather than other nutrients with antioxidant properties was the fact that DDGS source fed contained relatively high S content compared to the other feed ingredients. 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 level is expected to be concentrated by a factor of 3 in DDGS because of the removal of most of the starch during ethanol production. In addition, large amount of sulfuric acid is sometimes added in dry-grind ethanol production for pH adjustment, thereby greatly increasing the S content in DDGS. In the 31 DDGS sources that were analyzed before the animal experiments reported here, the total S content in DDGS varied from 0.27 to 0.95%, which was in agreement with previously published data (Spiehs et al., 2002; Kerr et al., 2008; Kim et al., 2012). The DDGS source used in the present study was found to contain the highest total S concentration (0.95%). By including 30% of this high S DDGS source in the diet, the total S content in DDGS diets was 2 times higher than CON (0.39 vs. 0.19%). This increase in total S content was largely contributed by high levels of inorganic S content in DDGS diets, while the organic S content, which was calculated from SAA, was similar between DDGS and CON diets (**Table 8**).

Effects of feeding DDGS with high S concentration on animal health and performance have been extensively evaluated in cattle (Sarturi et al., 2011; Uwituze et al., 2011a; Uwituze et al., 2011b). The maximum tolerable concentration of dietary S in diets fed to cattle is suggested to be 0.4% of DM (NRC, 1998), but the tolerance for S in diets fed to pigs has not been established. Indeed, Kim et al. (2012) concluded that high S content in DDGS diet did not influence growth performance of weanling or growing-finishing pigs, suggesting that high S content may not be the cause for reduced growth performance of pigs fed DDGS that was observed in some previous experiments (Whitney et al., 2006; Barbosa et al., 2008; Linneen et al., 2008).

However, the effect of feeding a high S DDGS source to pigs on SAA and other S-containing compounds *in vivo* has not been studied previously. In the present study, compared with pigs fed CON, feeding 30% DDGS increased Met and taurine concentrations in the serum of fed pigs by 55 and 37%, respectively. The increase in serum Met and taurine could be due to the combination effects of 24% higher level of Met (**Table 3**), 2.7 times higher level of inorganic S (**Table 8**), and improved S digestibility (**Table 7**) in DDGS

diets. As early as 1953, Charkey et al. (1953) and Denton et al. (1953) presented evidence that the concentration of any one amino acid in the blood is usually in agreement with the relative concentration of that amino acid in the diet, and the addition of supplemental amino acids to the diet results in an increase in the blood level of the corresponding amino acid. This statement was further confirmed by the study from Puchal et al. (1961), who found that the plasma concentration of essential amino acids, including Met, in young pigs were related to the amino acid composition in the diet. In the present study, elevated serum taurine and liver GSH concentrations were observed in pigs fed DDGS diets. The increase in taurine and GSH concentrations were likely due to the increase in Met, since Met can be rapidly converted to cysteine via the transsulfurylation pathway, and in turn, serves as a precursor for the synthesis of taurine and GSH (Atmaca, 2004; Bauchart-Thevret et al., 2009). In fact, dietary Met level was found to have a strong positive correlation with liver GSH concentration in this study ($r = 0.91$; $P = 0.01$; **Figure 1**). The observation in the present study is consistent with previous findings in rats that reducing dietary Met level resulted in a reduction of taurine concentration in serum and GSH content in the liver (Glazenburg et al., 1983). In another study published by Stockland et al. (1971) using growing pigs, the concentration of free taurine in plasma increased with dietary addition of Met, indicating an increased catabolism of Met and Cys due to Met supplementation. Similar findings were also reported from an *in vitro* study by Wang et al.(1997), where the authors observed increased intracellular GSH and GPX activity in rat hepatocytes cultured with increasing concentrations of Met. The GPX activity was also studied *in vivo*, and increased GPX activity was found in rats consuming additional Met (Hunter and Grimble, 1997) and in mice fed additional taurine (Ebrahim and Sakehisekaran, 1997). Although these effects have not previously evaluated in swine, there was a positive correlation between dietary SAA and GPX activity.

In addition to higher dietary Met, high inorganic S content in DDGS diets might be another reason for increased S-containing compounds observed in the present study. The inorganic S concentration was calculated to be 2.7 times higher in DDGS diets than CON, which is the major cause for elevated total S content in DDGS 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), however the capability of monogastric animals to utilize inorganic S to synthesize organic S is still unclear.

Biological S-containing compounds, including Met, Cys, taurine, and GSH, have been extensively studied for their antioxidant properties by mechanisms of radical scavenging, GPX activity, and metal-binding interactions (Fleischauer and Arab, 2001; Parcell, 2002; Atmaca, 2004; Battin and Brumaghim, 2009). For example, Met has been reported to have a free radical scavenging effect by being oxidized to methionine 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 has been to shown to scavenge reactive oxygen species and prevent changes in cell membrane permeability and thus, reduce lipid peroxidation (Alvarez and Storey, 1983; Hwang et al., 1998; Atmaca, 2004). In a study conducted by Hwang et al. (2000), feeding 5% taurine to rats increased BW and decreased liver TBARS caused by oxidized fish oil, suggesting that taurine could protect against lipid peroxidation. In the current study, an increased taurine concentration in serum occurred along with an increased hepatic GSH, which is the major cellular antioxidant. Hwang et al. (2000) also observed an increased liver GSH concentration in response to dietary supplementation of taurine, indicating that taurine may play an important role in the metabolism of GSH. Furthermore, SAA plays a role in determining the flux of cysteine between cysteine catabolism and GSH synthesis, and therefore, SAA supplementation appears to be an effective method of restoring GSH status (Atmaca, 2004). However, the detailed mechanism of this response has not been determined. Regardless of the mechanism, higher GSH concentration in pigs fed DDGS diets would be beneficial for increasing the ability of GSH to conjugate toxins or combat oxidative challenges encountered by the animal. Elevated 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 with a high degree of lipid peroxidation. 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 Wang et al. (1997), and Ebrahim and Sakehisekaran (1997), it may not be necessary to increase the levels of vitamin E higher than those recommended by NRC (1998) to protect pigs against oxidative stress when feeding DDGS.

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

Item	CON[1]			DDGS[2]		
	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%)	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
Analyzed nutrient composition						
ME,[6] kcal/kg	3,283	3,282	3,273	3,339	3,338	3,329
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

[1]CON = corn-SBM based control diet.
[2]DDGS = 30% inclusion of dried distillers grains with solubles
[3]No-E = No supplemental of vitamin E; 1X-E = NRC (1998) level of vitamin E, which is 13.2 IU/kg for BW = 9 kg; 10X-E = 10X NRC (1998) level of vitamin E, which is 132 IU/kg for BW = 9 kg.
[4]Vitamin-trace mineral premix (without vitamin E) provided the following nutrients per kilogram of diet: 11,023 IU of vitamin A as retinyl acetate; 2,756 IU of vitamin D3; 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 B12; 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 iodine as ethylenediamine dihydroiodide; 0.30 mg of selenium as sodium selenite; 90.39 mg of zinc as zinc oxide (SQM); 55.11 mg of iron as ferrous sulfate (SQM); 5.51 mg of copper as copper sulfate (SQM); and 17.64 mg of manganese as manganese oxide (SQM).
[5]Vitamin E was supplied as α-tocopheryl acetate with concentration of 20,000 IU/lb.
[6]ME values were calculated using NRC (1998) values for corn and soybean meal and 3,559 kcal/kg for DDGS from Pedersen et al. (2007).
[7]Calculated with analyzed Ca and P and relative P availability in DDGS from Whitney and Shurson (2001).

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

Item	CON[1]			DDGS[2]		
	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%)	–	–	–	30.00	30.00	30.00
DDGS	31.00	31.00	31.00	23.00	23.00	23.00
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
Analyzed nutrient composition						
ME,[6] kcal/kg	3,293	3,292	3,284	3,346	3,345	3,337
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

[1]CON = corn-SBM based control diet.
[2]DDGS = 30% inclusion of dried distillers grains with solubles
[3]No-E = No supplemental of vitamin E; 1X-E = NRC (1998) level of vitamin E, which is 11.0 IU/kg for BW = 18 kg; 10X-E = 10X NRC (1998) level of vitamin E, which is 110 IU/kg for BW = 18 kg.
[4]Vitamin-trace mineral premix (without vitamin E) provided the following nutrients per kilogram of feed: 11,023 IU of vitamin A as retinyl acetate; 2,756 IU of vitamin D3; 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 B12; 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 iodine as ethylenediamine dihydroiodide; 0.30 mg of selenium as sodium selenite; 90.39 mg of zinc as zinc oxide (SQM); 55.11 mg of iron as ferrous sulfate (SQM); 5.51 mg of copper as copper sulfate (SQM); and 17.64 mg of manganese as manganese oxide (SQM).
[5]Vitamin E was supplied as α-tocopheryl acetate with concentration of 20,000 IU/lb.
[6]ME values were calculated using NRC (1998) values for corn and soybean meal and 3,559 kcal/kg for DDGS from Pedersen et al. (2007).
[7]Calculated with analyzed Ca and P and relative P availability in DDGS from Whitney and Shurson (2001).

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

Item	CON[1]			DDGS[2]		
	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%)	–	–	–	30.00	30.00	30.00
DDGS	26.00	26.00	26.00	15.75	15.75	15.75
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
Analyzed nutrient composition						
ME,[6] kcal/kg	3,312	3,312	3,304	3,364	3,363	3,355
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

[1]CON = corn-SBM based control diet.
[2]DDGS = 30% inclusion of dried distillers grains with solubles
[3]No-E = No supplemental of vitamin E; 1X-E = NRC (1998) level of vitamin E, which is 11.0 IU/kg for BW = 37 kg; 10X-E = 10X NRC (1998) level of vitamin E, which is 110 IU/kg for BW = 37 kg.
[4]Vitamin-trace mineral premix (without vitamin E) provided the following nutrients per kilogram of feed: 11,023 IU of vitamin A as retinyl acetate; 2,756 IU of vitamin D3; 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 B12; 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 iodine as ethylenediamine dihydroiodide; 0.30 mg of selenium as sodium selenite; 90.39 mg of zinc as zinc oxide (SQM); 55.11 mg of iron as ferrous sulfate (SQM); 5.51 mg of copper as copper sulfate (SQM); and 17.64 mg of manganese as manganese oxide (SQM).
[5]Vitamin E was supplied as α-tocopheryl acetate with concentration of 20,000 IU/lb.
[6]ME values were calculated using NRC (1998) values for corn and soybean meal and 3,559 kcal/kg for DDGS from Pedersen et al. (2007).
[7]Calculated with analyzed Ca and P and relative P availability in DDGS from Whitney and Shurson (2001).

Table 4. Growth performance of pigs fed corn dried distiller's grains with solubles (DDGS) and increasing levels of vitamin E[1]

Item	CON[2]			DDGS[3]			SE	*P*-value		
	No-E[4]	1X-E[4]	10X-E[4]	No-E	1X-E	10X-E		Vit E	DDGS	Vit E × DDGS
Initial BW at weaning, kg	6.9	6.9	6.9	7.0	6.9	7.0	0.4	0.69	1.00	0.40
Final BW at wk 8, kg	42.0	41.3	43.8	39.9	39.9	40.0	1.5	0.67	0.05	0.70
ADG, kg	0.56	0.57	0.59	0.54	0.54	0.54	0.03	0.83	0.12	0.86
ADFI, kg	1.01	1.11	1.16	1.07	1.06	1.11	0.05	0.14	0.71	0.39
Gain:feed	0.57	0.52	0.51	0.50	0.51	0.49	0.02	0.26	0.14	0.39

[1]Values are least square means of four replicate pens per dietary treatment.
[2]CON = corn-SBM based control diet.
[3]DDGS = 30% dietary inclusion of dried distillers grains with solubles
[4]No-E = No supplemental of vitamin E; 1X-E = NRC (1998) level of vitamin E (11 IU/kg); 10X-E = 10X NRC (1998) level of vitamin E (110 IU/kg).

Table 5. Influence of DDGS and vitamin E supplementation on thiobarbituric acid-reactive substance (TBARS) and α-tocopherol concentration in serum[1]

Item	CON[2]			DDGS[3]			SE	*P*-value		
	No-E[4]	1X-E[4]	10X-E[4]	No-E	1X-E	10X-E		Vit E	DDGS	Vit E × DDGS
TBARS, μM	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 nine replicate pigs per dietary treatment.
[2]CON = corn-SBM based control diet.
[3]DDGS = 30% inclusion of dried distillers grains with solubles
[4]No-E = No supplemental of vitamin E; 1X-E = NRC (1998) level of vitamin E (11 IU/kg); 10X-E = 10X NRC (1998) level of vitamin E (110 IU/kg).

Table 6. Sulfur-containing antioxidants in pigs fed DDGS and increasing levels of vitamin E[1]

Item	CON[2]			DDGS[3]			SE	*P*-value		
	No-E[4]	1X-E[4]	10X-E[4]	No-E	1X-E	10X-E		Vit E	DDGS	Vit E × DDGS
Serum sulfur-AA in fed status, μM										
Methionine	53.8	41.1	42.4	73.6	77.1	61.7	4.8	0.12	<0.001	0.16
Taurine	138.9	135.9	156.3	194.4	206.8	190.6	19.3	0.94	0.002	0.64
Serum sulfur-AA in fasted status, μM										
Methionine	37.0	44.3	51.3	41.5	47.7	36.1	6.2	0.57	0.64	0.20
Taurine	144.1	120.3	160.0	176.0	172.9	201.0	17.5	0.14	0.006	0.85
Liver GSH, nmol/g	35.1	44.6	45.7	50.2	56.4	62.3	3.5	0.01	<0.001	0.78
Serum GPX activity, units/ml[5]	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-SBM based control diet.
[3]DDGS = 30% inclusion of dried distillers grains with solubles
[4]No-E = No supplemental of vitamin E; 1X-E = NRC (1998) level of vitamin E (11 IU/kg); 10X-E = 10X NRC (1998) level of vitamin E (110 IU/kg).
[5]One unit of activity equals 1 μmol NADPH oxidized per minute/mL serum.

Table 7. Daily S balance and apparent total tract digestibility (ATTD) in experimental diets (as-fed basis)[1]

Item	CON[2]			DDGS[3]			SE	*P*-value		
	No-E[4]	1X-E[4]	10X-E[4]	No-E	1X-E	10X-E		Vit E	DDGS	Vit E × DDGS
Total diet S, %	0.19	0.19	0.19	0.37	0.38	0.40	–	–	–	–
S intake, g	3.04	2.90	3.09	5.55	5.63	5.94	0.19	0.34	<0.001	0.64
S in feces, g	0.50	0.47	0.48	0.71	0.79	0.76	0.04	0.82	<0.001	0.30
S in urine, g	1.67	1.26	0.69	2.79	2.79	2.75	0.24	0.11	<0.001	0.16
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, %	84.3	84.0	85.4	87.3	85.9	87.3	0.70?	0.14	<0.001	0.68

[1]Values are least square means of nine replicate pigs per dietary treatment.
[2]CON = corn-SBM based control diet.
[3]DDGS = 30% inclusion of dried distillers grains with solubles
[4]No-E = No supplemental of vitamin E; 1X-E = NRC (1998) level of vitamin E (11 IU/kg); 10X-E = 10X NRC (1998) level of vitamin E (110 IU/kg).

Table 8. Total S content and calculated organic and inorganic S in Phase 3 experimental diets

Item	CON[1]			DDGS[2]		
	No-E[3]	1X-E[3]	10X-E[3]	No-E	1X-E	10X-E
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

[1]CON = corn-SBM based control diet.
[2]DDGS = 30% inclusion of dried distillers grains with solubles
[3]No-E = No supplemental of vitamin E; 1X-E = NRC (1998) level of vitamin E (11 IU/kg); 10X-E = 10X NRC (1998) level of vitamin E (110 IU/kg).

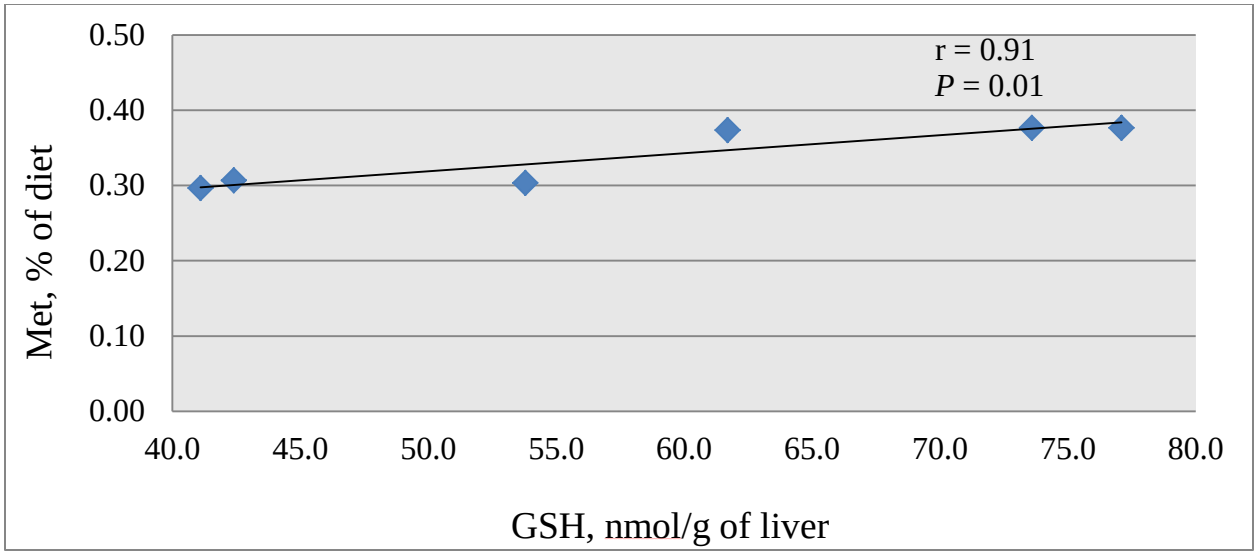

Figure 1. Correlation between dietary Met level and liver GSH concentration.