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# Vitamin E and selenium status of pigs fed DDGS diets and relationship to Mulberry Heart Disease

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## Abstract

Dietary inclusion of peroxidized lipids reduces ADFI and ADG, and compromises pig antioxidant status, leaving the pig susceptible to Mulberry Heart Disease (MHD). Levels of peroxidized lipid in dried distillers grains with solubles (DDGS) vary, but some sources can have more than 25 times higher levels of peroxidized lipid than corn as measured by the thiobarbituric acid reactive substances (TBARS) and peroxide value (PV) assays. This experiment evaluated if dietary inclusion of DDGS high in peroxidized lipid (Ox-DDGS) compromises vitamin E and Selenium (Se) status (as measured by serum and liver concentrations) and increases the incidence of MHD.

Sows (n = 12) were fed corn-soybean meal diets (0% DDGS) or diets with DDGS (40 and 20% in gestation and lactation, respectively) for 3 parities. In the third parity, 108 weaned pigs were penned (2 littermates/pen) and fed 1 of 3 nursery diets (ND): 1) 0% DDGS, 2) 30% Ox-DDGS, and 3) 30% Ox-DDGS with 5x NRC (1998) level of vitamin E for 7 weeks, resulting in a 2 x 3 factorial arrangement (n = 9 pens/treatment) of sow diet and ND. The concentration of TBARS and PV in Ox-DDGS was 25 and 27 times greater than a corn reference sample.

No evidence of MHD was found. Inclusion of DDGS in sow diets reduced ( $P < 0.01$ )  $\alpha$ -tocopherol ( $\alpha$ -T) in pig serum at weaning (5.6 vs.  $6.7 \pm 0.1$   $\mu\text{g/mL}$ ) compared with 0% DDGS. Liver  $\alpha$ -T concentration was greater ( $P < 0.01$ ) in pigs fed ND 3 than those fed ND 1 or 2 (2.4, 2.7,  $5.3 \pm 0.3$   $\mu\text{g/g}$  for ND 1, 2, and 3, respectively). Glutathione peroxidase activity and TBARS concentration of pig serum were not affected by ND. Regardless of nursery diet, vitamin E concentration of the pig serum decreased significantly ( $P < 0.05$ ) in the first four weeks after weaning. However, when pigs were fed Ox-DDGS+5xE, antioxidant status of pigs improved ( $P < 0.05$ ) at the end of the nursery period compared to pigs fed other nursery diets. The concentration of sulfur amino acids in serum from pigs fed Ox-DDGS or Ox-DDGS+5xE was 40 to 50% greater ( $P < 0.01$ ) than pigs fed 0% DDGS, which was likely due to greater ( $P < 0.01$ ) sulfur amino acid intake for pigs fed Ox-DDGS or Ox-DDGS+5xE compared to pigs fed 0% DDGS (4.4, 5.6,  $5.6 \pm 0.6$  g/d Met+Cys for ND 1, 2, and 3, respectively). Some sulfur amino acids act as antioxidants, which may have spared vitamin E and Se and masked any effect of the peroxidized lipid from DDGS. Therefore, increased vitamin E was unnecessary in nursery pig diets with Ox-DDGS. The inclusion of DDGS in sow diets reduced the Se and vitamin E status of pigs in lactation, but not after weaning when MHD is a concern. It remains unclear if antioxidant supplementation is needed in nursery diets containing peroxidized lipid without increased levels of sulfur amino acids.

## Introduction

Recent field reports (Weaver, 2010a; Weaver, 2010b) have suggested that the incidence of Mulberry Heart Disease (MHD) is increasing in the U.S. pork industry. Feeding diets low in the antioxidants vitamin E and/or selenium relative to the pig's requirement can result in oxidative stress, leading to MHD, which is a classic sign of deficiency of these nutrients. Vitamin E and/or selenium deficiency is often associated with the sudden death of nursery pigs, impaired immune system function, and reduced growth performance, all of which can contribute to significant economic losses for pork producers.

The use of dried distiller's grains with solubles (DDGS) in swine diets has increased dramatically in recent years. Concurrent with this increase has been an increase in field reports of MHD in nursery pigs. Some have theorized that DDGS inclusion in diets may be a contributing factor to the apparent increased occurrence of MHD. Concrete evidence linking high dietary DDGS to increased incidence of MHD is lacking, but some of the nutritional characteristics of DDGS may contribute to oxidative stress and ultimately MHD.

Dried distillers grains with solubles contain high levels of polyunsaturated fatty acids (PUFA) that can be highly peroxidized depending on drying temperature and time during the production of DDGS. Commonly, increased dietary concentrations of vitamin E are recommended with increased dietary PUFA (Harris and Embree 1963; Mahan, 1991; Mahan and Ullery, 2005). Interestingly, Harrell et al. (2010) demonstrated that dietary addition of an antioxidant (Santoquin) can improve growth performance of pigs fed diets containing peroxidized corn oil, but the vitamin E status of these pigs was not reported.

The dietary inclusion of peroxidized lipids impairs vitamin E status of animals, which may be alleviated with increased vitamin E supplementation (Engberg et al., 1996; Eder, 1999). The level of lipid peroxidation in DDGS (as measured by thiobarbituric acid reactive substances assay) varied considerably (1.0 to 5.2 ng malondialdehyde equivalents/mg oil) when measured in 31 DDGS sources, but the highest level measured was 26 times higher than a corn reference sample (Song et al., 2011).

Sulfur levels in DDGS are variable, but can be as high as 1% (Song et al., 2011), which is substantially greater than the level typically found in corn (0.1%). Increasing dietary levels of inorganic sulfur can be antagonistic to selenium utilization at high dietary selenium levels (Ganther and Baumann, 1962; Halverson et al., 1962; Ardüser et al., 1985), but such a relationship has not been established with typical levels of selenium (0.3 ppm). In addition to selenium, research in sheep suggests that the bioavailability of vitamin E (supplemented as dl- $\alpha$ -tocopheryl acetate) may be impaired as a result of excess dietary sulfur (Boyazoglu et al., 1967).

These data suggest that, depending on the inclusion level and quality of DDGS fed to pigs, the antioxidant status of the pig may be compromised, but no studies have been conducted to investigate this relationship. Therefore, the aim of this study was to determine if including DDGS in sow and nursery pig diets is a contributing factor to MHD, and whether supplementing diets with higher levels of vitamin E will reduce the incidence of MHD.

## Objectives

*Objective 1:* To assess the effects of dietary inclusion of DDGS in sow gestation and lactation diets on milk and colostrum composition, vitamin E and selenium status of progeny, pig growth performance, and occurrence of MHD in nursery pigs.

*Objective 2:* To evaluate the effects of dietary inclusion of DDGS high in peroxidized lipid and sulfur (Ox-DDGS) on growth performance, vitamin E and selenium status, and health of nursery pigs when supplemented with vitamin E at, or in excess of recommended levels.

## Procedures

The experimental design and procedures of this study were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Minnesota.

## Animals and Management

This experiment was conducted at the University of Minnesota's Southern Research and Outreach Center Swine Research Facility in Waseca. Two groups of 6 ( $n = 12$ ) second-parity pregnant sows (Landrace  $\times$  Yorkshire; Genetically Advanced Pigs, Winnipeg, Manitoba) bred to Duroc boars (Compart Boar Store, Nicollet, MN), were evaluated in this experiment. Sows were obtained from a separate experiment (Li et al., 2011) where DDGS was fed during gestation and lactation for 3 successive parities, and the focus of the current work is on the third parity. Progeny ( $n = 108$  pigs) from the third parity were evaluated.

Throughout gestation, sows were fed 2.04 kg/d of their assigned experimental diet. Feeding levels were adjusted to achieve a body condition score of 3 at farrowing. On d 109 of gestation, sows were moved into environmentally controlled farrowing rooms and placed in individual farrowing stalls (2.13 m long  $\times$  0.97 m high  $\times$  0.66 m wide) with fully slatted floors. Each farrowing stall was equipped with a feeder and a nipple waterer to provide sows with *ad libitum* access to water. Sows were fed 2.25 kg/d of their assigned lactation diet before farrowing. Feeding level was gradually increased after farrowing to achieve *ad libitum* access to their assigned diet within 5 d of lactation. A heat pad (Osborne Industries Inc., Osborne, KS) and a heat lamp were provided in each stall. Piglets were individually identified with ear notches at birth and weighed on d 1 and d 19 of age. Cross-fostering was performed within sow treatment group to adjust litter size to 10 to 11 piglets.

Pigs were weaned at d  $19.3 \pm 1.3$  of age. From weaning until seven weeks post-weaning, pigs were housed in pens (1.2 m  $\times$  1.2 m), and provided with *ad libitum* access to water and their assigned experimental nursery diets.

## Dietary Treatments

This experiment was conducted as a 2  $\times$  3 factorial arrangement of sow diet and nursery diet ( $n = 9$  pens/treatment). Sows were fed one of two gestation and lactation diets: corn-soybean meal diets (C) or diets with DDGS (40 and 20% in gestation and lactation) for three successive parities as part of a separate experiment (Li et al., 2011). In the third parity, 108 weaned pigs ( $n = 60$  and 48 for groups 1 and 2, respectively), half from each sow diet, were paired by similar BW within each litter and placed in nursery pens (2 littermates/pen). Within each BW block, pens were assigned to 1 of 3 nursery diets (ND): 1) Control (0% DDGS), 2) Ox-

DDGS (30% DDGS), and 3) Ox-DDGS+5xE (same as diet 2 with 5x NRC [1998] level of vitamin E as dl- $\alpha$ -tocopheryl acetate). Nursery diets were fed for 7 weeks.

### ***Diet Composition and DDGS Source***

Diet composition and nutrient concentrations of experimental diets are presented in Tables 1 and 2. Diets were fed in a 3-phase feeding program with feeding periods of one, two, and four weeks for phases 1, 2, and 3, respectively. Diets were provided in meal form and were formulated to contain similar standardized ileal digestible amino acids and available P within each phase. Nutrient concentrations of all diets met or exceeded NRC (1998) recommended nutrient requirements for nursery pigs.

The DDGS source used to formulate nursery diets was selected based on our evaluation of 31 corn DDGS sources produced by U.S. ethanol plants (Song et al., 2011). The selected DDGS source contained a greater thiobarbituric acid reactive substances (TBARS) value (5.2 ng MDA eq./mg oil), peroxide value (PV; 84.1 meq/kg oil), and total S concentration (0.95%) compared to 30 other DDGS sources sampled (mean values = 1.8 ng MDA eq./mg oil, 11.5 meq/kg oil, and 0.50%, respectively). The DDGS source used to formulate the sow diets was representative of a typical DDGS source in level of lipid peroxidation (TBARS = 1.6 ng MDA eq./mg oil).

### ***Data Collection***

On d 109 of gestation, within 24 h after farrowing, and at weaning, sows were weighed and backfat depth was determined ultrasonically (Lean-Meater, Renco Corp., Minneapolis, MN) at the last rib 6.5 cm off the dorsal mid-line on both the right and left side and averaged. Sow feed disappearance, pig mortality, and sow and pig weights at farrowing and weaning were collected.

Pigs were weighed individually the time of each nursery diet phase change, and pen feed disappearance was recorded to estimate ADFI of pigs on a pen basis. Pen ADG and ADFI were used to calculate G:F in each nursery phase and for the overall experimental period.

### ***Sample Collection***

Colostrum samples were collected on d 0 and milk samples were collected on d 7 and 19 of lactation. On d 7 and d 19, sows were given an intramuscular injection of oxytocin (30 USP) to stimulate milk release, but the d 0 colostrum samples were collected without oxytocin injection. Within 5 to 10 minutes, 20 to 30 mL of milk or colostrum was collected into plastic wide-mouthed containers by hand milking all functional glands. Samples were dispensed into a screw top plastic vial, and frozen at -20°C until further analysis.

Focal pigs (n = 2 to 3) were selected from each litter at birth and represented the first 2 apparently healthy pigs to be born regardless of sex. Blood was collected from pigs and sows via jugular venipuncture into a vacutainer tube (Becton Dickson, Franklin Lakes, NJ) on d 0 (within 24 h post farrowing), d 7, and d 19. On d 0, pigs were bled before suckling and 10 mL of blood was collected. Sows were bled while being restrained using a rope snout snare in the farrowing crate, and approximately 20 mL of blood was collected. Additional focal pigs were randomly selected to achieve one focal pig in each nursery pen, and blood (approximately 10 mL) was collected via jugular venipuncture into a vacutainer tube on d 19 (weaning), d 47, and d 68 of age. After collection blood samples were placed on ice, stored at 4°C, and centrifuged

at 1,400 x g for 10 min at room temperature. Serum was transferred into microcentrifuge tubes and frozen at -20° C until analysis.

After completion of the experiment, the focal pig from each nursery pen was transported to the University of Minnesota Veterinary Necropsy Lab (St. Paul) for sacrifice. Pigs were humanely sacrificed using a barbiturate overdose of sodium pentobarbital dosed at greater than 100 mg/kg BW. Liver samples were collected, temporarily placed on ice, and frozen at -20° C until further analysis. Heart muscle tissue was sampled at the right and left ventricles and septum. Samples were fixed in 10 % neutral buffered formalin overnight. Samples were then trimmed, embedded in paraffin, mounted onto slides, and slides were stained with haematoxylin and eosin. One trained pathologist blinded to treatments evaluated slides for presence of lesions characteristic of MHD. Before sampling, a trained technician blinded to treatments assigned each intact heart a score for gross heart lesions (0 = normal heart, 1 = abnormal heart, without characteristics of mulberry heart disease, 2 = abnormal heart with mild characteristics of MHD, and 3 = abnormal heart with severe characteristics of MHD).

### ***Laboratory Analysis***

#### ***Feed Samples***

Feed samples were retained and frozen at -20° C for subsequent nutrient analysis. Samples were submitted to commercial laboratories to analyze DM, crude fat, crude fiber, ash, nitrogen, calcium, phosphorus, sulfur, selenium,  $\alpha$ -tocopherol and amino acids.

#### ***Milk, Liver, and Serum Samples***

Concentrations of selenium and  $\alpha$ -tocopherol were measured in pig and sow serum, milk, and pig liver samples. To evaluate the metabolic oxidative status of pigs, pig serum was analyzed for TBARS, activity of the antioxidant enzyme glutathione peroxidase (GPX), and total glutathione concentration in liver. In addition, serum concentrations of amino acids were determined at d 67 of age (end of trial).

#### ***Statistical Analyses***

The MIXED procedure of SAS (SAS Inst. Inc., Cary NC) was used to evaluate the effect of sow dietary treatment on the measures obtained during the lactation period using group as a random effect. Sow and litter were the experimental unit for measures from the lactation phase. A separate analysis evaluated data collected in the nursery period using the MIXED procedure of SAS (SAS Inst. Inc., Cary NC). An ANOVA was used to evaluate the 2 x 3 factorial arrangement of sow and nursery pig diet in a split-plot design. Sow was the whole plot and nursery pen was the subplot. Pen was used as the experimental unit for data from the nursery phase. Group and nursery BW block were included as random factors in the model. The repeated measures option was used to evaluate the effect of time and its interaction with the other independent variables. Normality was evaluated using the UNIVARIATE procedure of SAS. The association of gross heart lesion score with dietary treatment was evaluated using Chi-square analysis. Results are reported as least squares means. Comparisons among treatments were performed using the PDIF option of SAS with the Tukey-Kramer

adjustment for multiple comparisons. Treatment effects were considered significant if  $P < 0.05$ , whereas values between  $0.05 \leq P \leq 0.10$  were considered statistical trends.

## **Results and Discussion**

### ***Sow and Litter Performance***

There were no effects of sow diet on the performance of sows and their litters (data not shown). However, the focus of this project was on the antioxidant status of progeny, and it was not meant to be an evaluation of sow reproductive performance.

### ***Nursery Pig Performance***

One pig died of a twisted gut and five were euthanized because of substantial body weight loss from refusing to eat after weaning, and these occurrences were unrelated to dietary treatment. None of the pigs developed Mulberry Heart Disease during the course of this experiment and gross heart lesion score was not associated with dietary treatment ( $P = 0.93$ ).

There were no interactive effects of sow and nursery diet on the growth performance of pigs. Including DDGS in sow diets did not affect growth performance of pigs during the nursery period (Table 3). However, pigs fed Ox-DDGS or Ox-DDGS+5x had improved ( $P = 0.04$  and  $0.08$ , respectively) ADFI compared to pigs fed control diets (Table 3). We may have underestimated the ME content of the DDGS source used in this experiment, which might have led to increased ADFI as the pigs attempted to fulfill their daily energy requirement. There was no effect on ADG, which substantiates this conclusion. Pigs fed control nursery diets tended ( $P = 0.08$ ) to have improved gain:feed compared to pigs fed Ox-DDGS, but gain:feed of pigs fed control diets did not differ from those fed Ox-DDGS+5x E. When evaluating dietary inclusion levels of DDGS ranging from 0 to 30%, other researchers have found variable effects on growth performance of nursery pigs. Most have found that including DDGS results in no difference in ADG (Gaines et al., 2006; Spencer et al., 2007; Barbosa et al., 2008; Burkey et al., 2008, Whitney and Shurson, 2004) or ADFI (Whitney and Shurson, 2004), but others have shown reduced ADFI (Gaines et al., 2006; Barbosa et al., 2008). However, results from other researchers may not be comparable to the present study because DDGS was not included in those diets immediately after weaning. Interestingly, we observed no effect from the inclusion of Ox-DDGS in nursery diets on ADG. Other researchers have consistently shown reduced ADG when swine (DeRouchey et al., 2004; Fernandez Dueñas, 2009; Harrell et al., 2010; Liu et al., 2012), poultry (Dibner et al., 1996), or rat (Liu and Huang, 1995) diets were supplemented with peroxidized lipids. DeRouchey et al. (2004) evaluated peroxidized choice white grease and suggested that there is a threshold point of 2.4 mEq peroxides/kg diet, above which growth performance is compromised. In the current experiment, nursery diets contained 1.7 mEq. peroxides/kg of diet (30% inclusion of DDGS [6.9% crude fat] with 84.1 mEq. peroxides/kg of oil). Therefore, it is possible that these diets did not have sufficient peroxidized lipid to elicit negative effects on pig performance reported by other researchers.

### ***Sow Vitamin E and Selenium Status***

The concentrations of  $\alpha$ -tocopherol in sow milk and serum were not affected by the inclusion DDGS in sow diets, but the concentrations of selenium in milk ( $P < 0.001$ ) and serum

( $P = 0.05$ ) were reduced compared to sows fed control diets (Table 4). The effect of sow diet on the selenium concentration of milk depended upon the day of lactation, with colostrum concentration being greater ( $P < 0.05$ ) than milk on d 7 and weaning. The concentration of  $\alpha$ -tocopherol in sow serum increased ( $P < 0.05$ ) after birth but decreased ( $P < 0.05$ ) in milk after birth. Similarly, the concentration of selenium in sow serum increased after parturition ( $P < 0.05$ ). The changes in milk selenium and vitamin E concentrations are likely a function of colostrum being more nutrient dense than milk produced later in lactation, and a similar relationship has been reported by others (Mahan et al., 2000).

### **Pig Metabolic Oxidation Status**

**Nursing Period.** The inclusion of DDGS in sow diets tended to reduce ( $P < 0.07$ ) the  $\alpha$ -tocopherol concentration of pig serum when measured during the nursing period (Table 5), and this may be linked to the reduction in the  $\alpha$ -tocopherol content of milk during lactation (Table 4) from sows fed DDGS compared to sows fed control diets. At birth, the concentration of  $\alpha$ -tocopherol in pig serum was lower ( $P < 0.05$ ) than when measured later in the nursing period. Other researchers have found similar results indicating that pigs have reduced  $\alpha$ -tocopherol status at birth (Mahan, 1991). Pigs are born with low reserves of vitamin E because transplacental transfer of this vitamin is poor (Mahan and Vallet, 1997).

Including DDGS in sow diets reduced ( $P < 0.001$ ) the selenium concentration of pig serum during the nursing period compared to sows fed control diets, and it is likely related to the reduced ( $P < 0.001$ ) selenium content of milk from these sows (Table 4). However, the relative magnitude of the difference is quite small. Interestingly, the selenium concentration of pig serum increased ( $P < 0.05$ ) from birth to weaning, despite the fact that the selenium concentration of milk declined over the course of lactation.

Serum glutathione peroxidase activity (GPX) of pigs during the nursing phase was not impacted by sow diet. However, the activity of this enzyme improved ( $P < 0.05$ ) by 67% during the nursing period. Other researchers (Loudenslager et al., 1986) have found that GPX activity increases in serum during the first weeks after birth, which is likely related to the development of the pig's ability to produce this enzyme as it matures.

The concentration of TBARS in nursing pig serum was not affected by sow diet. However, the concentration of TBARS in the serum of nursing pigs was elevated ( $P < 0.05$ ) at birth compared to d 7 and weaning. This is likely a function of the reduced vitamin E status of the pigs at birth. In other species, a negative relationship between vitamin E levels and TBARS has been reported in both serum (Yanik, 1999) and liver (Hossein Sadrzadeh et al., 1994).

**Post-weaning Period.** Pigs from sows fed DDGS began the post-weaning nursery period with lower serum  $\alpha$ -tocopherol concentrations compared with pigs from sows fed the control diet ( $6.7$  vs  $5.6 \pm 0.12$   $\mu\text{g}/\text{mL}$  for control and DDGS; Table 6). However despite this difference, average serum  $\alpha$ -tocopherol concentrations declined dramatically after weaning regardless of dietary treatment (Figure 1), but there was partial recovery of serum  $\alpha$ -tocopherol concentration by d 68 of age. There was a trend ( $P < 0.12$ ) for a sow diet x nursery diet interaction indicating that magnitude of reduction of serum  $\alpha$ -tocopherol was greater for pigs fed the DDGS diets when produced by sows fed the control diet than pigs from sows fed the DDGS diet. Interestingly, there tended ( $P < 0.08$ ) to be a sow diet x nursery diet x day

interaction suggesting that when supranutritional vitamin E was supplemented in the diets, the reduction in  $\alpha$ -tocopherol concentration appeared to be alleviated to a greater extent than pigs fed the unsupplemented Ox-DDGS diets.

There was a trend ( $P < 0.09$ ) for a sow diet x nursery diet interaction for pig serum selenium concentration (Table 6). This indicates that the magnitude of reduction in serum selenium was greater from pigs fed DDGS nursery diets from sows fed DDGS than for pigs fed DDGS nursery diets from sows fed the control diet. A sow diet x nursery diet interaction was observed ( $P < 0.05$ ) for serum TBARS (Table 6), indicating that pigs fed DDGS diets had increased serum TBARS when produced from sows fed the control diet but reduced TBARS when from sows fed the DDGS diets.

Including Ox-DDGS or Ox-DDGS+5x E in nursery pig diets increased ( $P < 0.05$ ) the total concentration of sulfur containing amino acids (sum of cystathione, cysteine, methionine, and taurine) in pig serum compared to pigs fed control diets (Table 7). This result, coupled with the improvements in the vitamin E status of the pigs fed Ox-DDGS, suggests that the increased sulfur containing amino acid content played a role in sparing the utilization of vitamin E. Sulfur containing amino acids (methionine, cysteine, and taurine) can work as important components of the antioxidant defense system.

The  $\alpha$ -tocopherol, selenium, and glutathione concentrations in pig liver were not affected by sow diet (Table 7). However, when DDGS was added to the nursery diets, liver  $\alpha$ -tocopherol increased ( $P < 0.05$ ). Feeding the DDGS nursery diet decreased ( $P < 0.05$ ) liver selenium concentration, but not when Ox-DDGS+5xE was fed. Total liver glutathione concentration was not affected by sow diet or pig diet.

### **Summary of Implications**

Long-term feeding of DDGS to sows (3 parities with dietary inclusion rates of 40% and 20% for gestation and lactation, respectively) did not compromise pig antioxidant status post-weaning. None of the pigs in this study developed Mulberry Heart Disease. Nursery diets containing a source of DDGS high in peroxidized lipid and sulfur content at levels up to 30% can be fed without compromising the vitamin E and selenium status of pigs or resulting in Mulberry Heart Disease. The lack of a negative effect on metabolic oxidation status of pigs fed DDGS diets is likely due to an increased intake of sulfur amino acids which have antioxidant properties and may have alleviated the negative effects of peroxidized lipid from DDGS. Inclusion of vitamin E levels in excess of those recommended by the NRC (1998) increased vitamin E status of pigs in the nursery, but may not be necessary.

**Table 1.** Composition of sow diets

<b>Ingredient, %</b>	<b>Gestation</b>		<b>Lactation</b>	
	<b>CON</b>	<b>DDGS</b>	<b>CON</b>	<b>DDGS</b>
Corn	74.5	54.4	61.6	51.9
DDGS	0.0	40.0	0.0	20.0
SBM, 46.5%	18.8	0.0	30.0	20.0
Choice White Grease	2.0	0.5	3.7	3.0
Dicalcium Phosphate	1.9	0.8	2.4	1.9
Limestone	1.4	2.3	1.3	1.8
Salt	0.4	0.4	0.4	0.4
Vitamin mineral premix <sup>1</sup>	0.5	0.5	0.5	0.5
Biotin <sup>2</sup>	0.2	0.2	0.2	0.2
Choline chloride, 50%	0.1	0.2	0.0	0.1
L-Lysine	0.0	0.4	0.0	0.2
L-Tryptophan	0.1	0.1	0.0	0.1
L-Threonine	0.2	0.2	0.0	0.0
DL-Methionine	0.1	0.1	0.0	0.0
Total	100.0	100.0	100.0	100.0
<b>Analyzed Nutrient Composition</b>				
ME, Kcal/kg <sup>3</sup>	3341	3351	3413	3417
CP, %	15.70	14.90	17.30	19.30
Crude fat, %	4.86	6.60	6.26	7.49
Crude fiber, %	2.47	3.72	2.37	2.78
Total Ca, %	1.28	1.21	1.20	1.34
Total P, %	0.72	0.64	0.82	0.82
Lysine, %	0.81	0.91	0.94	0.99
Cysteine, %	0.22	0.27	0.24	0.27
Methionine, %	0.22	0.27	0.24	0.26
Taurine, %	0.01	0.00	0.01	0.01
Threonine, %	0.57	0.56	0.65	0.67
Tryptophan, %	0.18	0.20	0.22	0.25
Sulfur, %	0.20	0.27	0.22	0.29
Se, mg/kg	0.56	0.46	0.55	0.68
Vitamin E, IU/kg	69.00	67.00	65.00	60.00

<sup>1</sup>Supplied the following per kilogram of diet: 12,114 IU of vitamin A (retinyl acetate); 2,753 IU of vitamin D (cholecalciferol); 66 IU of vitamin E (dl- $\alpha$ -tocopheryl acetate); 4.4 mg of vitamin K; 1 mg thiamine; 10 mg of riboflavin; 55 mg of niacin; 33 mg of pantothenic acid; 2.2 mg of pyridoxine; 1.6 mg of folic acid; 0.06 mg of vitamin B12; 0.5 mg of Iodine (ethylenediamine dihydriodide); 0.3 mg of Se (sodium selenite); 548mg of choline (chloride); 125 mg of Zn (metal polysaccharide complex of zinc sulfate); 125 mg of Fe (iron sulfate); 40 mg of Mn (manganese sulfate); and 15 mg of Cu (copper sulfate)

<sup>2</sup>Supplied 0.51 mg of biotin (JBS United Inc., Sheridan, IN) per kg of diet

<sup>3</sup>Calculated values according to NRC (1998).

**Table 2.** Composition of nursery diets

Item	Phase 1 (d 19 - d 25)			Phase 2 (d 26 - d 40)			Phase 3 (d 41 - d 68)		
	C	D	D+5xE	C	D	D+5xE	C	D	D+5xE
<b>Ingredient, %</b>									
Corn	43.4	26.4	26.3	63.5	42.7	42.6	67.9	47.5	47.4
DDGS	0.0	30.0	30.0	0.0	30.0	30.0	0.0	30.0	30.0
Soybean meal (46.5%)	23.5	10.4	10.4	32.0	23.0	23.0	29.0	19.5	19.5
Fish meal, menhaden	10.0	10.0	10.0	0.0	0.0	0.0	0.0	0.0	0.0
Whey powder	20.0	20.0	20.0	0.0	0.0	0.0	0.0	0.0	0.0
Limestone	1.0	1.4	1.4	1.2	1.6	1.6	0.9	1.3	1.3
Monocalcium phosphate	0.7	0.0	0.0	1.4	0.7	0.7	1.0	0.3	0.3
Salt	0.3	0.3	0.3	0.4	0.4	0.4	0.4	0.4	0.4
Vitamin mineral premix <sup>1</sup>	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Antibiotic <sup>2</sup>	0.5	0.5	0.5	0.5	0.5	0.5	0.0	0.0	0.0
Zinc oxide	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0
$\alpha$ -tocopheryl acetate <sup>3</sup>	0.03	0.03	0.16	0.03	0.03	0.15	0.03	0.03	0.13
L-Lysine	0.0	0.3	0.3	0.3	0.5	0.5	0.2	0.4	0.4
DL-Methionine	0.1	0.0	0.0	0.1	0.0	0.0	0.1	0.0	0.0
L-Threonine	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
<b>Analyzed Nutrient Composition</b>									
ME <sup>4</sup> , kcal/kg	3274	3322	3318	3241	3296	3292	3291	3345	3342
CP, %	23.3	23.9	24.6	20.6	22.8	23.3	19.3	20.4	21.5
Crude fat, %	2.7	3.7	3.7	2.4	3.5	3.6	2.2	3.9	3.9
Total Ca, %	1.6	1.5	1.4	1.0	1.1	1.2	0.8	0.6	0.6
Total P, %	1.0	0.9	0.9	0.7	0.7	0.8	0.6	0.6	0.6
Lysine, %	1.4	1.6	1.5	1.2	1.3	1.4	1.3	1.1	1.3
Met+Cys, %	0.7	0.8	0.8	0.6	0.7	0.6	0.5	0.7	0.8
Sulfur, %	0.3	0.6	0.7	0.2	0.5	0.6	0.2	0.4	0.5
Se, mg/kg	0.7	0.7	0.8	0.6	0.6	0.5	0.5	0.4	0.4
Vitamin E, IU/kg	14.0	19.0	60.0	11.0	15.0	67.0	12.0	13.0	44.0

<sup>1</sup> Premix supplied the following nutrients per kilogram of diet: 11,023 IU of vitamin A (retinyl acetate); 2,756 IU of vitamin D3; 4.41 mg of vitamin K (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 (ethylenediamine dihydroiodide); 0.30 mg of selenium (sodium selenite); 90.39 mg of zinc (zinc oxide, SQM); 55.11 mg of iron (ferrous sulfate, SQM); 5.51 mg of copper (copper sulfate, SQM); and 17.64 mg of manganese (manganese oxide, SQM)

<sup>2</sup> Mecadox (Carbadox 5.51 g / kg), Phibro Animal Health, Teaneck, NJ

<sup>3</sup> Concentration: 44,090 IU vitamin E / kg

<sup>4</sup> ME values were calculated using NRC (1998) values for corn and soybean meal and 3,559 kcal/kg was used for DDGS (Pedersen et al., 2007).

**Table 3.** Main effects of sow diet and nursery diet on growth performance

Item	Sow Diet <sup>1</sup>		Nursery Diet <sup>2</sup>			PSEM <sup>3</sup>	P-values	
	CON	DDGS	C	D	D+5xE		Sow diet	Nursery diet
<b>Overall</b>								
Weaning wt., kg	6.6	6.7	6.7	6.6	6.6	0.36	0.91	0.64
Final BW, kg	28.7	29.1	28.2	29.2	29.3	1.39	0.65	0.33
ADFI, g	794	793	734 <sup>a,x</sup>	830 <sup>b</sup>	816 <sup>y</sup>	82.4	0.97	0.03
ADG, g	456	469	456	463	468	28.8	0.47	0.81
Gain:Feed	0.58	0.60	0.63 <sup>x</sup>	0.57 <sup>y</sup>	0.58 <sup>x,y</sup>	0.03	0.37	0.06

<sup>1</sup>CON = corn-soybean meal diets; DDGS = gestation and lactation diets contained 20 and 40% DDGS, respectively.

<sup>2</sup>C = corn-soybean meal diets; D = diets contained 30% DDGS; D+5xE = diets containing 30% DDGS and 5 times the recommended (NRC, 1998) level of vitamin E as dl- $\alpha$ -tocopheryl acetate.

<sup>3</sup>Pooled standard error of the mean.

<sup>a,b</sup>Means within a row without a common superscript differ ( $P < 0.05$ ).

<sup>x,y</sup>Means within a row without a common superscript differ ( $P < 0.1$ ).

**Table 4.** The effect of sow diet on  $\alpha$ -tocopherol and selenium in sow serum and milk

Item	Sow Diet <sup>1</sup>			P-values	
	CON	DDGS	SEM <sup>2</sup>	Sow Diet	Sow Diet
<b>Sow serum <math>\alpha</math>-tocopherol, ug/mL<sup>3</sup></b>					
Birth <sup>a</sup>	1.51	1.35	0.258		
d 7 <sup>b</sup>	2.53	2.55	0.258		
Weaning <sup>b</sup>	2.95	2.87	0.258		
Average	2.27	2.17	0.149	0.62	0.87
<b>Milk <math>\alpha</math>-tocopherol, ug/mL<sup>3</sup></b>					
Birth <sup>a</sup>	5.13	5.01	0.999		
d 7 <sup>b</sup>	3.25	1.75	0.999		
Weaning <sup>b</sup>	1.76	1.63	0.999		
Average	3.23	2.60	0.577	0.41	0.66
<b>Sow serum selenium, ppm<sup>3</sup></b>					
Birth <sup>a</sup>	0.21	0.19	0.009		
d 7 <sup>b</sup>	0.24	0.22	0.009		
Weaning <sup>b</sup>	0.24	0.23	0.009		
Average	0.23	0.21	0.005	0.05	0.86
<b>Milk selenium, ppm<sup>3</sup></b>					
Birth <sup>a</sup>	0.40 <sup>c</sup>	0.30 <sup>d</sup>	0.008		
d 7 <sup>b</sup>	0.11	0.09	0.008		
Weaning <sup>b</sup>	0.11	0.09	0.008		
Average	0.20	0.16	0.004	< 0.001	< 0.001

<sup>1</sup>CON = corn-soybean meal diets; DDGS = gestation and lactation diets contained 20 and 40% DDGS, respectively.

<sup>2</sup>Pooled standard error of the mean.

<sup>3</sup>Time effect (P < 0.05).

<sup>a,b</sup>Means within each variable without a common superscript differ (P < 0.05).

<sup>c,d</sup>Means within a row without a common superscript differ (P < 0.05).

**Table 5.** The effect of sow diet on serum  $\alpha$ -tocopherol, selenium, glutathione peroxidase, and TBARS in nursing pigs

Item	Sow Diet <sup>1</sup>			P-values	
	CON	DDGS	SEM <sup>2</sup>	Sow diet	Sow Diet x Day
<b>Pig serum <math>\alpha</math>-tocopherol, ug/mL<sup>3</sup></b>					
Birth <sup>a</sup>	0.41	0.36	0.492		
d 7 <sup>b</sup>	6.39	5.28	0.492		
Weaning <sup>b</sup>	6.47	5.43	0.492		
Average	4.42	3.69	0.307	0.07	0.46
<b>Pig serum selenium, ppm<sup>3</sup></b>					
Birth <sup>a</sup>	0.07	0.06	0.003		
d 7 <sup>b</sup>	0.10	0.09	0.003		
Weaning <sup>c</sup>	0.12	0.11	0.003		
Average	0.09	0.09	0.002	< 0.001	0.85
<b>Pig serum glutathione peroxidase activity, units<sup>4</sup>/mL<sup>3</sup></b>					
Birth <sup>a</sup>	0.11	0.10	0.019		
d 7 <sup>b</sup>	0.15	0.14	0.019		
Weaning <sup>c</sup>	0.18	0.17	0.011		
Average	0.14	0.14	0.009	0.36	0.98
<b>Pig serum thiobarbituric acid reactive substances, uM malondialdehyde eq.<sup>3</sup></b>					
Birth <sup>a</sup>	6.17	7.52	0.779		
d 7 <sup>b</sup>	1.22	1.39	0.744		
Weaning <sup>b</sup>	2.25	2.04	0.779		
Average	3.21	3.65	0.440	0.49	0.58

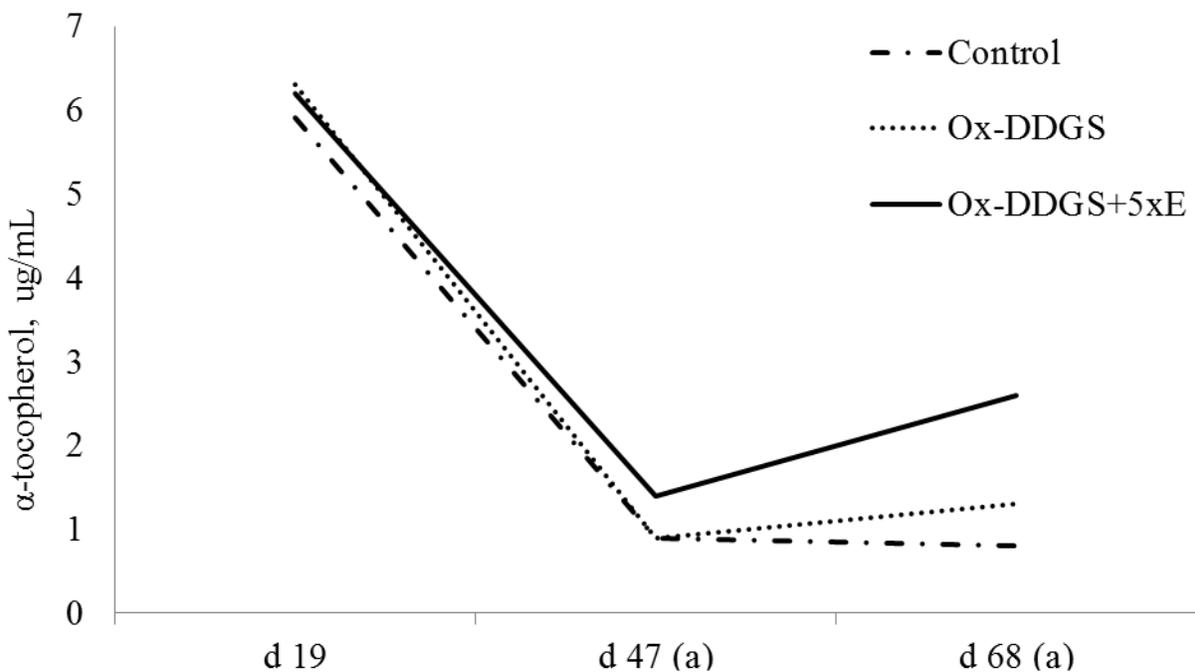
<sup>1</sup>CON = corn-soybean meal diets; DDGS = gestation and lactation diets containing 20 and 40% DDGS,

<sup>2</sup> Pooled standard error of the mean.

<sup>3</sup>Time effect (P < 0.05).

<sup>4</sup>Unit = umol NADPH oxidized/minute/mL of serum

<sup>a,b,c</sup>Means within each variable without a common superscript differ (P < 0.05).



**Figure 1.** Effect of dietary dried distiller’s grains with solubles (DDGS) on the concentration of  $\alpha$ -tocopherol in serum from nursery pigs. Control = corn-soybean meal (0% DDGS); Ox-DDGS = diet containing 30% DDGS high in peroxidized lipid; Ox-DDGS+5xE = diet containing 30% DDGS high in peroxidized lipid supplemented with 5 times the recommended (NRC, 1998) level of vitamin E as dl- $\alpha$ -tocopheryl acetate. Time effect ( $P < 0.01$ ). Diet x Time effect ( $P < 0.01$ ). (a) Within each day, pigs fed Ox-DDGS+5xE > other 2 diets ( $P \leq 0.05$ ; PSE = 0.12).

**Table 6.** Effect of sow diet x nursery diet over time on serum  $\alpha$ -tocopherol, selenium, and TBARS of nursery pigs

Item	Sow diet <sup>1</sup> : CON			Sow diet: DDGS			SEM <sup>3</sup>	P-values	
	Nursery Diet <sup>2</sup> : C	Ox-D	Ox-D +5xE	C	Ox-D	Ox-D +5xE		Sow Diet x Nursery Diet	Sow Diet x Nursery Diet x Day
Pig serum $\alpha$ -tocopherol, $\mu\text{g}/\text{mL}$ <sup>4</sup>									
Wean, d19 <sup>a</sup>	6.48	6.52	6.96	5.42	6.08	5.37	0.194		
d 47 <sup>b</sup>	0.96	0.90	1.35	0.81	0.95	1.49	0.176		
d 68 <sup>c</sup>	0.91	1.29	2.57	0.69	1.28	2.58	0.176		
Average	2.78	2.90	3.62	2.30	2.77	3.15	0.123	0.12	0.08
Pig serum selenium, ppm <sup>4</sup>									
Wean, d19 <sup>a</sup>	0.12	0.12	0.11	0.11	0.11	0.10	0.006		
d 47 <sup>b</sup>	0.15	0.15	0.14	0.14	0.16	0.13	0.006		
d 68 <sup>c</sup>	0.16	0.15	0.16	0.16	0.17	0.16	0.006		
Average	0.14	0.14	0.16	0.16	0.11	0.11	0.005	0.09	0.45
Pig serum thiobarbituric acid reactive substances, $\mu\text{M}$ malondialdehyde eq. <sup>4</sup>									
Wean, d19 <sup>x</sup>	0.68	0.84	0.74	0.79	0.74	0.74	0.080		
d 47 <sup>y</sup>	0.55	0.71	0.59	0.76	0.69	0.71	0.080		
d 68 <sup>x</sup>	0.65	0.66	0.74	0.95	0.79	0.79	0.080		
Average	0.62 <sup>d</sup>	0.74 <sup>d,e</sup>	0.69 <sup>d,e</sup>	0.83 <sup>e</sup>	0.74 <sup>d,e</sup>	0.75 <sup>d,e</sup>	0.050	0.05	0.82

<sup>1</sup>CON = corn-soybean meal diets; DDGS = gestation and lactation diets containing 20 and 40% DDGS, respectively.

<sup>2</sup>C = corn-soybean meal diets; Ox-D = diets containing 30% DDGS; D = diets containing 30% DDGS and 5 times the recommended (NRC, 1998) level of vitamin E as dl- $\alpha$ -tocopherol acetate

<sup>3</sup>Pooled standard error of the mean.

<sup>4</sup>Age effect ( $P < 0.05$ ).

<sup>a-c</sup>Means within a variable without a common superscript differ ( $P < 0.05$ ).

<sup>x,y</sup>Means within a variable without a common superscript differ ( $P < 0.1$ ).

<sup>d,e</sup>Means within a row without a common superscript differ ( $P < 0.05$ ).

**Table 7.** Effect of sow diet and nursery diet on serum amino acids and liver  $\alpha$ -tocopherol, selenium, and glutathione content of nursery pigs

Item	Sow Diet <sup>1</sup>		Nursery Diet <sup>2</sup>				P-values	
	CON	DDGS	C	D	D+5xE	PSEM <sup>3</sup>	Sow diet	Nursery Diet
<b>Serum amino acids<sup>4</sup>, umol/L</b>								
Cystathione	5.4	5.8	5.2 <sup>a</sup>	5.6 <sup>a,b</sup>	6.2 <sup>b</sup>	0.39	0.230	0.032
Cystine	2.5	2.9	2.4	2.8	2.8	0.86	0.125	0.100
Methionine	58.8	54.6	48.3 <sup>x</sup>	59.1 <sup>x,y</sup>	62.6 <sup>y</sup>	5.50	0.488	0.089
Taurine	234.4	278.6	198.6 <sup>a</sup>	256.2 <sup>a,b</sup>	314.6 <sup>b</sup>	56.98	0.225	0.001
Total S-amino acids <sup>5</sup>	315.3	343.0	247.6 <sup>a</sup>	343.3 <sup>b</sup>	396.6 <sup>b</sup>	49.55	0.496	<0.001
<b>Liver<sup>6</sup></b>								
$\alpha$ -Tocopherol, ug/g	3.5	3.2	2.1 <sup>a</sup>	2.6 <sup>b</sup>	5.2 <sup>b</sup>	0.21	0.272	<0.001
Selenium, ug/g	0.6	0.6	0.64 <sup>a</sup>	0.50 <sup>b</sup>	0.58 <sup>a,b</sup>	0.05	0.841	0.023
Total glutathione, nmol/g	97.8	99.6	94.4	87.7	114.1	8.19	0.863	0.094

<sup>1</sup>CON = corn-soybean meal diets; DDGS = gestation and lactation diets containing 20 and 40% DDGS, respectively.

<sup>2</sup>C = corn-soybean meal diets; D = diets containing 30% DDGS D = diets containing 30% DDGS and 5 times the recommended (NRC, 1998) level of vitamin E as dl- $\alpha$ -tocopheryl acetate.

<sup>3</sup>Pooled standard error of the mean.

<sup>4</sup>Final serum sample, d 68 of age.

<sup>5</sup>Sum of Cystathione, Cys, Met, and Tau; Sow Diet x Nursery Diet interaction ( $P = 0.02$ ).

<sup>6</sup>Liver data are on "as-is" basis.

<sup>a,b</sup>Means within a row without a common superscript differ ( $P < 0.05$ ).

<sup>x,y</sup>Means within a row without a common superscript differ ( $P < 0.1$ ).

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