LIPID PEROXIDATION IN CORN DRIED DISTILLERS GRAINS WITH SOLUBLES (DDGS) AND EFFECTS OF FEEDING A HIGHLY OXIDIZED DDGS SOURCE TO SWINE

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS i
DEDICATION ii
TABLE OF CONTENTS iii
LIST OF TABLES vi
LIST OF FIGURES viii
CHAPTER I. Literature Review 1
I. Introduction 1
II. Corn DDGS in Swine Diets 4
1. Energy Content and Digestibility in DDGS 4
2. Amino Acid Digestibility in DDGS 6
3. Lipid Peroxidation in DDGS 10
4. Sulfur in DDGS 12
5. Feeding DDGS to Swine 13
- Effects on Growth Performance 14
- Effects on Carcass Characteristics 16
- Effects on Pork Fat Quality 17
- Effects on Loin Muscle (LM) Oxidation and Food Safety Concerns 18
III. Lipid Peroxidation 20
1. Free Radicals and Oxygen Species 21
2. Process of Lipid Peroxidation – A Free Radical Chain Reaction 22
- Formation of Hydroperoxides from Linoleic Acid 24
- Decomposition of Hydroperoxides 25
1) Decomposition of Hydroperoxides by Hemolytic Cleavage 26
2) Decomposition of Hydroperoxides by Heterolytic Cleavage 27
- Formation of Malondialdehyde 30
3. Measurement of Lipid Peroxidation Level in Oils and Fats 30
- Predictive Tests 31
1) Active Oxygen Method (AOM) 31
2) Oxidative Stability Index 32
3) Oxygen Bomb Test 32
4) Iodine Value 33
- Indicator Tests 34
1) Peroxide Value 34
2) Thiobarbituric Acid Reactive Substances 35
3) Anisidine Value 36
4) Measurements of 4-Hydroxy-2-trans-nonenal 37
5) Hexanal Value 38
IV. Oxidative Stress and Dietary Oxidized Lipids 38
1. Use of Fat in Swine Diets 39
2. Characteristics of Thermally Oxidized Lipids 42

3. Feeding Oxidized Lipids to Animals 44
- Effects on Growth Performance 44
- Effects on Metabolic Oxidation Status 45
- Effects on Lipid Metabolism via PPARα 46
- Effects on Nutrient Digestibility 47
- Effects on Gut Barrier Function 48
V. Antioxidants in Swine Nutrition 52
1. Antioxidant System 52
2. Vitamin E 53
- Structure and Sources 53
- Antioxidant Functions of Vitamin E 55
- Accumulation of Vitamin E in Tissues 57
- Supplementation of Vitamin E under Dietary Oxidative Challenge 58
- Effects of Supplementation of Vitamin E on Pork Quality 60
3. Sulfur-Containing Compounds 63
- Structure and Sources 63
- Metabolism and *De Novo* Synthesis 64
- Antioxidant Properties 66
VI. Summary 67

CHAPTER II. Technical note: Evaluation of lipid peroxidation level in corn dried distillers grains with solubles (DDGS) 69
INTRODUCTION 70
MATERIALS AND METHODS 71
RESULTS 73
DISCUSSION 74

CHAPTER III. High Sulfur Content in Corn Dried Distillers Grains With Solubles (DDGS) Protects Against Oxidized Lipids in DDGS by Increasing Sulfur-Containing Antioxidants in Nursery Pigs 81
INTRODUCTION 82
MATERIALS AND METHODS 83
RESULTS 90
DISCUSSION 93

CHAPTER IV. Impact of Highly Oxidized Dried Distillers Grains With Solubles at Increasing Dietary Vitamin E Levels on Energy and Nutrient Digestibility and Metabolic Oxidation Status in Growing Pigs 113
INTRODUCTION 114
MATERIALS AND METHODS 115
RESULTS 120
DISCUSSION 123

CHAPTER V. Effects of Feeding Diets Containing Highly Oxidized Dried Distillers Grains with Solubles and Increasing Vitamin E Levels to Wean-Finish Pigs on Growth Performance, Carcass Characteristics, and Pork Fat Composition 141
INTRODUCTION 142

MATERIALS AND METHODS 144
RESULTS 149
DISCUSSION 153
LITERATURE CITED 184

LIST OF TABLES

Table 1-1. Concentrations of energy in corn and 10 sources of corn dried distillers grains with solubles (DDGS) fed to growing pigs (Adapted from Pedersen et al., 2007) 5

Table 1-2. Energy content and digestibility in 10 sources of corn dried distillers grains with solubles (DDGS) fed to growing pigs (Adapted from Stein et al., 2006)..... 6

Table 1-3. Concentration and standardized ileal digestibility (SID) of CP and AA in distillers dried grains with solubles (DDGS) fed to growing pigs (Stein and Shurson, 2009) 7

Table 1-4. Calibration and validation statistics for predicting amino acid content of DDGS (Shurson, 2011)..... 10

Table 1-5. Fatty acid composition of vegetable oils..... 11

Table 1-6. Maximum recommended dietary inclusion for DDGS in swine diets (Adapted from Stein and Shurson, 2009) 14

Table 1-7. Typical fatty acids found in swine diets and tissue depots (Adapted from Azain, 2001)..... 40

Table 1-8. Summary of effects of supplemental dietary fat on growth performance and carcass characteristics in growing-finishing pigs (Xu, 2007)..... 42

Table 1-9. Reactive oxygen species scavenging enzymes (Blokhina et al., 2003)..... 53

Table 1-10. Tocopherol and tocotrienol contents (mg/kg) in some seed oils (Adapted from Gunstone et al., 1994) 55

Table 1-11. Mean a-tocopherol content of plasma (mg α-tocopherol/mL) and tissues (ng α-tocopherol/mg of protein) from pigs fed a control diet (containing 30 mg α-tocopheryl acetate/kg of feed) or a diet supplemented with vitamin E (200 mg α-tocopheryl acetate/kg of feed) for approximately 125 days prior to harvest (Monahan et al., 1990). 58

Table 1-12. Influence of dietary vitamin E supplementation on muscle vitamin E deposition and refrigerated pork quality and storage stability (Jensen et al., 1998)..... 62

Table 2-1. Lipid peroxidation and color in corn dried distillers grains with solubles (DDGS)..... 79

Table 3-1. Composition and nutrient analysis of Phase 1 diets (7 - 11 kg BW, as-fed basis) 103

Table 4-1. Analyzed composition of dried distillers grains with solubles (DDGS) source used in this experiment (as-fed basis)..... 133

Table 4-2. Composition and nutrient analysis of experimental diets (as-fed basis) 134

Table 4-3. Influence of DDGS and vitamin E supplementation on serum thiobarbituric acid-reactive substance (TBARS) and α-tocopherol concentration[1] 136

Table 4-5. Energy and N balance for pigs fed experimental diets (as-fed basis)[1] 138

Table 4-6. Daily C and S balance for pigs fed experimental diets (as-fed basis)[1] 139

Table 4-7. Apparent total tract digestibility (ATTD) of GE, N, C and S of experimental diets[1] 140

Table 5-1. Composition and nutrient analysis of Phase 1 diets (6 - 9 kg BW, as-fed basis) 164

Table 5-2. Composition and nutrient analysis of Phase 2 diets (9 - 16 kg BW, as-fed basis) 166

Table 5-3. Composition and nutrient analysis of Phase 3 diets (16 - 27 kg BW, as-fed basis) 168

Table 5-4. Composition and nutrient analysis of Phase 4 diets (27 - 45 kg BW, as-fed basis) 170

Table 5-5. Composition and nutrient analysis of Phase 5 diets (45 - 68 kg BW, as-fed basis) 172

Table 5-6. Composition and nutrient analysis of Phase 6 diets (68 - 91 kg BW, as-fed basis) 174

Table 5-7. Composition and nutrient analysis of Phase 7 diets (91 - 113 kg BW, as-fed basis) 176

Table 5-8. Effects of dietary dried distillers grains with solubles (DDGS) and increasing levels of vitamin E on growth performance[1] 178

Table 5-9. Effects of dietary dried distillers grains with solubles (DDGS) and increasing levels of vitamin E on carcass and ultrasound measurements[1] 180

Table 5-10. Effects of dietary dried distillers grains with solubles (DDGS) and increasing levels of vitamin E on fatty acid profile of belly fat and backfat samples[1] 181

Table 5-11. Influence of dietary dried distillers grains with solubles (DDGS) and vitamin E supplementation on thiobarbituric acid-reactive substance (TBARS) and α-tocopherol concentration in LM[1] 183

LIST OF FIGURES

Figure 1-1. Hypothetical free-radical induced autoxidation reaction (Seppanen, 2005).. 23

Figure 1-2. General process of lipid peroxidation (Seppanen, 2005) 23

Figure 1-3. Formation of 9- and 13- hydroperoxides from linoleic acid (Adapted from Seppanen, 2005)..... 25

Figure 1-4. Decomposition of fatty acid hydroperoxides (Seppanen 2005)..... 26

Figure 1-5. Hemolytic cleavage of fatty acid hydroperoxide (Seppanen, 2005)..... 27

Figure 1-6. Secondary oxidation products from the hemolytic cleavage of methyl linoleate hydroperoxides (Seppanen, 2005)..... 28

Figure 1-7. Heterolytic cleavage of fatty acid hydroperoxide (Seppanen, 2005)..... 29

Figure 1-8. Changes of peroxide value (PV) with development of lipid peroxidation..... 35

Figure 1-9. Simple and mixed triglycerides (adapted from Food Fats and Oils, 2006) ... 40

Figure 1-10. Tocopherol content of soybean oil heated at 185 ℃ for 0–10 h (Seppanen and Csallany, 2002) 43

Figure 1-11. Normal (left) and "leaky" (right) small intestine (Redelmeier, 2009)..... 49

Figure 1-12. Chemical structure of tocopherols and tocotrienols..... 55

Figure 1-13. Chemical structure of α-tocopheryl acetate 55

Figure 1-14. Lipid peroxidation process and effect of α-tocopherol (Burton and Traber, 1990) 57

Figure 1-15. Structures of the sulfur-containing amino acids (Brosnan and Brosnan, 2006) 63

Figure 1-16. Structure of glutathione (The Merck Index, 1989) 63

Figure 1-17. Major pathways of sulfur-containing amino acid metabolism (Brosnan and Brosnan, 2006)..... 65

Figure 2-1. Correlation between peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) in corn dried distillers grains with solubles (DDGS)..... 79

Figure 2-2. Correlation between thiobarbituric acid reactive substances (TBARS) and Minolta color (A and B), and peroxide value (PV) and Minolta color (C and D)_in corn dried distillers grains with solubles (DDGS)..... 80

CHAPTER I. Literature Review

I. Introduction

Within the past decade, the United States has experienced a rapid growth in corn ethanol production. With every gallon of ethanol produced, approximately 2.6 kg of distillers grains are produced (RFA, 2012). In 2011, the United States ethanol industry produced 35.7 million metric tons of distillers grains for use in livestock and poultry feeds (RFA, 2012), among which approximately 3.9 million metric tons of dried distillers grains with solubles (DDGS) were used as a feed ingredient in pork production (RFA, 2012). Due to the cost competitiveness of DDGS relative to the recent high prices for corn and soybean meal, pork producers have saved between $3 to 9 per market hog by adding up to 40% DDGS to grower-finisher diets. However, the long-term sustainability of this level of usage, and the potential to further increase DDGS usage in grower-finisher swine diets is in jeopardy. Concerns about the potential negative impact of oxidized corn oil in DDGS on health and antioxidant status of pigs, and the impact of feeding high concentrations PUFA present in DDGS lipid on pork fat quality may limit the use of high levels (20 to 30%) of DDGS in swine diets.

Corn DDGS contains approximately 10% corn oil. Corn oil contains high levels of polyunsaturated fatty acids (particularly linoleic acid; NRC, 1998) that are vulnerable to lipid peroxidation. Drying temperatures used by ethanol plants can vary substantially, and increased drying time and temperature used during the drying process also accelerate lipid peroxidation. Lipid peroxidation, also called lipid oxidation, refers to the oxidative degradation of lipids (Frankel, 1987). Lipid peroxidation proceeds by a sequential free

radical chain-reaction mechanism that involves the production of a series toxic secondary lipid peroxidation product (Tappel, 1977). The occurrence of lipid peroxidation in foods or feed ingredients leads to quality and nutritional losses, while it can also take place *in vivo* leading to several negative effects, including damage of cellular and subcellular bio-membranes, growth depression of animals, and undesirable meat and fat quality. Oxidative damage in feedstuffs reduces feeding values of the ingredients. Lipid peroxidation in animal feed has been shown to negatively affect pig health and growth performance (Miller and Brzezlnska-Slebodzlnska, 1993; Pfalzgraf et al., 1995), resulting in more days on feed to reach market weight. Furthermore, changes in body composition, pork quality, and shelf-life stability of fresh and ground pork may occur, leading to a decrease in overall acceptance of pork from animals fed under these dietary conditions.

Vitamin E, supplemented in diets, can be deposited in fat associated with muscle tissue as α-tocopherol. Vitamin E is the most important natural antioxidant to protect against lipid peroxidation and increase pork shelf-life stability (Jensen et al., 1998). Feeding DDGS containing oxidized lipids to pigs may require supplementation of higher levels of antioxidants (e.g. vitamin E) than currently being fed. For example, supplementation of additional antioxidants improved growth performance in pigs fed diets containing 20% DDGS or 5% oxidized corn oil (Harrell et al., 2010). Therefore, the objectives of the following experiments were to evaluate the effects of feeding DDGS containing oxidized lipids on growth performance, metabolic oxidation status, nutrient digestibility, carcass characteristics, pork fat composition, and loin peroxidation status, as well as to determine if any negative effects could be overcome by increasing the dietary

level of vitamin E (α-tocopheryl acetate).

II. Corn DDGS in Swine Diets

With the expansion of the ethanol industry, the utilization of corn co-products, such as DDGS, in swine feeds has increased dramatically due to increased availability and cost competitiveness compared with corn and soybean meal. However, often with DDGS, limits on dietary inclusion rates occur because of inconsistent growth performance when high dietary levels of DDGS (20 to 30%) are fed to growing-finishing pigs (Whitney et al., 2006a; Linneen et al., 2008; Stein and Shurson, 2009). Potential risk factors, such as low net energy levels, poor amino acid (**AA**) balance and digestibility, excess dietary nitrogen (crude protein), oxidized lipids, and high S content may contribute to the inconsistent growth responses reported when adding DDGS to swine diets. Although many experiments have been conducted to evaluate the use of DDGS in swine diets, none of them have focused on lipid peroxidation in DDGS and effects on pig growth performance and meat quality. Oxidized lipids in DDGS may not only be a contributing factor for reduced growth performance, but could also lead to increased peroxidation level and decreased shelf-life stability in meat products due to high levels of PUFA in muscle tissues as a result of feeding DDGS (Xu et al., 2010). Additionally, the toxic aldehydes produced from peroxidation of PUFA in meat products may potentially be a human health concern. This review will discuss some of the concerns related to feeding DDGS to growing-finishing pigs as well as the possible effects of oxidized lipids in DDGS on growth performance, carcass characteristics and meat quality.

1. Energy Content and Digestibility in DDGS

Pedersen et al. (2007) measured GE, DE and ME, and the apparent total tract

digestibility of energy from 10 DDGS samples, and the results, as well as the comparisons to corn are shown in **Table 1-1**. The average concentration of GE in DDGS is approximately 5,434 kcal/kg of DM, which is greater than that in corn (4,496 kcal/kg of DM). However, the digestibility of energy in DDGS, measured as percentage of GE, is 15% lower than that in corn. The average DE and ME content of 10 DDGS samples were 4,101 and 3,897 kcal/kg DM, respectively, with a small variation (DE CV = 5.0 %, and ME CV = 5.4 %) among sources. These values are similar to the DE and ME in corn (4,088 and 3,989 kcal/kg of DM, respectively). Stein et al. (2009) reported similar values of GE (mean value = 4,992 kcal/kg or 5,592 kcal/kg of DM), ATTD of GE (mean value = 75.1%), DE (mean value = 4,072 kcal/kg of DM) and ME (mean value = 3,751 kcal/kg of DM) in corn DDGS by analyzing four sources of DDGS produced from corn grown within a narrow geographical area. Moreover, Anderson et al. (2012) also reported energy content of corn DDGS by analyzing a six DDGS sources. The mean values of GE, DE and ME were 5,420, 4,029, and 3,790 kcal/kg of DM, respectively, which were similar to those reported by Pedersen et al. (2007) and Stein et al. (2009).

Table 1-1. Concentrations of energy in corn and 10 sources of corn dried distillers grains with solubles (DDGS) fed to growing pigs (Adapted from Pedersen et al., 2007)

Item	Corn	DDGS			
		Average	Lowest value	Greatest value	CV, %
GE, kcal/kg of DM	4,496	5,434	5,272	5,592	2.0
ATTD[1] of energy, %	90.4	76.8	73.9	82.8	3.6
DE, kcal/kg of DM	4,088	4,140	3,947	4,593	5.0
ME, kcal/kg of DM	3,989	3,897	3,674	4,336	5.4

[1]ATTD: apparent total tract digestibility

Additionally, Stein et al. (2006) also analyzed 10 DDGS samples and reported GE, DE, and ATTD of GE of these DDGS samples as shown in **Table 1-2**. Compared with the data from Pedersen et al. (2007) and Stein et al. (2009), the average GE, ATTD of GE and DE reported by Stein et al. (2006) were lower. The differences in these values could be due to the variation of DDGS sources, and/or differences in analytical and experimental procedures they used. For example, the ATTD of GE in the Pedersen et al. (2007) study was calculated using the GE concentration in feces, while Stein et al. (2006) used the GE concentration in ileal digesta, which contains higher GE than feces. Thus, Stein et al. (2006) obtained a lower ATTD of GE. Furthermore, in the study of Stein et al. (2006), the DE in each of the DDGS samples was calculated by subtracting one-third of the DE in the N-free diet from the DE in each of the DDGS-containing diets according to the difference procedure (Adeola, 2001), while Pedersen et al. (2007) did not include an N-free diet.

Table 1-2. Energy content and digestibility in 10 sources of corn dried distillers grains with solubles (DDGS) fed to growing pigs (Adapted from Stein et al., 2006)

Item	DDGS			
	Average	Lowest value	Greatest value	CV, %
GE, kcal/kg	4,451	4,366	4,522	1.0
ATTD[1] of GE, %	66.0	62.7	70.5	3.8
DE, kcal/kg of DM	3,556	3,382	3,811	4.0

2. Amino Acid Digestibility in DDGS

The concentration and standardized ileal digestibility (SID) of AA from 39 sources of corn DDGS, 1 source of sorghum DDGS and 2 sources of wheat DDGS were

summarized by Stein and Shurson (2009). As shown in **Table 1-3**, the digestibility of most AA in DDGS is approximately 10 percentage units less than in corn. In addition, a large variation (55.7 to 68.7%) of AA digestibility among difference sources of DDGS also exists, especially for lysine (Urriola, 2009). The reason for the low lysine digestibility and high variation is believed to be that some sources of DDGS have been heat-damaged during the drying process. Excessive heat during the drying process not only darkens the color of DDGS (Parsons et al., 1992), but also impairs availability and digestibility of lysine resulting from the Maillard reactions between the amino residues and carbohydrate moieties (Fastinger and Mahan, 2006). The bound and converted lysine is apparently not available for use by the animal, nor does all of it seem to be easily released during acid hydrolysis conditions used in AA analysis (Hurrell, 1983), thereby leading to the reduced digestibility of lysine in DDGS.

Table 1-3. Concentration and standardized ileal digestibility (SID) of CP and AA in distillers dried grains with solubles (DDGS) fed to growing pigs (Stein and Shurson, 2009)

Item	Concentration of CP and AA, %			SID of CP and AA, %		
	Corn DDGS	Sorghum DDGS	Wheat DDGS	Corn DDGS	Sorghum DDGS	Wheat DDGS
CP	27.38	31.50	40.67	72.8	71.4	72.2
Indispensable AA						
Arg	1.14	1.06	1.53	81.1	78.2	83.4
His	0.71	0.68	0.92	77.1	70.6	74.2
Ile	1.00	1.31	1.35	75.3	72.7	77.2
Leu	3.11	4.02	2.66	83.5	76.3	81.4
Lys	0.76	0.66	0.65	60.6	62.0	47.7
Met	0.54	0.51	0.53	81.8	75.4	79.5
Phe	1.32	1.62	1.92	80.8	75.8	85.1
Thr	1.04	1.03	1.21	70.4	68.6	70.4
Trp	0.21	0.34	0.40	69.6	70.4	80.4
Val	1.34	1.59	1.70	74.4	72.3	80.1
Dispensable AA						

Ala	1.89	2.79	1.48	78.1	73.4	68.0
Asp	1.80	2.09	1.92	68.1	68.0	56.7
Cys	0.52	0.47	0.73	72.5	65.6	71.6
Glu	4.24	6.08	9.81	80.4	75.5	86.3
Gly	1.02	0.99	1.62	63.2	66.9	67.8
Pro	2.06	2.41	4.11	74.1	83.1	81.0
Ser	1.14	1.35	1.88	75.9	72.5	77.0
Tyr	1.00	-	-	80.9	-	-

The large variation in AA digestibility among sources of DDGS creates a challenge for animal nutritionists attempting to formulate diets containing DDGS for swine. To reduce the risk of under feeding digestible lysine and for more accurate diet formulation, we need rapid, accurate, and inexpensive "nutritional tools" to estimate total and SID AA values among DDGS sources. One of these methods of estimating digestible AA in DDGS is color measurement with Minolta or HunterLab spectrophotometers. In theory, darker-colored DDGS may represent a greater degree of Maillard reactions during processing, and thus have a lower AA digestibility compared with a lighter-colored DDGS (Cromwell et al., 1993; Fastinger and Mahan, 2006). However, Urriola et al. (2007) analyzed SID of AA from 36 DDGS samples, and showed that color, as measured by L*, or b*, was poorly correlated to AA digestibility in DDGS. Instead, the same authors suggested that a front face fluorescence method may be a more accurate way to predict SID of amino acids and digestible nutrient content, but this approach involves complex calculations using principle components analysis, which may not be applicable in practical DDGS digestible AA evaluations. Furthermore, studies have also been conducted to develop *in vitro* procedures to estimate SID Lys concentration in corn DDGS (Pahm et al., 2008; Kim et al., 2010). The digestibility of Lys in DDGS may be estimated using the following equation (Pahm et al., 2008):

Standardized ileal digestible Lys (%) = 0.023 + 0.637 × reactive Lys (%)

Reactive Lys can be calculated from the concentration of furosine that is analyzed in DDGS after acid hydrolysis of the sample using the following equation (Pahm et al., 2008):

Reactive Lys (%) = analyzed Lys (%) – furosine (%) / 0.32 × 0.40

The concentration of SID Lys can also be calculated without including reactive Lys by using the concentration of total Lys and CP in the equation (Stein, 2011):

Standardized ileal digestible Lys (%) = -0.636 + [0.858 × Lys (%)] × [0.12 × (100 × Lys (%) / CP (%))]

By using one of the above equations, it is possible to predict the concentration of digestible Lys in a given source of DDGS, and this value can then be used in diet formulations.

In addition to digestible AA, total AA content in DDGS can also be estimated. Near infrared spectroscopy (NIR) is a popular method that can be considered to estimate AA content in DDGS. The NIR technology has been used many years for estimating nutrient and chemical composition of a variety of foods and feed ingredients. This technology is based on a simple principle: absorption of infrared light by organic matter. A sample can partially or selectively absorb this radiation, and thus provides the chemical composition of the organic molecules present (Gady et al., 2007). Researchers from the University of Minnesota (Shurson, 2011) recently developed NIR calibrations for proximate analysis components, minerals, and AA among DDGS sources using a common Perten NIR instrument. As shown in **Table 1-4**, the predicted values of AA content in DDGS are close to the reference values, and the standard errors of calibration

and prediction are relatively low. These results suggest that NIR can be a valuable tool for estimating AA content of DDGS sources, and thus a good method of choice for qualifying the current and evolving DDGS variability.

Table 1-4. Calibration and validation statistics for predicting amino acid content of DDGS (Shurson, 2011)

Amino acid, %	SEC[a]	R[b]	Bias[c]	SEP[d]
Arginine	0.11	0.87	-0.04	0.14
Cysteine	0.23	0.83	-0.09	0.29
Histidine	0.06	0.82	-0.01	0.08
Isoleucine	0.07	0.73	0.05	0.08
Leucine	0.20	0.82	0.05	0.23
Lysine	0.55	0.81	-0.07	0.65
Methionine	0.09	0.84	0.00	0.11
Phenylalanine	0.10	0.80	0.07	0.11
Threonine	0.06	0.80	0.00	0.07
Tryptophan	0.18	0.80	-0.03	0.23
Valine	0.16	0.90	-0.01	0.22

[a]SEC = standard error of the calibration
[b]R = correlation coefficient
[c]Bias = mean of differences between reference and predicted values
[d]SEP = standard error of prediction

3. Lipid Peroxidation in DDGS

Corn DDGS contains approximately 10% corn oil (Stein and Shurson, 2009). Compared to other vegetable oils, corn oil contains relatively high levels of PUFA (particularly linoleic acid; **Table 1-5**; NRC, 1998) that are vulnerable to lipid peroxidation (Seppanen, 2005). In addition, drying temperatures used by ethanol plants vary substantially (371 to 593 ºC inside the dryer during heating), and increased drying time and temperature during the production of DDGS may accelerate lipid peroxidation by oxidizing unsaturated lipids in DDGS.

Table 1-5. Fatty acid composition of vegetable oils (United States Department of Agriculture Nutrient database http://ndb.nal.usda.gov/)

Type	SFA[1]	MUFA[2]	PUFA[3]		
			Total	C18:2[4]	C18:3[5]
Corn	12.9	27.6	54.7	58	1
Canola	7.4	63.3	28.1	22	10
Coconut	86.5	5.8	1.8	2	-
Cottonseed	35.9	17.8	51.9	54	1
Olive	13.8	73.0	10.5	10	1
Palm	49.3	37.0	9.3	10	-
Peanut	16.9	46.2	32.0	32	-
Soybean	15.7	22.8	57.7	54	7
Sunflower	10.1	45.4	40.1	39.8	0.2

[1]SFA = saturated fatty acids.
[2]MUFA = monounsaturated fatty acids.
[3]PUFA = polyunsaturated fatty acids.
[4]C18:2 = linoleic acid.
[5]C18:3 = linolenic acid.

In a preliminary evaluation comparing the levels of secondary lipid peroxidation products in two DDGS sources (Shurson and Csallany, 2006), a dark colored (overheated) DDGS sample contained 40% more total polar aldehydes and 12.5% more total nonpolar aldehydes than a light colored (less heat damaged) DDGS sample. These results suggest that there are significant differences in lipid peroxidation during processing and drying among DDGS sources. Similarly, another study was conducted by our laboratory (Song et al., 2011) to evaluate the lipid peroxidation level in 31 DDGS samples from ethanol plants in the U.S. In this study, two commonly used indicators of lipid peroxidation, thiobarbituric acid reactive substances (**TBARS**) and peroxide value (**PV**), were measured. 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 suggests 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 of TBARS and PV in a corn reference sample (0.2 ng MDA/mg oil and 3.1 meq/kg oil, respectively).

4. Sulfur in DDGS

Corn DDGS contains a relatively high S content (0.33 to 1.04%, DM basis, Kim et al., 2012) compared to other feed ingredients. Organic S, mainly in the form of sulfur-containing AA (Met and Cys), is present in DDGS intrinsically because the corn kernel contains approximately 0.1% S (Kerr et al., 2008). 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, during the dry-grind ethanol production process, a significant quantity of sulfuric acid is added to the distillation column continuously for cleaning purposes to remove the insoluble precipitates of organometallic salts, i.e. calcium oxalate (beer stone), and calcium/magnesium phytate, left from the fermentation beer. These insoluble precipitates coat the surface of the distillation column, and can impair heat transfer and cause production interruptions if they are allowed to accumulate. Thus, sulfuric acid is added to prevent and decrease the formation of these deposits (Kim et al., 2012). Inorganic S can also enter into the product stream from water containing significant sulfate concentrations, sulfuric acid used for pH adjustment of fermentation, and bisulfites for air scrubbers (Kim et al., 2012).

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; Uwituze et al., 2011b). The high S level in cattle feed can lead to polioencephalomalacia. 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. Kerr et al. (2011) reported that growing pigs can tolerate relatively high amounts of dietary inorganic S (1.21% of diet), but high dietary S content alters inflammatory mediators and intestinal bacteria. Kim et al. (2012) conducted four experiments to evaluate the effects of feeding high S diets on growth performance in weanling and growing-finishing pigs. Additional $CaSO_4$ (0.74 or 1.10%) was added to the diets containing 20 or 30% DDGS to mimic the feeding of a high S DDGS source. The authors did not observe any negative effects on growth performance from feeding the high S content, DDGS-containing diets, and thus they concluded that the high S content in DDGS may not be the cause for reduced growth performance of pigs observed in some previous experiments conducted by Whitney et al. (2006a), Barbosa et al. (2008), and Linneen et al. (2008). One should note that, although the increased S concentration in DDGS is largely due to the addition of inorganic S during the production process (Song et al., 2012b), about 33% of the total S is in the form of organic S (mainly from Met and Cys) in DDGS, which is approximately 3 times higher than the level in corn (Kim et al., 2012). Further studies are needed to determine the effects of high organic S content in DDGS on pig growth performance.

5. Feeding DDGS to Swine

Stein and Shurson (2009) reviewed several studies regarding the effects of feeding corn DDGS to weanling pigs, grower-finisher pigs, and sows. They concluded a maximum recommended dietary inclusion rate of DDGS for different swine production

stages without negative effects on growth and reproductive performance (**Table 1-6**). More recently, Cromwell et al. (2011) fed up to 45% DDGS with supplemented lysine and tryptophan to grow-finish pigs, and did not observe major effects on growth performance, although softer bellies occurred with increasing dietary levels of DDGS. With the ethanol industry expanding rapidly in recent years, more experiments have been conducted to evaluate the effects of feeding DDGS to pigs in many aspects of their growth, reproduction as well as quality of meat products.

Table 1-6. Maximum recommended dietary inclusion for DDGS in swine diets (Adapted from Stein and Shurson, 2009)

Production Phase	Maximum Dietary Inclusion Rate
Weanling (> 7 kg)	25%
Grow-finish	30%
Gestation	50%
Lactation	30%

- **Effects on Growth Performance**

Inconsistent results in ADG and ADFI have been reported in studies evaluating the effects of feeding 20 or 30% DDGS to growing-finishing pigs (Stein and Shurson, 2009). Results from some experiments showed that feeding diets containing 20 or 30% DDGS leads to similar growth performance (Cook et al., 2005; DeDecker et al., 2005; Gaines et al., 2007), whereas other experiments (Whitney et al., 2006a; Linneen et al., 2008) showed reduced ADG and ADFI in pigs fed diets containing DDGS compared with those fed standard corn-soybean meal diets.

The reasons for inconsistent growth performance in response to increasing dietary levels of DDGS are still unclear. These differences are likely due to a combination of

several factors including the quality of DDGS sources (i.e. AA digestibility, lipid peroxidation level) and other ingredients, formulation methods (i.e. SID vs. total AA and CP basis), and mycotoxin levels in the diet (Stein, 2007). By comparing the previous studies, experiments that showed positive or unaffected growth performance responses all used a DDGS source with known analyzed nutrient values, and not values from NRC (1998). In addition, diets used in these studies were formulated on a digestible lysine basis. However, experiments that showed negative growth performance either used NRC (1998) nutrient and AA digestibility values for DDGS when formulating the diets (e.g. Linneen et al., 2008), or diets were formulated on a total lysine basis (e.g. Whitney et al., 2006a). As discussed above, nutrient levels, especially lysine digestibility and fat quality, can vary significantly among different DDGS sources due to processing (heat damage) and storage conditions (high temperature and moisture accelerate the peroxidaiton of fat). Therefore, using the nutrient values from NRC (1998) may not be accurate for a specific source of DDGS. In addition, formulating diets containing DDGS based on total lysine basis and not on a SID lysine basis may overestimate the digestible lysine concentration in DDGS containing diets, since the lysine digestibility is lower in DDGS than that in corn and soybean meal (Stein and Shurson, 2009). Considering these relationships, pork producers should know the exact nutrient concentration, especially for AA, in the DDGS source being fed, and use that information when formulating the diets to achieve acceptable growth performance. Furthermore, diets should be formulated on SID lysine basis, and crystalline lysine should be added as needed to avoid a high CP concentration in the final diet. Excessively high dietary protein concentrations reduce pig growth performance. Chen et al. (1995, 1999) reported that ADG and ADFI were reduced

linearly in pigs fed corn-soybean meal based diets containing greater than 16% CP, which is the maximum level of dietary CP required by mixed-sex growing-finishing pigs with 350 g lean gain/day (NRC, 1998). In addition, an excessive quantity of CP in the diet increases plasma urea concentration (Goerl et al., 1995; Chen et al., 1999). This happens because when pigs are fed high-protein diets, organ weights increase and activities of many tissue enzymes (i.e. pancreas and liver) are elevated to process the greater amounts of nitrogenous compounds (Chen et al, 1999). Urea synthesis in the liver may increase the energy expenditure for the liver and further reduce the amount of energy available for growth. In general, pigs fed high-protein diets may require more energy for metabolizing excessive amounts of protein or AA, and hence, less energy is available for body growth.

- **Effects on Carcass Characteristics**

In addition to growth performance, feeding high levels of DDGS has also been studied extensively for its effects on pig carcass characteristics. Again, the responses have been inconsistent. Hot carcass weight and dressing percentage were unaffected in some studies (Linneen et al., 2008; Widmer et al., 2008). However, Whitney et al. (2006a) and Fu et al. (2004) reported reduced HCW when feeding 30% DDGS to growing-finishing pigs. Dressing percentage was also found to be linearly reduced in pigs fed diets containing 0 to 30% DDGS (Cook et al., 2005; Whitney et al., 2006a; Xu et al., 2010). The reduction in HCW and dressing percentage could be due to the high levels of NDF in DDGS, which can potentially increase gut fill (Pond et al., 1988). In particular, Fu et al. (2004) observed an increased fecal mass in pigs fed increasing dietary DDGS, which indicated a reduced HCW at the same slaughter BW in pig fed DDGS

containing diets.

Backfat depth was unchanged in many studies when feeding 30% DDGS to pigs (Fu et al., 2004; Cook et al., 2005; Whitney et al., 2006). However, in a more recent study conducted by Xu et al. (2010), last-rib backfat depth was reduced linearly with increasing dietary DDGS from 0 to 30%. The authors explained that the reduction in backfat depth might be due to the reduction in ADFI, which resulted in a reduction in total daily energy intake, and thus leaving less energy available for fat deposition.

The reasons for these inconsistencies in carcass weight, dressing percentage and backfat depth are still not very clear. Effects on loin eye area and percentage of fat-free carcass lean are more consistent. Gaines et al. (2007) reported reduced carcass lean percentage due to reduced loin muscle area, but most of the studies reported that loin muscle area and carcass lean percentage were not affected by including up to 30% DDGS in the diet (Fu et al., 2004; Xu et al., 2007; Widmer et al., 2008).

- **Effects on Pork Fat Quality**

Soft pork fat is one of the major problems in the meat industry, because it creates challenges during cutting, grinding, and slicing operations (Schinckel et al., 2002). Belly firmness is perhaps the most notable fat quality concern because soft bellies result in bacon slicing difficulties (Schinckel et al., 2002). Soft bellies also result in lower processing yields and thus, lead to economic losses.

Soft pork fat is caused by a high proportion of polyunsaturated fatty acids, especially linoleic acid, in belly fat and backfat (Wood et al., 1989; Maw et al, 2003). The fatty acid composition in animal tissues depends on (1) *de novo* fatty acid synthesis,

and (2) fatty acid composition in the diet (Wiseman and Agunbiade, 1998). Particularly in pigs, *de novo* fatty acid synthesis, relative to the direct deposition of dietary fatty acid in adipose tissue, is effectively inhibited by dietary fat (Farnworth and Kramer, 1987; Chilliard, 1993). Therefore, fatty acid composition of adipose tissue triglycerides, particularly essential PUFA such as C18:2 and C18:3 which cannot be synthesized by animals, is influenced significantly by the composition of dietary fat (Gatlin et al., 2002).

Feeding DDGS has quite a profound effect on pork fat quality by reducing pork fat firmness. Corn DDGS contains approximately 10% corn oil, which is high in unsaturated fatty acids (**UFA**, 81% of total) and C18:2 (54% of total; Xu et al., 2010). In the study conducted by Xu et al. (2010), the concentration of dietary C18:2 increased by 58% (from approximately 1.7 to 2.7% of the diet) with an increase in dietary DDGS from 0 to 30%, which resulted in a linear increase in C18:2 content of body fat in pigs fed diets containing 0 to 30% DDGS. In addition, many studies have confirmed that feeding an increasing level of DDGS leads to a decrease in belly firmness, as measured by the distance from skin to skin when the belly was draped skin-side down on a stainless-steel rod (Widmer et al., 2008; Leick et al., 2010; Xu et al., 2010). Furthermore, increasing dietary DDGS concentration also increased the iodine value (**IV**) in belly fat (White et al., 2009; Xu et al., 2010), backfat (Xu et al., 2010), and jowl fat (Benz et al., 2007).

- **Effects on Loin Muscle (LM) Oxidation and Food Safety Concerns**

Lipids in muscle tissues undergo autoxidation through free-radical chain reactions after harvest, and the susceptibility of fatty acids to peroxidation increases with their degree of unsaturation (Shahidi, 1998; Skidsted et al., 1998). The susceptibility of PUFA

to peroxidation is the main reason for reduced meat shelf-life stability, increased rancidity flavor, and meat discoloration during storage, as observed in several studies when feeding pigs vegetable oils (Leszczynski et al., 1992; Ahn et al., 1996; Leskanich et al., 1997) or DDGS (Leick et al., 2010) that contain high levels of PUFA. Additionally, dietary oxidized lipids may also promote lipid peroxidation and discoloration of LM under commercial storage conditions (Fernández-Dueñas, 2009).

Strong evidence has shown that dietary ω-3 PUFA (i.e. α-linolenic acid) may minimize the risk of cardiovascular disease in humans (Kinsella et al., 1990; Lee and Lip, 2003), however if they are oxidized, the remaining secondary lipid peroxidation products in the meat products can be highly toxic for humans. One class of the highly reactive aldehydes generated from peroxidation of PUFA are α-, β-unsaturated hydroxyalkenals. These substances in food can be absorbed during digestion and react readily at low concentrations with biomolecules, leading to damaging effects, such as mutagenic, cytotoxic, and carcinogenic effects (Witz, 1989).

One group of the extremely toxic hydroxyaldehydes is 4-hydroxyalkenals, including 4-hydroxy-2-trans-nonenal (HNE), 4-hydroxy-2-trans-octenal (HOE) and 4-hydroxy-2-trans-hexenal (HHE; Esterbauer et al., 1988). 4-hydroxy-2-trans-nonenal and HOE are produced from lipid peroxidation of ω-6 fatty acids, such as linoleic acid and γ-linolenic acid, while HHE results from ω-3 polyunsaturated fatty acids, such as α-linolenic acid (Esterbauer et al., 1988). Among these 4-hydroxyalkenals, HNE is the most toxic (Seppanen, 2005). Formation of HNE is related to many diseases in humans. For example, patients with Parkinson's disease were found to have significantly higher concentration of HNE bound proteins in substrantia nigra than those of normal subjects

(Yoritaka et al., 1996). Furthermore, HNE was also found to mediate amyloid β-peptide-induced apoptotic cell death which is related to Alzheimer's disease (Mark et al., 1997). In addition, HNE also performs various cytotoxic effects, such as genotoxicity, cytoskeletal modifications, alteration of membrane fluidity, inactivation of enzymes, inhibition of DNA synthesis and induction of cataracts in the lens (Seppanen, 2005). Therefore, lipid peroxidation of PUFA in meat products could have negative effects on human health.

Since HNE has many negative biological effects, it has been widely used as a biomarker for oxidative damage in tissues and oxidized oils/fats in addition to TBARS and PV (Seppanen, 2005). In a recent study conducted by our laboratory (Liu et al., 2012), HNE concentration increased from 0 to 194 μmol/kg when corn oil was heated at 95 ºC for 72 h. Similarly, in soybean oil heated at 185 ºC for 6 h, the concentration of HNE increased from 0 to 256 μmol/kg (Seppanen, 2005). These results indicate that significant amounts of HNE can be produced in thermally-oxidized oil containing PUFA. Although we were not able to detect the HNE concentration in oils extracted from DDGS, it is likely that feeding DDGS containing oxidized lipids may cause negative effects on shelf-life stability of LM as well as formation of some toxic aldehydes. Therefore, one of the objectives of the following studies was to determine the effects of feeding DDGS on LM oxidation, particularly TBARS and α-tocopherol concentration.

III. Lipid Peroxidation

Lipid peroxidation, also called lipid oxidation, refers to the oxidative degradation of lipids (Frankel, 1987). Lipid peroxidation proceeds by a sequential free radical chain-

reaction mechanism that involves the production of semi-stable peroxides (Tappel, 1977). Lipid peroxidation occurs in foods or feed ingredients containing fat/oil and leads to quality and nutritional losses. Lipid peroxidation can also take place *in vivo* leading to several negative effects, including damage of cellular and subcellular bio-membranes, growth depression of animals, and undesirable meat and fat quality.

1. Free Radicals and Oxygen Species

Free radicals cause lipid peroxidation of fatty acids in the presence of molecular oxygen in an autocatalytic process (Seppanen, 2005). Free radicals are highly reactive atoms or groups of atoms with an odd (unpaired) number of electrons, which can initiate hydrogen abstraction (Seppanen 2005). A free radical is formed easily when a covalent bond between entities is broken and one electron remains with each newly formed atom (Seppanen 2005). Additionally, lipid peroxidation also requires the presence of oxygen, which can generate exceedingly reactive intermediates through a series of single electron transfers when reduced to water (Pryor, 1976). These active oxygen species include superoxide anion (O_2-), hydroxyl radical (HO·), and singlet oxygen ($^1O_2$).

Superoxide anions are present in most biological systems through autoxidation. Through a proton induced reaction, O_2- is converted to oxygen and hydrogen peroxide, which is the most stable of the products of oxygen reduction (Halliwell and Gutteridge, 1984).

$$2O_2\text{-} + 2H^+ \rightarrow 2H_2O_2 + O_2$$

When hydrogen peroxide accumulates in a cell, it can also react with O_2- and produce HO·. The hydroxyl radical is the most potent oxidant generated by the

hydrolysis of water. The reactions of HO· generate secondary radicals by adding to C-C double bonds and form organic free radicals by hydrogen abstraction (Halliwell and Gutteridge, 1984).

$$O_2\text{-} + H_2O_2 \rightarrow O_2 + OH\text{-} + HO\cdot$$

Singlet oxygen is another unstable oxygen species, which is produced from the O_2- by photo-sensitized reactions or reaction with HO· (Halliwell and Gutteridge, 1984).

2. Process of Lipid Peroxidation – A Free Radical Chain Reaction

Lipid peroxidation is processed through a free radical chain reaction, including initiation, propagation and termination (Seppanen, 2005). The initiators are free radicals, such as hydroxyl radical, which extract a hydrogen atom from a fatty acid to form water and a lipid radical. The lipid radical is not a stable molecule and it reacts readily with oxygen to generate more radicals as propagation continues. Termination occurs when free radicals combine to generate products that do not participate in the propagation reactions and therefore free radicals are removed from the pool of reactants. A hypothetical autoxidation mechanism is shown in **Figure 1-1**. The general process of lipid peroxidation is summarized in **Figure 1-2**, which suggests that a wide variety of products could be formed in the process of lipid peroxidation.

Initiation	RH	→	R· + H·
Propagation	R· + O_2	→	ROO·
	ROO· + R'H	→	ROOH + R'·
Termination	2 R·	→	RR
	RO· + R·	→	ROR
	ROO· + R·	→	ROOR
	2 ROO·	→	ROOR + O_2

Figure 1-1. Hypothetical free-radical induced autoxidation reaction (Seppanen, 2005)

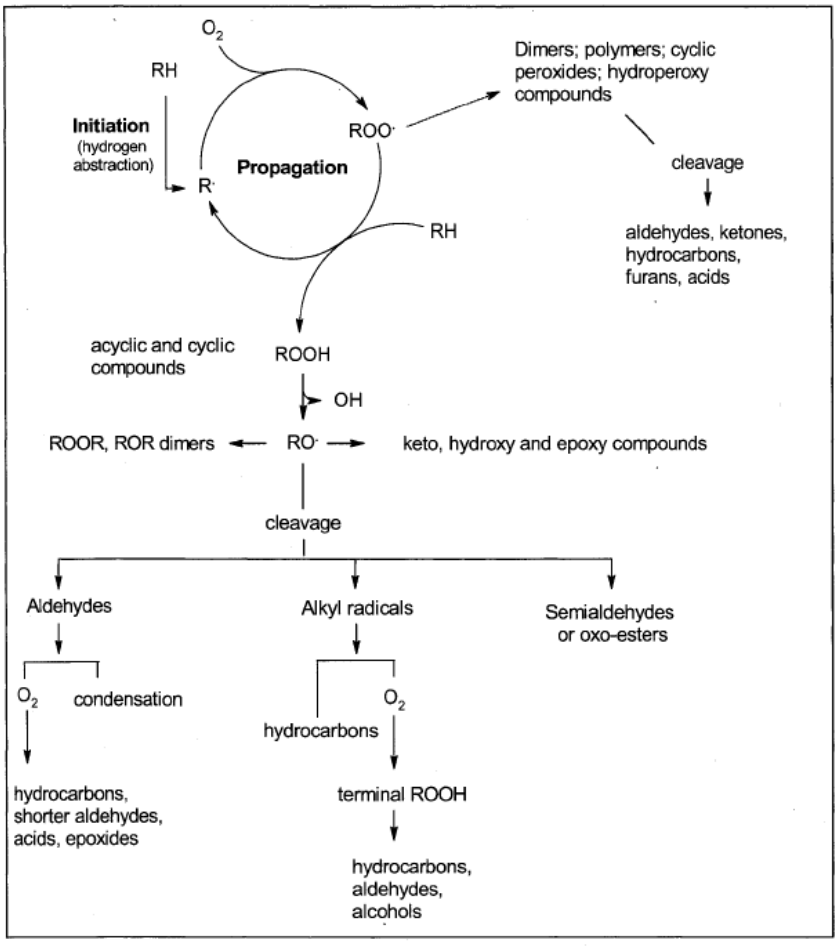


Figure 1-2. General process of lipid peroxidation (Seppanen, 2005)

- **Formation of Hydroperoxides from Linoleic Acid**

The main products of lipid peroxidation are fatty acid hydroperoxides, which are formed when hydrogen is abstracted from an unsaturated fatty acid by a free radical and molecular oxygen is attached at the alkyl radial (Chan and Levett, 1977). Linoleic acid (C18:2) is used as an example in this literature review to illustrate how hydroperoxides are produced and decomposed from lipid peroxidation of unsaturated fatty acids.

Linoleic acid (cis,cis-9,12-octadecadienoic acid) is very susceptible to peroxidation because of the methylene-interrupted double bond structure (Belita and Grosch, 1999). The abstraction of hydrogen from the methylene is easy when two carbon double bonds are present adjacent at each side of the methylene. In linoleic acid, C11 is the interrupted methylene with double bonds on the left (C9) and right (C13). The hydrogen from C11 can be abstracted easily by free radicals and then produces a pentadienyl radical intermediate in which the double bond has shifted position resulting in a *cis,trans* or a *trans,cis* conjugated double bond system. As shown in **Figure 1-3**, with the reaction of this intermediate with molecular oxygen, 9-diene hydroperoxides and 13-diene hydroperoxides can be formed equally if the -CH·- on C11 shifts to the right (to 9 position) and if the -CH·- shifts to the left (to 13 position), respectively. Therefore, two major hydroperoxides could be formed from the peroxidation of linoleic acid. The carbons at positions 8 and 14 are also susceptible to hydrogen extraction. However, since those methylene groups are not adjacent with two carbon double bonds at each side, the possibility of hydrogen to be extracted by free radicals is much less.

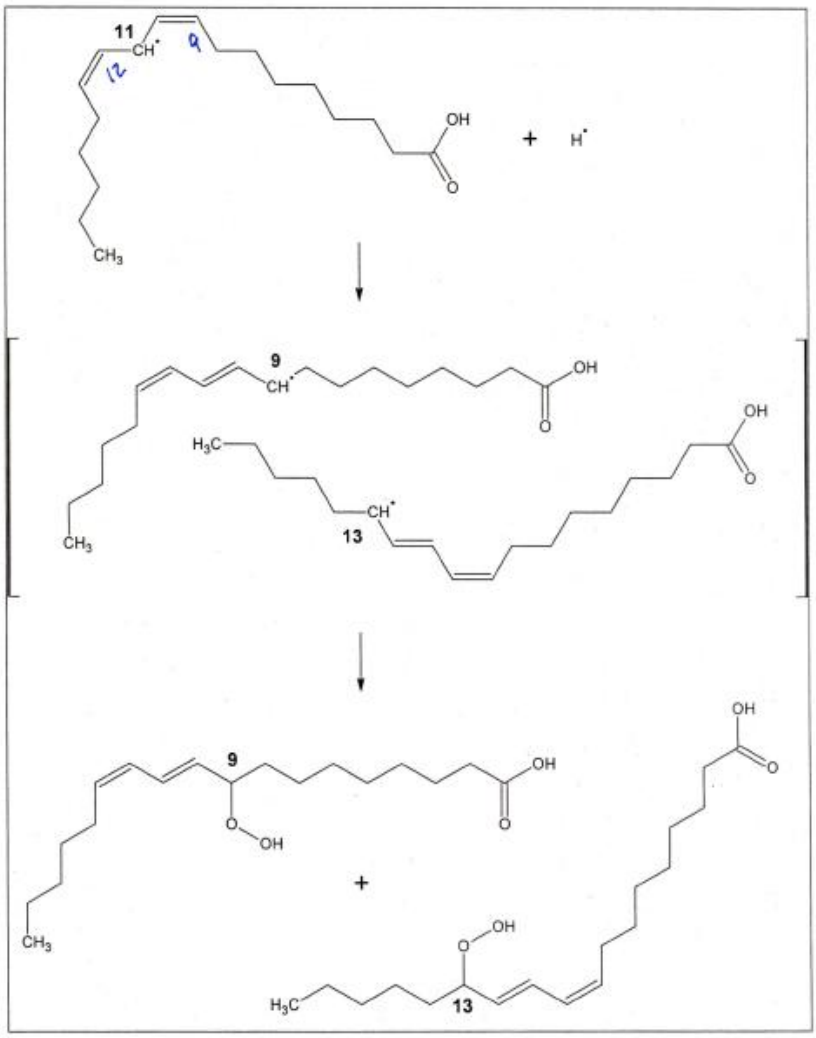


Figure 1-3. Formation of 9- and 13- hydroperoxides from linoleic acid (Adapted from Seppanen, 2005)

- **Decomposition of Hydroperoxides**

The hydroperoxides (ROOH) generated by lipid peroxidation begin to decompose as soon as they are formed, resulting in the production of fatty acid free radicals and hydroxyl free radicals. The initial breakdown of hydroperoxide by scission of the oxygen-oxygen bond yields a fatty acid alkoxy radical and a hydroxyl radical (**Figure 1-4**). Further decomposition of fatty acid oxygen free radicals in several steps yields a variety of smaller molecular weight compounds, such as aldehydes, ketones, acids, esters,

hydrocarbons, lactones, and aromatic compounds (Belita and Grosch, 1999).

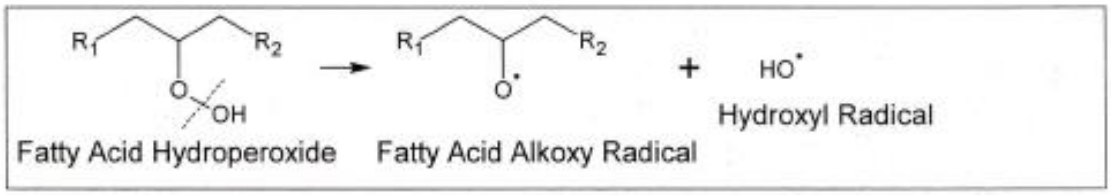


Figure 1-4. Decomposition of fatty acid hydroperoxides (Seppanen 2005)

1) Decomposition of Hydroperoxides by Hemolytic Cleavage

After the fatty acid alkoxyl radical is formed, hemolytic cleavage could happen at the carbon-carbon bond on either side of the alkoxyl group as oxidation proceeds further (Frankel, 1983). As shown in **Figure 1-5**, hemolytic cleavage that happens on the hydrocarbon side (option "A") results in the formation of a hydrocarbon and an oxoacid (or oxoester), while cleavage on the acid side (option "B") yields an aldehyde and an acid (or ester). Take the 9-, and 13-OOH methyl linoleate hydroperoxides as an example (**Figure 1-6**), hemolytic cleavage of 9-OOH methyl hydroperoxides yields (*3E*)-non-3-enal and methyl 9-oxononanoate (option "A"), and (*2E,4E*)-deca-2,4-dienal and methyl octanoate (option "B"), while hemolytic cleavage of 13-OOH hydroperoxides produces pentane and methyl (*9E,11E*)-13-oxotrideca-9,11-dienoate (option "A"), and hexanal and methyl (*9E*)-12-oxododec-9-enoate (option "B"). In general, a wide variety of secondary oxidation products could be formed by cleavage of different sides of different methyl hydroperoxides, most of which are volatile compounds due to the presence of carbonyl groups and short chain lengths.

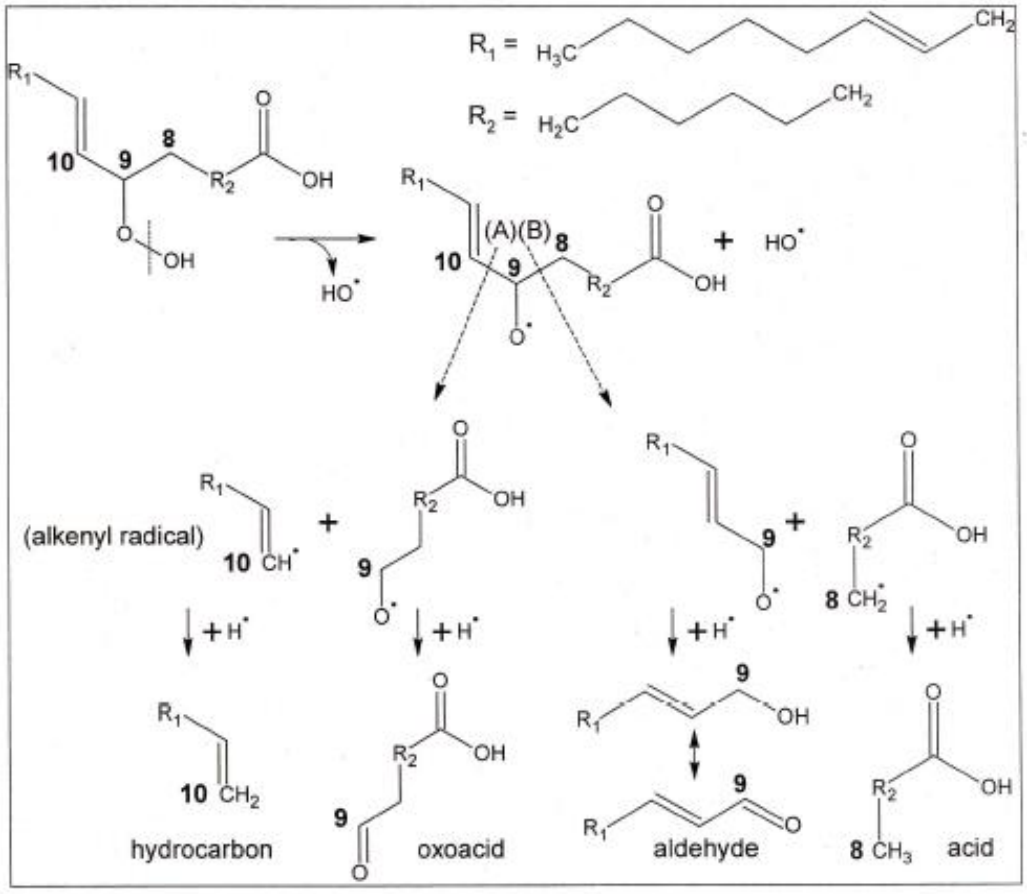


Figure 1-5. Hemolytic cleavage of fatty acid hydroperoxide (Seppanen, 2005)

2) Decomposition of Hydroperoxides by Heterolytic Cleavage

Another pathway of hydroperoxide decomposition is through heterolytic cleavage, which occurs between the hydroperoxide group and the allylic double bond in the presence of water (**Figure 1-7**). This reaction is initiated by the combination of a proton with the hydroperoxide group, followed by elimination of molecular water, resulting in an oxygen atom inserted in the C-C linkage adjacent to the double bond. The carbonium ion is then broken down to oxoacid and aldehyde.

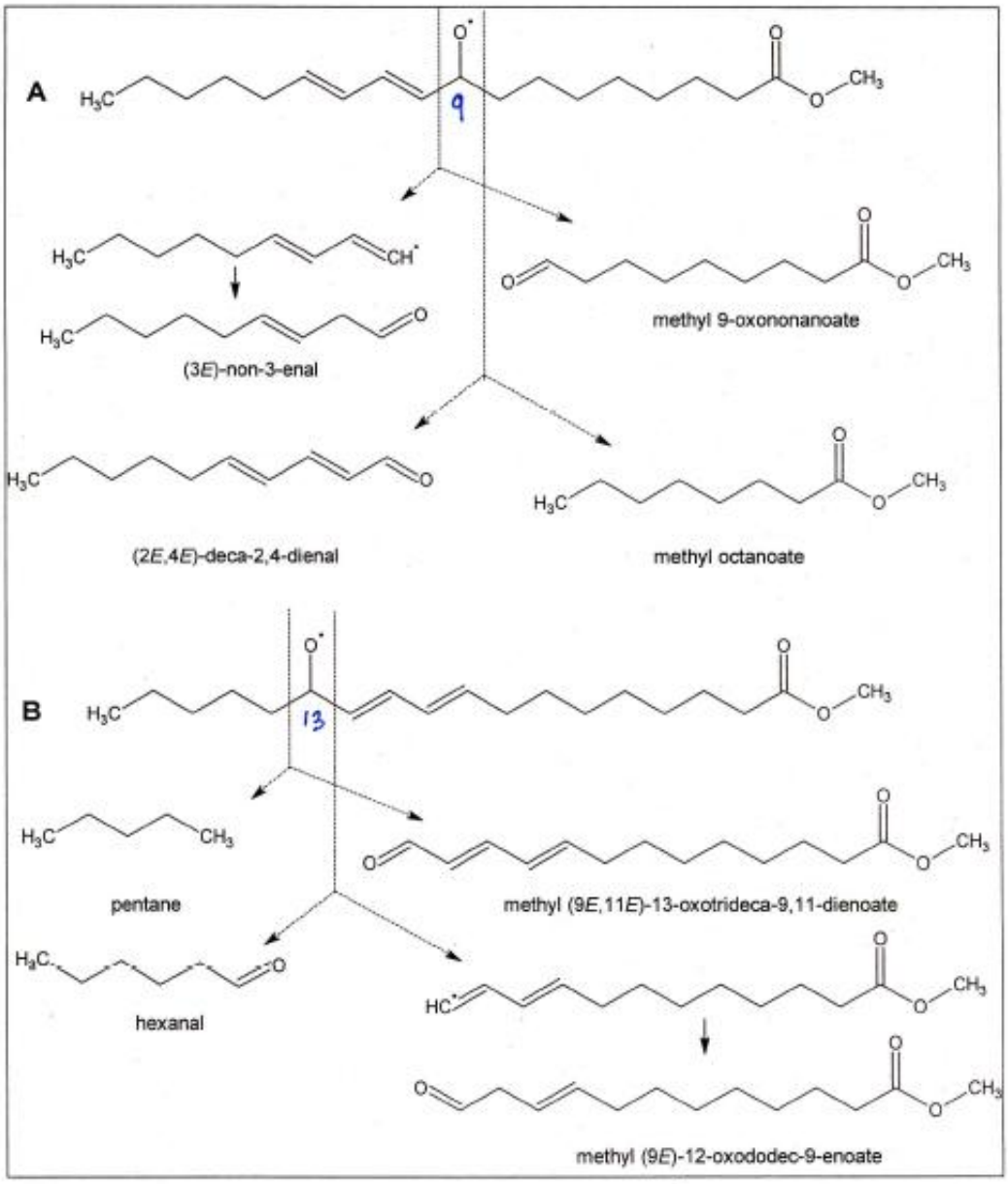


Figure 1-6. Secondary oxidation products from the hemolytic cleavage of methyl linoleate hydroperoxides (Seppanen, 2005)

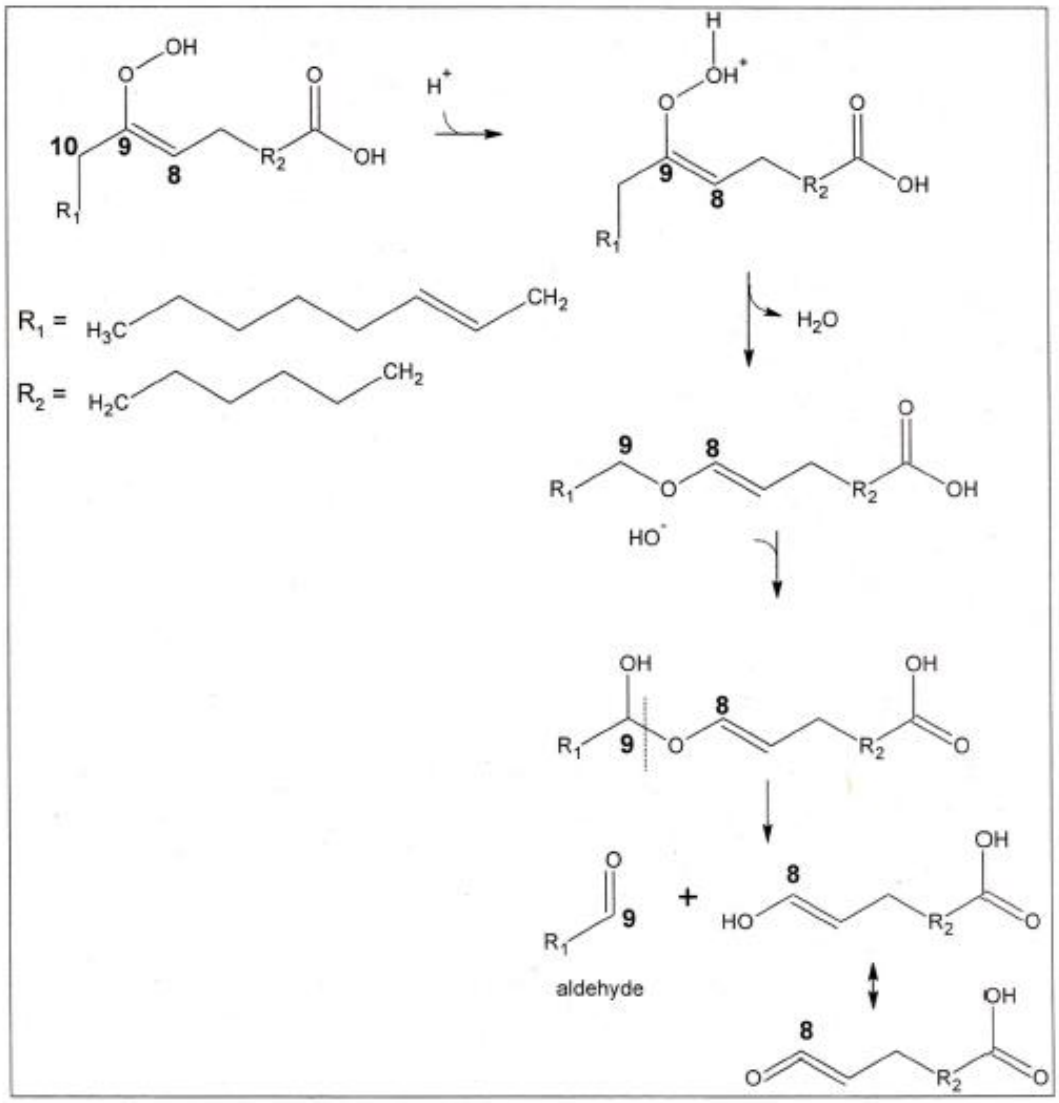


Figure 1-7. Heterolytic cleavage of fatty acid hydroperoxide (Seppanen, 2005)

The differences between hemolytic and heterolytic cleavage of hydroperoxides are mainly from the reaction condition and the degradation products formed. Since heterolytic cleavage requires the participation of a proton, it is more likely to occur in aqueous solutions (Belita and Grosch, 1999). However, hemolytic cleavage of hydroperoxides is the predominant reaction in oil (Belita and Grosch, 1999). If heterolytic cleavage occurs, the production of carbonyl compounds will be greater than

those produced by hemolytic cleavage, and thus resulting in more selective distribution of carbonyl compounds (Kimoto and Gaddis, 1968).

- **Formation of Malondialdehyde**

Unsaturated aldehydes can undergo further oxidation and generate more free radicals and peroxidation products by hemolytic cleavage, such as short-chain hydrocarbons, aldehydes, and dialdehydes, one of which is malondialdehyde (MDA). Malondialdehyde is highly reactive, which is formed from the simple peroxidation of unsaturated aldehydic products of linolenic acid, or from fatty acids with three or more double bonds through formation of hydroperoxide-epidioxide (Dahle et al., 1962).

3. Measurement of Lipid Peroxidation Level in Oils and Fats

Due to the complexity of the lipid peroxidation process, and the wide variety of secondary lipid peroxidation products, it is not easy to select an optimal method to determine lipid peroxidation level in oils and fats. Since many compounds produced during the termination phase (i.e. aldehydes, ketones, hydrocarbons) are volatile (Frankel, 1983), their concentrations in the tested samples may decrease over time. Therefore, any method to determine the lipid peroxidation level in oils or fats should consider the stage of peroxidation as constantly changing. For example, when a low peroxide value is discovered, it could be due to a low oxidation status, and/or the decreased concentrations of hydroperoxides over time because of decomposition. Similarly, a low aldehyde concentration, as measured by TBARS, may be due to limited lipid peroxidation, and/or because the aldehydes may have already been volatilized. Therefore, it is not reliable to

use just one indicator of lipid peroxidation. Conducting multiple tests is likely necessary to better determine the lipid peroxidation in fat or oil samples.

A variety of measurements of lipid peroxidation have been developed, which include predictive tests and indicator tests. Predictive tests use accelerated conditions to measure the stability of a fat/oil, and they can be used to determine ingredient quality, measure effectiveness of preservatives, or estimate product shelf-life stability (Wanasundara, 2002). Indicator tests are designed to quantify products generated during the initiation, propagation or termination phases of lipid peroxidation. Some of the more commonly used tests are described briefly in the following paragraphs.

- **Predictive Tests**

1) Active Oxygen Method (AOM)

This method predicts the stability of a fat/oil by bubbling air into a solution of the fat using a specific flow rate, temperature and concentration. As described by American Oil Chemists' Society (**AOCS**, Method Cd 12-57, 1998), oil is heated at 100 ºC. Peroxides and hydroperoxides produced during heating are determined by titration with iodine at various time intervals. The AOM value is then defined as the amount of time required for the peroxidation concentration to reach 100 meq/kg of fat. The more stable the fat, the longer it will take to reach that level. This method is very time-consuming since a stable fat/oil may take 48 hours or longer before reaching the required peroxide concentration. While still used today, the AOM method is being supplanted by faster automated techniques, such as Oxidative Stability Index (**OSI**).

2) Oxidative Stability Index

Although the principle of Oxygen Stability Index (OSI; AOCS, Method Cd 12b-92, 1998) is similar to the AOM method, OSI is much faster and more automated, and therefore has been used as a replacement for the AOM. Briefly, air is passed through an oil/fat sample held at 110 ºC at 2.5 ml/s, and bubbled into a reservoir of deionized water. Volatile organic acids produced by lipid peroxidation are dissolved in the water increasing its conductivity, which is monitored continuously by a computer or strip chart recorder. The OSI value is defined as the hours required for the rate of conductivity change to reach a predetermined value. Multiple samples can be tested simultaneously. However, since it requires special equipment and software, it is not feasible for all laboratories.

3) Oxygen Bomb Test

The Oxygen Bomb Test is used to predict stability and evaluate antioxidant systems in fat/oil or finished products. Standardized protocols for this procedure have been developed by Reiners (1972). Oxygen uptake of the sample is measured in a closed chamber. The rate at which oxygen is consumed indicates the oxidative stability of the tested product. Compared with the AOM and OSI, an advantage of this technique is its ability to measure stability of the complete product without extracting the lipid. However, results from this test are not always consistent, because several factors, such as sample size, reaction temperature, pressure of oxygen, and heating time can influence the rate the oxygen uptake (Peng, 2010). Due to the high variability and complicated equipment required, the oxygen bomb test is not used widely to determine quality and stability of oil commercially (Peng, 2010).

4) Iodine Value

Iodine value (**IV**) measures the reaction between iodine with the double bonds of unsaturated fatty acids. Iodine value is one of the most common methods to measure the degree of unsaturation of the fatty acids in a fat source. Fatty acids containing a greater number of double bonds provide more sites for peroxidation. Therefore, although IV is not a specific measurement of lipid peroxidation, it could predict the potential of a fat to be oxidized.

Various methods are available to determine the IV of fats and oils. One of the methods is called "Hanus reaction" (Paquot, 1979). In this reaction, iodine monochloride reacts with the double bonds in unsaturated fatty acids to produce a di-halogenated single bond. After this reaction is finished in a dark room, potassium iodide is added for titration with 0.1 mol/L sodium thiosulfate. The IV of the fatty acid is then calculated from the titration volume of sodium thiosulfate.

$$4I_2 + Na_2S_2O_3 + 5H_2O \rightarrow 2NaI + 2H_2SO_4 + 6HI$$

However, the traditional direct IV determination in the laboratory is expensive and time-consuming. As a result, IV is most commonly calculated from the fatty acid composition of the target product. The IV can be calculated based on the equation from AOCS (1998) using the percentage of several unsaturated fatty acids in total fatty acids: IV = (C16:1 × 0.95) + (C18:1 × 0.86) + (C18:2 × 1.732) + (C18:3 × 2.616) + (C20:1 × 0.785). Recently, Apple (2010) questioned the accuracy of IV in quantifying fat firmness because longer chain fatty acids are not included in the equation developed by AOCS. Meadus et al. (2010) amended the AOCS (1998) equation by adding seven additional long-chained fatty acid, which may provide a better prediction: IV = (C16:1 × 0.95) +

(C18:1 × 0.86) + (C18:2 × 1.732) + (C18:3 × 2.616) + (C20:1 × 0.785) + (C20:2 × 1.57) + (C20:3 × 2.38) + (C20:4 × 3.19) + (C20:5 × 4.01) + (C22:4 × 2.93) + (C22:5 × 3.68) + (C22:6 × 4.64).

In pork carcass evaluation, IV is commonly used as an indicator of belly firmness. Iodine value increases linearly with increasing PUFA concentration, and thus, an increase in IV suggests a decrease in belly fat firmness (Apple et al., 2008). However, Apple et al. (2010) indicated some limitations of using IV to determine belly firmness. Belly softness is a combination of increased PUFA and increased moisture content of lean and fat (Sather et al., 1995). Therefore, a high IV may not necessarily indicate a firm belly if the moisture content is high. Furthermore, IV does not distinguish between the location of the double bonds nor the degree of fatty acid isomerization. Thus, two fats with the same IV may be structurally different because of intramolecular distribution and location of double bonds (Apple et al., 2010). For example, a triglyceride with two SFA and one PUFA can have the same IV as a triglyceride with 3 MUFA. As a result, using IV as the only indicator of belly firmness may not be sufficient and accurate. Other measurements, such as belly flop, compression and puncture test, and a number of mechanical firmness-measuring devices, should also be considered when determining belly firmness (Apple et al., 2010).

- **Indicator Tests**

1) Peroxide Value

Peroxide value is one of the most commonly used measurements of lipid peroxidation in the oil industry. Peroxide value measures the concentrations of peroxides

and hydroperoxides formed during the initial stages of lipid peroxidation (Palmquist and Jenkins, 2003). The PV is defined as the amount of hydroperoxides per 1 kilogram of fat or oil, and normally is expressed as milliequivalents (mEq)/kg of oil. As previously mentioned, hydroperoxides generated by lipid peroxidation begin to decompose as soon as they are formed. Therefore, using PV as the only indicator of lipid peroxidation may not be accurate or sufficient. As shown in **Figure 1-8**, the change in PV with increasing lipid peroxidation level is not linear, but follows a bell shaped curve. A high PV definitely indicates a high lipid peroxidation status, whereas a moderate value may be caused by low lipid peroxidation level, or the depletion of hydroperoxides after reaching high concentrations.

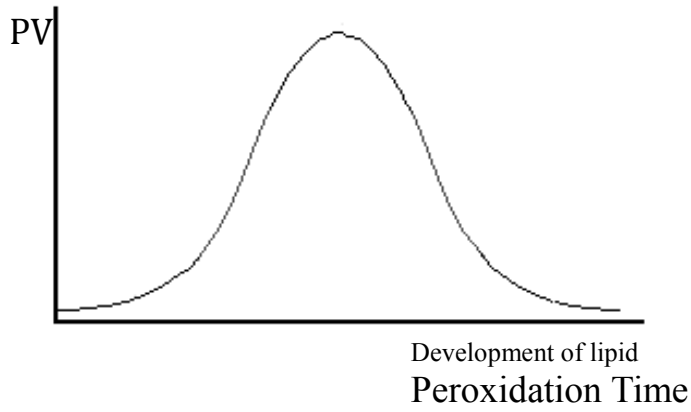


Figure 1-8. Changes of peroxide value (PV) with development of lipid peroxidation

2) Thiobarbituric Acid Reactive Substances

This test is a measure of saturated aldehydes (i.e. malonaldehyde, **MDA**), 2-enals, and 2-dienals produced in the termination phase of lipid peroxidation (Palmquist and Jenkins, 2003). Basically, the saturated aldehyde can react with 2-thiobarbituric acid (**TBA**) and produces a pink color which can be measured using a spectrophotometer at 532 nm. Since MDA is not the only aldehyde present in a peroxidized lipid sample, and

other aldehydes are capable of producing the same pink pigment with TBA when the conditions are favorable, the result of TBARS assay is expressed as mg MDA equivalents/kg sample. Although the classical TBARS assay is one of the most frequently employed methods for determining lipid peroxidation in fats/oil because of its simplicity and relative speed, there are some limitations of this method. First, similar to PV, a low TBARS value is not an absolute indicator of lipid peroxidation, because aldehydes may have not yet been produced or volatile aldehydes may have been already lost during processing and storage of the oil or fat. In addition, the TBARS value obtained from the color reaction should not be interpreted as absolute levels of lipid peroxidation. In addition to MDA and other aldehydes, a number of non-oxidation substances such as soluble proteins, peptides, amino acids, and pigments in food samples can interfere with the TBA reagent and give falsely high readings (Pegg, 2001). Therefore, compared to TBARS assay, a direct quantification of aldehydes using HPLC or liquid chromatography–mass spectrometry (**LC-MS**) can be a more accurate measurement of lipid peroxidation level in fat/oil samples, although these methods are relatively complex and expensive.

3) Anisidine Value

As hydroperoxides break down by chain cleavage, volatile aldehydes are produced, leaving a non-volatile portion of the fatty acid that remains as a part of the glyceride molecule (Seppanen, 2005). These non-volatile carbonyl compounds can be measured by reaction with ansidine. Basically, anisidine value (**AV**) is a measurement of past lipid peroxidation, and essentially reflects the freshness of the oil (Palmquist and Jenkins,

2003). High AVs may indicate that fat has been oxidized even when TBA or PV are low. However, similar to PV assay, AV measurements also follow a bell shaped curve as a function of peroxidation time. Therefore, only one measurement of AV may not provide reliable information of the degree of lipid peroxidation.

4) Measurements of 4-Hydroxy-2-trans-nonenal

As discussed previously HNE is one of the most toxic 4-hydroxyalkenals produced during the termination phase of lipid peroxidation, and has been used widely as an indicator for oxidative damage in tissues and oxidized lipids in addition to TBARS and PV (Seppanen 2005). A number of methods have been developed to detect and quantify HNE in oxidized oil or biological samples. Traditionally, HNE was measured using a HPLC-spectrophotometric detector system monitoring at 223 nm (Seppanen 2005). However, HNE is not stable, and many compounds absorb near 220 nm, resulting in inaccurate results. In addition, the experimental procedures of this method are very complex and time-consuming, which limit its use in practical analysis.

Uchida et al. (2002) developed a fast and sensitive method to quantify HNE, which is solid-phase microextraction (SPME). This is a method to concentrate volatile or nonvolatile components from a liquid, solid, or gas for GC or HPLC analysis. The SPME fiber is exposed in the sample to absorb the components of interest, and then injected directly into the GC or HPLC. One of the advantages of using the SPME method is that it can concentrate analytes without using any solvent and does not require any complicated apparatus. More recently, Liu et al. (2012; unpublished) used LC-MS to quantify HNE concentration in oxidized corn oil, canola oil, poultry fat and beef tallow.

Results from that study showed that using LC-MS allowed detection of HNE in all of these oxidized oils with a high sensitivity of 2 μmol/kg. These results suggest that LC-MS could also be considered a fast and sensitive method when measuring HNE.

5) Hexanal Value

Hexanal is one of the major secondary products formed during the peroxidation of linoleic and other ω6 fatty acids, and has served as a reliable indicator of lipid peroxidation in foods rich in ω6 fatty acids (Shahidi and Pegg, 1994). Hexanal content is highly correlated with TBARS values and sensory scores (Shahidi and Pegg, 1994). Hexanal can be measured by gas chromatography (**GC**; Elisia and Kitts, 2011). Generally, a portion of sample is heated moderately in a sealed septum tube. A gas syringe is used to withdraw a small portion of the headspace over the sample. The headspace sample is then injected onto a GC column to separate hexanal from other volatile components. However, this method may require volatilization of hexanal, whereas hexanal volatilization may be hindered due to covalent or other types of binding between hexanal and proteins in foods, and thus, may affect accurate hexanal quantifications (Goodridge et al., 2003). More recently, an indirect enzyme-linked immunosorbant assay (**ELISA**) has been developed for monitoring lipid peroxidation level through quantification of hexanal-protein adducts (Goodridge et al., 2003).

IV. Oxidative Stress and Dietary Oxidized Lipids

The antioxidant-oxidant balance depends on the equilibrium between antioxidant systems and the rate and amount of free radical production in tissues. The excess

systemic manifestation of reactive oxygen species (ROS), and/or a deficiency in the biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage will produce an imbalance in the antioxidant-oxidant status which leads to oxidative stress (Droge, 2002). Oxidative stress, in terms of disturbances in the normal redox state of cells, can cause toxic effects through the production of free radicals and peroxides that damage all components of cells, including proteins, lipids, and DNA (Miller et al., 1993). Furthermore, some reactive oxidative species function as cellular messengers in redox signaling. Thus, oxidative stress can also lead to disruptions in normal mechanisms of cellular signaling (Miller et al., 1993). All of the endogenous and exogenous sources triggering the production of ROS may be the cause of oxidative stress. Some of the exogenous contributors for the remarkable increase of ROS include fungal toxins, disease, environmental pollutants, ultraviolet (UV) light, ionizing radiation, and dietary oxidants (Apel and Hirt, 2004; Puthran et al., 2009). This literature review focuses on the oxidative stress induced by oxidized lipids in diets fed to swine.

1. Use of Lipids in Swine Diets

Dietary fat refers to the lipid component of the diet, primarily triglycerides. Triglycerides consist of three fatty acids linked to glycerol via an ester bond. Triglycerides can be either simple, where the fatty acids are identical, or mixed, where two or three fatty acids are different (**Figure 1-9**). Since free fatty acids can be toxic at high levels (Canakci and Gerpen, 2001) and also corrosive to feeding equipment, most feed-grade fats have a requirement of a maximum level of free fatty acids and minimum level of total fatty acids to meet acceptable lipid quality standards. For example, good

quality tallow requires a maximum of 15% free fatty acids and a minimum of 90% total fatty acids (Azain, 2001).

The major fatty acids found in typical swine diets and fat depots, as well as the chain length, number of double bonds and melting point are listed in **Table 1-7**. Generally, vegetable fats (i.e. corn oil, soybean oil, and canola oil) contain more long-chain unsaturated fatty acids, and are liquid at room temperature, whereas animal fats (i.e. beef tallow and poultry fat) have more saturated fatty acids, and most are solid at room temperature.

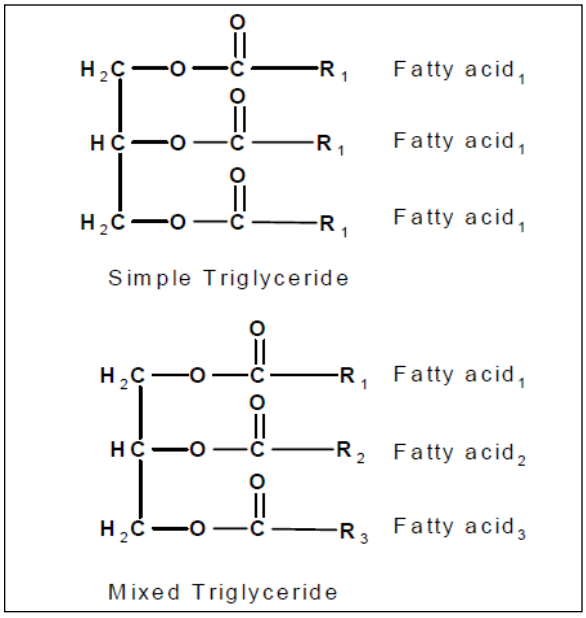


Figure 1-9. Simple and mixed triglycerides (adapted from Food Fats and Oils, 2006)

Table 1-7. Typical fatty acids found in swine diets and tissue depots (Adapted from Azain, 2001)

Fatty Acid	Chain length	Melting Point, °C
Caprylic	C8:0	16.5

Capric	C10:0	31.4
Lauric	C12:0	44
Myristic	C14:0	58
Palmitic	C16:0	63
Palmitoleic	C16:1	1.5
Stearic	C18:0	71.5
Oleic	C18:1	16.3
Linoleic	C18:2	-5.0
Linolenic	C18:3	-11.3
Arachidic	C20:0	75.4
Arachidonic	C20:4	-49.5

Fats and oils are added to commercial swine diets for 4 main reasons: 1) to supply energy for maintenance and production; 2) serve as a source of essential fatty acids, which can only be supplied by the diet and serve as building blocks for hormones; 3) carry fat-soluble vitamins; and 4) act as an integral portion of cell membranes (Pond et al 1995). Xu (2007) analyzed and summarized data involving 10 studies evaluating the effects of supplemental fat in growing-finishing (20- to 100- kg) pig diets on growth performance and carcass characteristics. As shown in **Table 1-8**, 5, 7, and 9 out of 10 studies showed an increase in ADG, reduction in ADFI, and improvement in G:F with supplemental fat, respectively. Dressing percentage and loin eye area were not affected by dietary fat supplementation in any of these studies. Only 2 out of 10 studies showed that backfat thickness increased with supplemental fat (<5%). These data, together with a review by Pettigrew and Moser (1991) comparing 92 studies involving feeding supplemental fat to growing-finishing pigs, show that feed intake is reduced and G:F is improved. The effect of dietary fat on ADG is less consistent and may depend on fat source, dietary inclusion rate, and pig age. Carcass characteristics including dressing percentage, loin pH, carcass length, and loin muscle area are not affected by supplemental fat, whereas backfat thickness may be increased in pigs fed diets containing

additional fat.

Table 1-8. Summary of effects of supplemental dietary fat on growth performance and carcass characteristics in growing-finishing pigs (Xu, 2007)

Source	Fat source	Inclusion rate, %	ADG	ADFI	G:F	Backfat depth	Dressing	Loin eye area
Azain et al., 1991	Poultry fat	10	+	-	+	NS	ND	NS
Azain et al., 1992	Poultry fat	10	NS	-	+	NS	NS	NS
Myer et al., 1992	Canola oil	10	+	-	+	NS	ND	NS
Williams et al., 1994	CWG	5	NS	-	+	NS	NS	NS
Smith et al., 1999	CWG	6	+	-	+	NS	NS	ND
De la Llata et al., 2001	CWG	6	+	NS	+	NS	NS	ND
Engel et al., 2001	CWG	6	NS	-	+	NS	NS	NS
Van de Ligt et al., 2002	Corn oil	5	+	ND	ND	+	ND	ND
Gatlin et al., 2002	Soybean oil	5	NS	-	+	NS	NS	NS
Apple et al., 2004	Unknown	5.6	NS	NS	+	+	NS	NS

Note: CWG = choice white grease; + = positive effect; - = negative effect; NS = no significant difference; ND = no data.

2. Characteristics of Thermally Oxidized Lipids

Approximately 2.5 billion pounds of waste vegetable oils and fats are produced annually from large food processing plants and restaurants, which are then rendered and sold almost exclusively to the animal feed industry because of the low cost (Canakci, 2007). Thermally oxidized oil contains several toxic lipid peroxidation products (i.e. hydroperoxides and aldehydes) due to repeated heating processes (Ringseis et al., 2007; Adam et al., 2008). For example, soybean oil heated at 185˚C for 8 h contains up to 60

times more total secondary lipid peroxidation products than unheated soybean oil (Csallany et al., 2002). Additionally, some natural antioxidants, such as tocopherols, that exist in fresh vegetable oils can also be lost during the heating process. As shown in **Figure 1-10**, tocopherol concentrations are reduced significantly in soybean oil heated at 185°C from 0 to 10 h (Seppanen and Csallany, 2002).

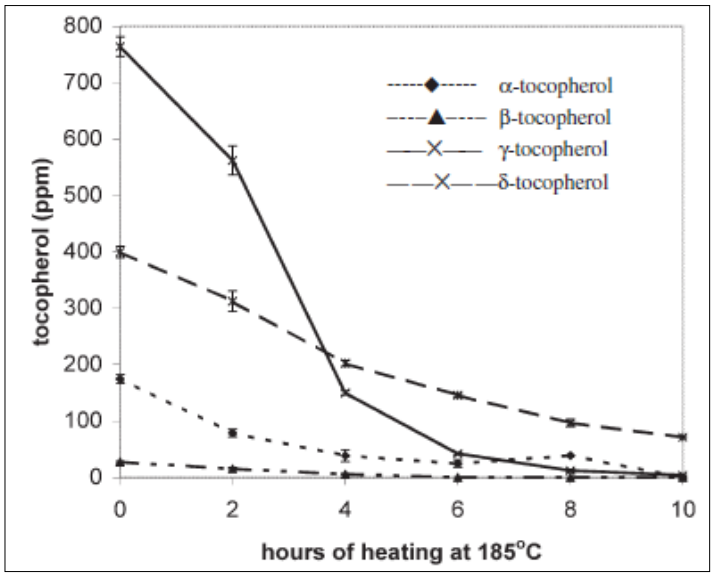


Figure 1-10. Tocopherol content of soybean oil heated at 185°C for 0–10 h (Seppanen and Csallany, 2002)

Different mechanisms have been hypothesized related to the metabolic pathways that oxidized lipids follow after ingested. Some studies (Aw and Williams, 1992; Aw et al., 1992) suggested that absorption of lipid peroxides depends on the activities of antioxidant enzymes that metabolize lipid peroxides in mucosa of the digestive tract. The same authors also reported that increased lipid peroxides were related to decreased glutathione in liver and kidney. Furthermore, lipid peroxides are absorbed and peroxide metabolic sub-products are released into lymph associated to lipoproteins (Aw, 2005). Suomela et al. (2005) reported that pigs fed oxidized sunflower oil showed oxidized

triacylglycerol in chylomicrons and very low density lipoproteins (VLDL).

3. Feeding Oxidized Lipids to Animals

Feeding diets containing thermally oxidized oils/fats causes economical loss because of the negative impacts on animal health and performance, resulting in more days on feed to reach market weight. In this literature review, effects of feeding oxidized lipids to animals on growth performance, metabolic oxidation status, fatty acid metabolism, nutrient digestibility, and gut barrier function will be discussed.

- Effects on Growth Performance

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 high amounts of oxidized lipids 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 growth performance (Logani and Davies, 1979; Comporti, 1993). For example, reduced BW gain was 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, 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/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 x 6%), might not result in decreased growth performance in nursery pigs. However, as mentioned earlier in this literature review, 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. The breakdown of hydroperoxide by cleavage yields a variety of smaller molecular weight compounds, such as aldehydes, ketones, acids esters, hydrocarbons, and aromatic compounds (Gray, 1978), which cannot be detected using PV. Therefore, a low PV could be due to either minimal oxidation or decomposition of hydroperoxides that has already begun.

- **Effects on Metabolic Oxidation Status**

Metabolic oxidation status in animals can be determined by measuring TBARS and concentrations of antioxidants in blood and tissues. Engberg et al. (1996) observed that plasma concentration of TBARS was higher, and α-tocopherol was lower in broilers fed 11% oxidized vegetable oil (9% rapeseed oil, 2% soybean oil; PV = 156 meq/kg oil) with dietary PV equal to 17.6 meq/kg feed. Similarly in swine, feeding oxidized corn oil (PV = 180 meq/kg oil) with dietary PV equal to 9 meq/kg feed increased plasma TBARS, and decreased α-tocopherol concentrations in plasma and liver (Fernández-Dueñas, 2009). In

contrast, in another study conducted by the same authors (Fernández-Dueñas et al., 2008), feeding 5% oxidized canola oil (PV = 7.98 meq/kg oil) with dietary PV = 0.4 meq/kg feed did not change plasma TBARS. These results suggest that the lack of effect on blood TBARS may be due to the insufficient dietary oxidative challenge, as measured by PV of the oil and feed. Therefore, there seems to be a threshold level above which oxidized lipids can elevate metabolic oxidation status when feeding to animals.

- **Effects on Lipid Metabolism via PPARα**

Feeding thermally oxidized oil/fat to rats (Koch et al., 2007; Sulzle et al., 2004) and pigs (Luci et al., 2007; Liu et al., 2012) alter *in vivo* lipid metabolism by activating the peroxisome proliferator-activated receptor α (PPARα) via up-regulation of some target genes of PPARα, such as acyl CoA oxidase, catalase, and carnitine palmitoyltransferase-1. The transcription factor PPARα controls the expression of fatty acid oxidative metabolism in many aspects, including fatty acid uptake through membranes, fatty acid activation, intracellular fatty acid trafficking, fatty acid oxidation, ketogenesis, and triglyceride storage and lipolysis (Cabrero et al., 2001). Some mechanisms regarding these regulatory roles of PPARα in lipid metabolism have been studied, while most of them are still unknown. In general, the activation of PPARα stimulates catabolic pathways of fatty acids (Desvergne and Wahli, 1999), by increasing activities of fatty acid transportation, and enhancing β-oxidation capacity, gluconeogenesis, and ketogenesis. Therefore, up-regulation of the target genes of PPARα involved in the oxidation of fatty acids is expected to reduce concentrations of triglycerides in the liver, plasma and adipose tissue (Sulzle et al., 2004). Taken together, one may theorize that

dietary oxidized oil/fat could promote fatty acid catabolism by up-regulating the target genes of PPARα, and thus leaving less fatty acids available for deposition as fat in the adipose tissue. However, this effect has not been studied. Fernández-Dueñas (2009) reported reduction in both 10th-rib and last-rib backfat depth in pigs fed 5% oxidized corn oil, indicating that dietary oxidized lipids or their components might lead to reduced body fat, compared with feeding fresh oil. Further studies focusing on PPARα gene expression in adipose tissue of animals fed oxidized versus fresh oil is needed to better understand this effect.

- **Effects on Nutrient Digestibility**

Previous studies found that feeding oxidized lipids may negatively affect nutrient digestibility and utilization in animals due to localized oxidative damage to the intestinal epithelial cells. Ringseis et al. (2007) reported that feeding pigs oxidized sunflower oil, which contains high levels of linoleic acid similar to corn oil, increased TBARS in intestinal epithelial cells, which indicates an increased localized oxidative stress within the intestine. Additionally, Dibner et al. (1996) observed increased intestinal epithelial cell turnover and decreased lymphocyte proliferation in follicles of the lamina propia in broilers fed oxidized poultry fat, indicating that nutrient absorption and digestion may be compromised due to the impairment of intestinal cells.

More direct evidence could be found in some metabolism studies focusing on the digestibility of DM, CP, fat and energy in animals fed oxidized lipids. However, the results have been inconsistent. Yuan et al. (2007) reported decreased digestibility of DM, CP, fat, and ME in nursery pigs fed oxidized fish oil. In broilers fed oxidized vegetable

oil, retention of fat, and energy was reduced (Engberg et al., 1996). Similarly, reduced digestibility of fat and GE were also reported in mink fed oxidized fish oil (Borsting et al., 1994). In contrast, DeRouchey et al. (2004) reported that digestibility of GE, DM, N, fat and/or fatty acids were not affected by feeding thermally oxidized choice white grease to nursery pigs. These contradictory findings might be due to differences in quality and lipid peroxidation level in the dietary lipids fed. In general, feeding oxidized lipids with a higher peroxidation level is likely to induce negative effects on nutrient digestibility in animals.

- **Effects on Gut Barrier Function**

On a daily basis, the intestine is exposed to an unlimited number and variety of intraluminal entities, including foreign antigens, microorganisms, and their toxins, which are derived from ingested food, xenobiotics, bacteria, and viruses (Walker and Sanderson, 1992). Some of these antigens pose no threat to the host, while others are harmful and may lead to disorders affecting the nervous and immune systems.

The intestinal barrier consists of an intrinsic layer, including epithelial cells and tight junctions, and an extrinsic layer, which is comprised of bacteria and a coating of mucus with high concentrations of secretory IgA (Walker and Sanderson, 1992). Proper intestinal barrier function is essential for maintaining optimal health and balance throughout the body. The intestinal barrier is the first line of defense against the toxic environment. As shown in **Figure 1-11**, the normal gut barrier (shown in the left half of the figure) shows intact mucosal lining cells of the small intestine with ongoing submucosal vascular perfusion and minimal loss of plasma constituents in the lumen of

the gastrointestinal (GI) tract. While in a "leaky" gut with impaired gut barrier function (shown in the right half of the figure), luminal contents such as bacteria, fungi, and other potential toxins pass through the endothelial gap into the blood, leading to infection, inflammation, and a series of disease. At the same time, plasma constituents, such as proteins, immunoglobulins, and other middle sized molecules, also pass through the endothelial gap into the lumen of the GI tract. The increased losses of plasma constituents provide a substrate for aerobic and anaerobic bacterial proliferation in the lumen, resulting in an imbalanced microflora distribution.

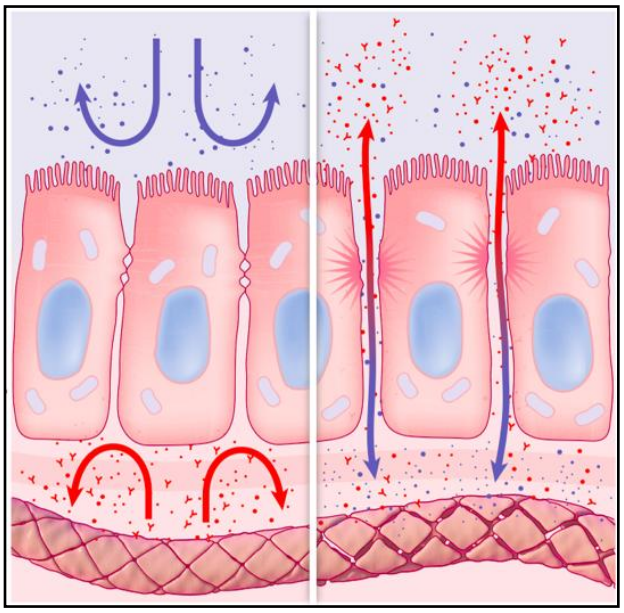


Figure 1-11. Normal (left) and "leaky" (right) small intestine (Redelmeier, 2009)

Intestinal barrier function can be measured using the probes of mannitol and lactulose. This dual sugar test was first introduced by Menzies (1974). The principle of the test is based on the fact that orally administered mannitol, a monosaccharide, will be passively absorbed through the intestinal epithelium, while lactulose, a disaccharide, normally does not pass the intestinal epithelium through the paracellualr route unless the

barrier function is compromised. However, there are several pre-mucosal (e.g., bacterial degradation) and post-mucosal (e.g., completeness of urine collection) factors (Bjarnason et al., 1995) that affect the test results, and this test assumes that both probes are affected by these factors to a similar degree. Therefore, the L/M ratio, instead of the urinary concentration of these compounds, is a more accurate indicator to determine intestinal barrier function (Bjarnason et al., 1995). For example, an increased L/M ratio indicates an increased intestinal permeability and an impaired intestinal barrier function, whereas a decreased L/M ratio indicates a decreased intestinal permeability and an improved intestinal barrier function.

Another method that has been used to determine gut barrier function *in vivo* is through the measurement of circulating endotoxin concentrations in the blood (Sharma et al., 2007). Endotoxins are the complex lipopolysaccharide components of the outer cell wall membrane of Gram-negative bacteria, and are considered to be a major virulence factors that are responsible for the lethal effects and clinical manifestations of diseases in humans and animals (Swain et al., 2008). Normally, Kupffer cells in the liver detoxify endotoxins by phagocytosis (Rao, 2009). When the flux of endotoxins overwhelms the phagocytotic capacity of Kupffer cells, the endotoxins spill into the systemic circulation. Impaired gut barrier function is associated with increased bacterial translocation and endotoxin absorption (Walker and Sanderson, 1992). Based on this theory, elevated endotoxin concentration in the blood has been used to indicate impaired gut barrier function in some studies (Sharma et al., 2007; Rao, 2009). Compared with the lactoluse-mannitol method, the measurement of endotoxin in blood samples is easier, faster, and does not require any special feeding to the animals. However, there are some

disadvantages of this method. First, this method is not a direct measurement of intestinal permeability. Many factors, other than change in intestinal permeability could influence the level of endotoxin in the blood, such as disease challenge, malnutrition, thermal injury, and an unsanitary environment (Saadia et al., 1990). Second, Kupffer cells in the liver normally detoxify endotoxins by phagocytosis (Rao, 2009). When the flux of endotoxins overwhelms the phagocytotic capacity of Kupffer cells, the endotoxins spill into the systemic circulation. As a result, the increased concentration of endotoxin in the circulating system can be a combination effect of reduced detoxification capacity of the liver and gut barrier function. Furthermore, sample contamination with bacteria during collection and analysis may also lead to increased production of endotoxin, which overestimates the endotoxin level in blood samples. Due to these limitations, measurement of endotoxin is not as widely used as the lactoluse-mannitol method when determining gut barrier function *in vivo*.

Dibner and Knight (2008) stated that oxidative stress in the gastrointestinal system, regardless of its cause, is associated with loss of barrier function due to the denaturation of the protein component of the enterocyte junctional complexes (Musch et al., 2006), which results in failure of both cell-cell adhesion and apical membrane integrity (Blikslager et al., 2007). Additionally, intestinal epithelial cells contain relatively high concentrations of PUFA, which are particularly effective in enhancing intestinal epithelia barrier integrity by improving natural resistance (Willemsen et al., 2008). Long chain PUFA are susceptible to lipid peroxidation (Tappel, 1962). Oxidation of PUFA present in intestinal epithelial cell membranes may lead to cell injury, and thus, impair epithelial barrier function due to the disruption of the normal membrane structure

and function (Lauridsen et al., 1999). In a human study, lipid hydroperoxides increased intestinal cell apoptosis (Wang et al., 2000), suggesting that dietary oxidants could impair the integrity of the intestinal epithelium. However, little research has been conducted to evaluate the effects of feeding oxidized lipids to animals on gut barrier function. In a recent study, Liu et al. (2012; unpublished) evaluated the effects of feeding oxidized lipids, including oxidized corn oil, canola oil, beef tallow, and poultry fat, respectively, to nursery pigs on intestinal barrier function. However, none of these four oxidized lipids had any effect on intestinal barrier function as measured by the lactoluse-mannitol protocol.

V. Antioxidants in Swine Nutrition

1. Antioxidant System

To control the level of reactive oxygen species (ROS) and to protect cells and tissues under oxidative stress, animals have several enzymes scavenging ROS (i.e. superoxide dismutase, catalase, and glutathione peroxidase), detoxifying lipid peroxidation products (i.e. glutathione S-transferases, phospholipid-hydroperoxide glutathione peroxidase and ascorbate peroxidase), a network of low molecular mass antioxidants (i.e. ascorbate, glutathione, phenolic compounds and tocopherols), and a whole array of enzymes regenerating the active forms of the antioxidants (i.e. monodehydroascorbate reductase, dehydroascorbate and glutathione reductase; Blokhina et al., 2003). These compounds constitute the important and complex antioxidant system also known as the oxidation defense system in the animals.

Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX)

are three protective antioxidant enzymes that have been widely studied in swine. **Table 1-9** shows the reactions they catalyze to scavenge ROS. In addition, there are several nutritionally essential minerals incorporated into these protective antioxidant enzymes. Zinc and Cu are required for the activity of SOD in the cytosol (Cu-Zn-SOD), and Mg is the co-factor for the SOD in the mitochondria (Mg-SOD). Selenium, an essential component of GPX, is important in the decomposition of hydrogen peroxide and lipid peroxides. Catalase, a heme protein, contains Fe and catalyzes the decomposition of hydrogen peroxide (Lawrence et al., 1987). In addition to these essential minerals, there are three essential vitamins that can directly scavenge free radicals, including vitamin E, vitamin C (ascorbic acid) and β-carotene or vitamin A. The level of dietary intake of all of the antioxidant micronutrients directly affects the circulating level of these nutrients and the activity of the antioxidant enzymes. Thus, low intakes of one or more of these antioxidant nutrients could reduce the body's antioxidant system and increase the susceptibility to negative effects associated with free radical damage.

Table 1-9. Reactive oxygen species scavenging enzymes (Blokhina et al., 2003)

Enzyme	Reaction catalyzed
Superoxide dismutase	$O_2^- + O_2^- + 2H^+ \rightarrow 2H_2O_2 + O_2$
Catalase	$2H_2O_2 \rightarrow O_2 + 2H_2O$
Glutathione peroxidase	$2GSH + ROOH \rightarrow GSSG + RH + 2H_2O$

2. Vitamin E

- Structure and Sources

Vitamin E refers to a group of eight fat-soluble compounds, tocopherols and tocotrienols, which are mainly found in plant tissues and vegetable oils. As shown in **Figure 1-12**, tocopherols constitute a series of related benzopyranols (or methyl tocols),

and a saturated side chain of a 20-carbon phytyl tail (including the pyranol ring). Tocotrienols contain a 20-carbon geranyl tail with double bonds at the 3', 7' and 11' positions attached to the benzene ring. The side-chain methyl groups have *R,R,R* stereochemistry. The four main constituents of the two classes are termed - *alpha* (5,7,8-trimethyl), *beta* (5,8-dimethyl), *gamma* (7,8-dimethyl) and *delta* (8-methyl).

Tocopherols and tocotrienols are only synthesized by plants and other oxygenic, photosynthetic organisms (Brigelius-Flohe and Traber, 1999). Seed oils are a major source for natural vitamin E (**Table 1-10**). Sunflower and olive oils are good sources of α-tocopherol and palm oil is a good source of the tocotrienols. In general, tocotrienols tend to be abundant only in seeds and fruits, especially of monocots such as wheat, rice and barley, although a major commercial source is palm oil (Brigelius-Flohe and Traber, 1999). Among these 8 forms of vitamin E, α-tocopherol is the most abundant form in nature, and has the most biological activity in the body (Traber and Atkinson, 2007). In swine, vitamin E cannot be synthesized and is always provided as an essential nutrient in the diet. The most commonly used commercial form of vitamin E for animal supplementation is the acetate ester of *all-rac*-α-tocopherol (*all-rac*-α-tocopheryl acetate; **Figure 1-13**). Since the esters are resistant to oxidation and display no antioxidant activity, α-tocopherol acetate is more stable than α-tocopherol in feed. The ester is hydrolyzed in the gut, releasing the native α-tocopherol and thus, regains its antioxidant activity. In the following review and chapters, vitamin E specifically refers to α-tocopherol (α-TOH).

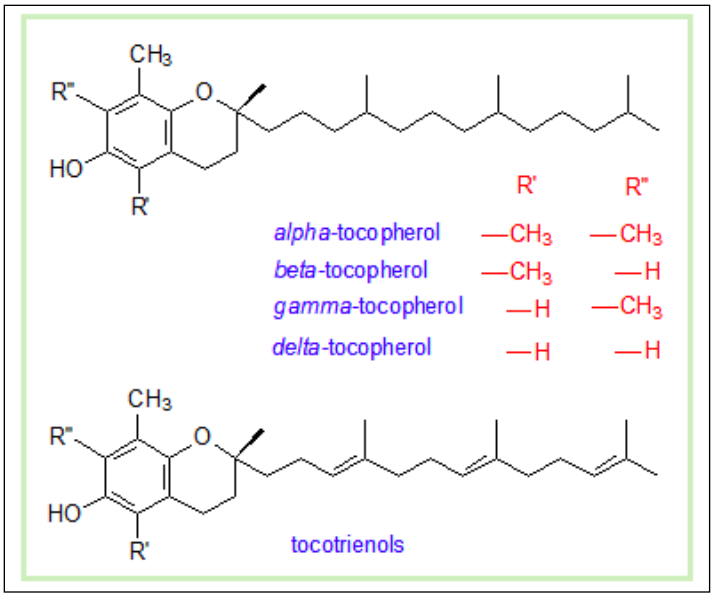


Figure 1-12. Chemical structure of tocopherols and tocotrienols
(http://lipidlibrary.aocs.org/lipids/tocol/index.htm)

Table 1-10. Tocopherol and tocotrienol contents (mg/kg) in some seed oils (Adapted from Gunstone et al., 1994)

Seed oil	α-T	β-T	γ-T	δ-T	α-TT	β-TT	γ-TT	δ-TT
Palm	89	-	18	-	128	-	323	72
Soybean	100	8	1021	421	-	-	-	-
Maize	282	54	1034	54	49	8	161	6
Sunflower	670	27	11	1	-	-	-	-
Rapeseed	202	65	490	9	-	-	-	-

Note: T - tocopherol; TT – tocotrienol

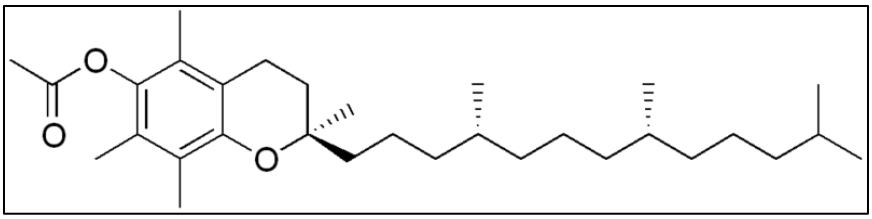


Figure 1-13. Chemical structure of α-tocopheryl acetate
(The Merck Index, 1989)

- **Antioxidant Functions of Vitamin E**

Extensive research documents that vitamin E functions as a chain-breaking antioxidant to prevent the propagation of free radical reactions (Tappel, 1962; Burton, et al., 1983; Traber and Atkinson, 2007). As discussed at the beginning of this literature review, lipid peroxidation is a chain reaction that proceeds in three stages, initiation, propagation and termination. Ideally, this undesirable process can be prevented by stopping the initial production of free radicals via the activities of compounds referred to as preventive antioxidants (Burton and Ingold, 1989). Catalase and glutathione peroxidase, for example, convert the free radical precursors H_2O_2 and hydroperoxides (ROOH), respectively, into harmless products (Burton and Traber, 1990). However, in the event that peroxyl radicals (ROO•) do form, the chain-breaking antioxidants, which typically are sterically hindered phenols such as α-TOH, are required to inhibit the propagation phase. Specifically, α-TOH donates its phenolic hydrogen atom to the radical and converts it to a hydroperoxide product. At the same time, it is converted to the tocopheroxyl radical (α-TO•) which is sufficiently stable to be prevent continuation of the chain and, instead, is removed from the cycle by reaction with another peroxyl radical to form inactive, nonradical products. Since α-TOH can compete for peroxyl radicals much faster than can PUFA, it protects the PUFA from lipid peroxidation by preventing H atom abstraction by free radicals. The dynamic interplay of these factors is illustrated in **Figure 1-14**, which shows the three phases of the free radical chain mechanism of lipid peroxidation and the chain-breaking effect from α-TOH.

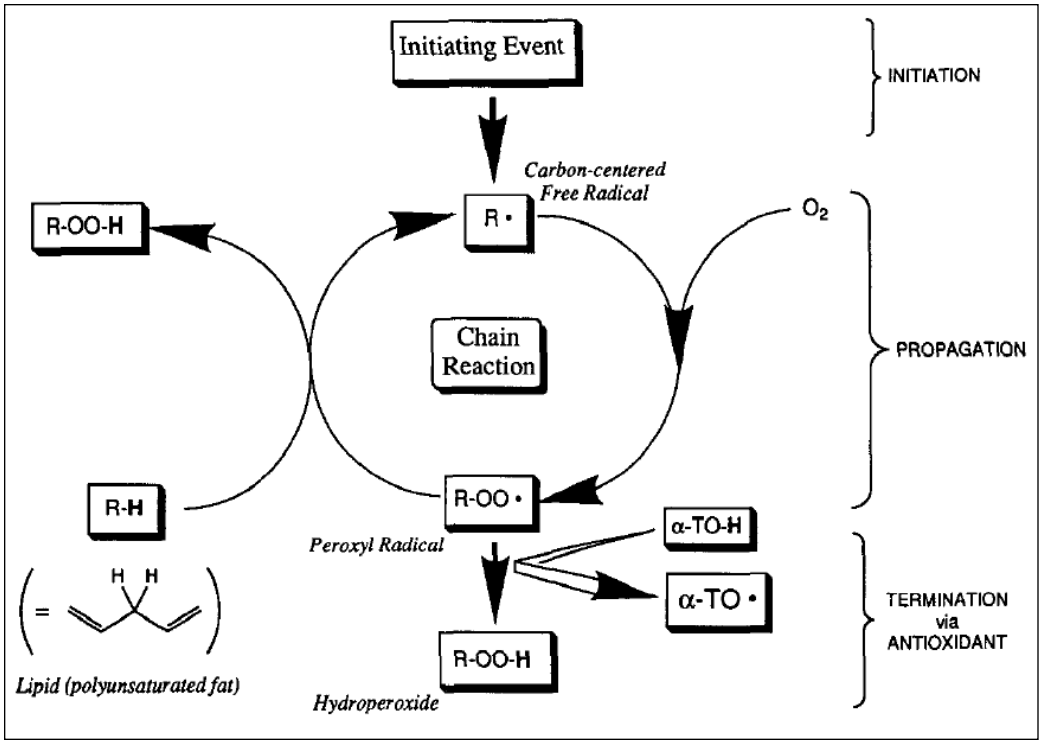


Figure 1-14. Lipid peroxidation process and effect of α-tocopherol (Burton and Traber, 1990)

- **Accumulation of Vitamin E in Tissues**

Since vitamin E cannot be synthesized by animals, the presence of the vitamin in animal tissues reflects its dietary availability. Because vitamin E is fat soluble, absorption of vitamin E is dependent on the animal's ability to digest and absorb fat.

Monahan et al. (1990) evaluated the effects of feeding pigs α-tocopheryl acetate at either basal (30 mg/kg of feed) or supplemental (200 mg/kg of feed) levels from weaning until harvest. As shown in **Table 1-11**, plasma and tissue levels of α-tocopherol were 2.5 to 3.0 times higher in pigs fed the supplemented diet than in those fed the control diet. The response to dietary vitamin E intake of different organs varies depending on their metabolic activities, and α-tocopherol concentrations increase in the following order: liver > heart > lungs > kidney (Buckley et al., 1995). Furthermore, α-tocopherol levels in

porcine tissues also depend on the duration of dietary supplementation. For example, the α-tocopherol concentrations in all tissues examined, except for heart and plasma, were greater in pigs fed vitamin E at 200 mg/kg in diets from weaning to slaughter than those in pigs just fed supplemented diets for 35 d before slaughter (Sisk et al., 1994).

Table 1-11. Mean a-tocopherol content of plasma (mg α-tocopherol/mL) and tissues (ng α-tocopherol/mg of protein) from pigs fed a control diet (containing 30 mg α-tocopheryl acetate/kg of feed) or a diet supplemented with vitamin E (200 mg α-tocopheryl acetate/kg of feed) for approximately 125 days prior to harvest (Monahan et al., 1990)

Sample	Dietary vitamin E content	
	Control, 30 mg/kg	Supplemented, 200 mg/kg
Plasma, ug/mL	2.0	5.5
Lung, ng/mg of protein	25.3	71.4
Liver, ng/mg of protein	39.2	98.7
Heart, ng/mg of protein	27.4	79.9
Kidney, ng/mg of protein	10.3	28.3
Longissimus muscle, ng/mg of protein	7.6	21.8
Muscle mitochondria, ng/mg of protein	45.3	124.2
Muscle microsomes, ng/mg of protein	62.4	164.8

- **Supplementation of Vitamin E under Dietary Oxidative Challenge**

As discussed in this review, feeding oxidized lipids to different species can result in reduced appetite, decreased growth, and health problems. Fatty acid peroxidation products and cholesterol oxides are implicated in mutagenesis and carcinogenesis, and in the etiology of coronary heart disease in humans (Buckley et al., 1995). Buckley et al. (1989) suggested that the animal's natural oxidation defense system, especially glutathione peroxidase, may become overwhelmed by high dietary oxidative challenge from peroxides, and therefore, allow the passage of lipid peroxides through the intestinal mucosa. The same researchers also showed that oxidized dietary lipids have a

destabilizing effect on muscle microsomes and lead to remarkable changes in membrane structure. Furthermore, dietary oxidative challenge clearly results in significant loss of antioxidants in animal tissues. Alpha-tocopherol is likely degraded in the intestinal lumen, tissues, and/or membranes by the secondary lipid peroxidation products that are absorbed (Izakl et al., 1984). For example, reductions in the concentration of α-tocopherol were found in pig muscle and liver (Murphy et al., 1991), in pig plasma and muscle microsomal membranes (Monahan et al., 1993), and in poultry plasma (Sheehy et al., 1993) when these animals were fed oxidized oil. Because of the depletion of antioxidants and the overwhelmed oxidation defense system resulting from dietary oxidized lipids, supplementation of additional vitamin E in the diet may be important to compensate for the oxidative stress and maintain a favorable antioxidant/prooxidant balance in animals as well as in meat products.

The effects of increasing vitamin E level or other antioxidants when feeding diets containing oxidized lipids have been evaluated in several studies, but the results are inconsistent. Harrell et al. (2010) reported that feeding a blend of antioxidants improved ADG, ADFI, and BW but not G:F in nursery pigs fed 5% oxidized corn oil (PV = 7.5 meq/kg of diet). However, other studies did not observe any beneficial effects from the addition of dietary antioxidants in finishing pigs fed 5% oxidized corn oil (PV = 9 meq/kg of diet; Fernández-Dueñas, 2009), in broilers fed 3% oxidized soybean oil (PV = 1.5 meq/kg of diet; Anjum et al., 2002), or in rainbow trout fed 7.5% oxidized fish oil (PV = 9 meq/kg of diet; Hung et al., 1981). The inconsistent response may be due to several factors, such as differences in the amount of peroxides in the oxidized oil, lipid type, dietary inclusion rate, length of feeding time of antioxidants, animal age, and

antioxidant system status. For example, pigs may respond less to dietary vitamin E due to the protective effects from other antioxidants (i.e. sulfur-containing antioxidants) that already exist in the body (Song et al., 2012).

- **Effects of Supplementation of Vitamin E on Pork Quality**

Oxidation of lipids is a major cause of deterioration in the quality of meat products and can directly affect many quality characteristics such as flavor, color, texture, nutritive value, and safety. Use of supranutritional levels of vitamin E in the diet is an efficient approach to improve the quality and storage stability of pork (Jensen et al., 1998). The measurement of TBARS is commonly used as a marker for the development of rancid off-flavors in meat and is, therefore, chosen as the marker for oxidative deterioration of meat in several studies (Lin et al., 1989; Lanari et al., 1995; Dirinck et al., 1996). Tarladgis et al. (1960) and Lanari et al. (1995) suggested a TBARS value of 0.50 mg MDA equivalents (MDA eq) /kg as the threshold concentration for detection of off-flavor by trained sensory panels. Jensen et al. (1998) summarized of 16 studies that evaluated the effects of dietary vitamin E on refrigerated pork quality (**Table 1-12**). Among these studies, 15 of 16 experiments showed reduction in muscle TBARS when higher levels of *all-rac*-α-tocopheryl acetate were provided in the diet. In particular, when pigs were fed 0 -10 mg α-tocopheryl acetate/kg feed, TBARS values in the muscle reached the critical level of 0.50 mg MDA eq/kg after 6 d of refrigerated storage. However, increasing dietary α-tocopheryl acetate levels to 200 mg/kg kept the TBARS values below 0.50 mg MDA eq/kg in most studies for the same storage period, suggesting that eating quality is still acceptable after 6 d of refrigerated storage.

Furthermore, dietary supplementation with α-tocopheryl acetate improves color stability of pork and poultry meat products (Jensen et al., 1998). The mechanism by which endogenous vitamin E improves color stability is not clear, but it is well accepted that discoloration and lipid peroxidation in fresh meat are closely related (Dirinck et al., 1996). Additionally, decreased myoglobin oxidation has been observed in meat where lipid peroxidation was lowered due to high levels of endogenous vitamin E (Monahan et al., 1994). The effect of dietary vitamin E on color stability of refrigerated pork chops was also summarized by Jensen et al. (1998), and is shown in **Table 1-12**. The efficacy of supplemental vitamin E to control color deterioration in pork meat is inconsistent. Five out of 10 experiments that evaluated the meat color reported improved color stability when feeding 200 mg/kg vitamin E, whereas the other 5 experiments showed no differences.

Table 1-12. Influence of dietary vitamin E supplementation on muscle vitamin E deposition and refrigerated pork quality and storage stability (Jensen et al., 1998)

Storage conditions	Muscle	Feed Vitamin E[a]	Muscle vitamin E[b]	Reduction in lipid oxidation[c]	Improved colour[d]
Atmospheric air,	M. *longissimus*	0	1.7		
Illuminated, 4°C, 6 days	*lumborum*	200	7.9	73%	106%
Atmospheric air, 4°C,	M. *longissimus*	60			
8 days	*dorsi*	100	NA	NA	64%
Atmospheric air, illuminated,	M. *longissimus*	10	0.8		
4°C, 8 days	*dorsi*	100	2.7	76%	NS
		200	4.1	77%	44%
Atmospheric air, illuminated,	M. *longissimus*	10	0.8		
4°C, 8 days[e]	*dorsi*	100	2.6	87%	NS
		200	4.1	88%	64%
Atmospheric air	M. *longissimus*	10	0.5		
illuminated, 4°C, 6 days[e]	*dorsi*	100	2.6	68%	33%
		200	4.7	80%	43%
Atmospheric air, 4°C,	M. *longissimus*	0	0.2		
5 days	*dorsi*	100	1.9	35–45%	NS
Atmospheric air, illuminated,	M. *longissimus*	100	5.2		
4°C, 6 days	*dorsi*	200	7.9	31%	NS
		700	11.1	52%	NS
Atmospheric air, illuminated,	M. *psoas*	100	5.9		
4°C, 6 days	*major*	200	11.7	22%	NS
		700	15.7	52%	NS
Atmospheric air, 4°C,	M. *longissimus*	10			
6 days	*dorsi*	200	NA	58%	NA
Atmospheric air, illuminated,	M. *longissimus*	0			
4°C, 6 days	*dorsi*	200	NA	45%	NA
Atmospheric air, 4°C,	M. *longissimus*	0			
6 days	*dorsi*	200	NA	67%	NA
Atmospheric air, illuminated,	M. *longissimus*	10			
4°C, 8 days	*dorsi*	200	NA	78%	NA
MAP (80% O_2/20% CO_2),	M. *longissimus*	0	1.0		
illuminated, 4°C, 6 days	*lumborum*	200	6.9	53%	74%
MAP (80% O_2/20% CO_2),	M. *longissimus*	40	2.5		
Illuminated, 4°C, 9 days	*dorsi*	200	4.0	52%	NA

[a] Mg *all-rac*-α-tocopheryl acetate/kg feed
[b] Mg α-tocopherol/kg muscle
[c] Decrease in TBARS numbers in supplemented animals relative to control animals
[d] Increase in the a-value (indicative of the amount of red colour) in supplemented animals relative to control animals
[e] Loins were pre-frozen prior to cutting and refrigerated storage
[f] Loins were vacuum-packed and stored at 4°C for 58 days prior to cutting and refrigerated storage
NA, Parameter was not analysed
NS, The effect of dietary vitamin E did not result in significant changes in meat quality

3. Sulfur-Containing Compounds

- Structure and Sources

Sulfur is an essential component in normal physiological functions of animals and is incorporated into amino acids, proteins, enzymes and micronutrients (Atmaca, 2004). Sulfur occurs in its native or elemental state and combines with iron, base metals and sulfide minerals (Atmaca, 2004). Methionine (Met) and cysteine (Cys) may be considered the principal sulfur-containing amino acids because they are 2 of 20 amino acids that are incorporated into proteins. However, homocysteine and taurine also play important physiological roles in the body (**Figure 1-15**). Glutathione (GSH) is a sulfur-containing tripeptide, which consists of Cys, glutamate and glycine (**Figure 1-16**).

Figure 1-15. Structures of the sulfur-containing amino acids (Brosnan and Brosnan, 2006)

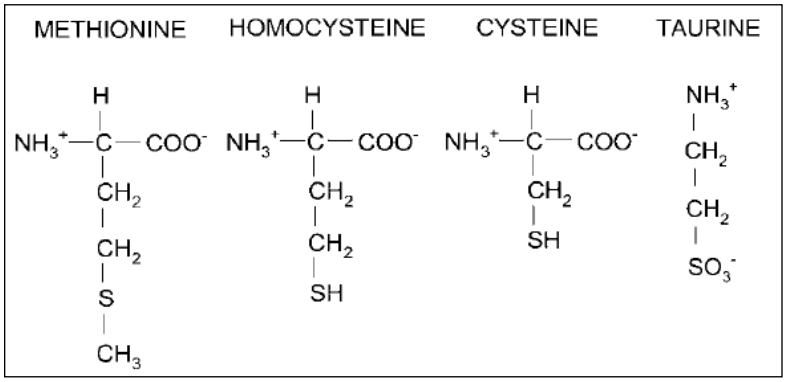
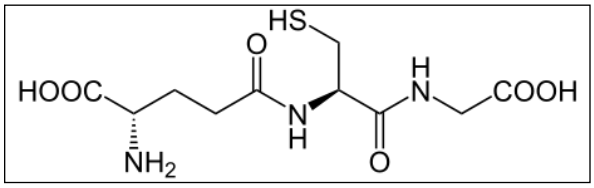


Figure 1-16. Structure of glutathione (The Merck Index, 1989)



Unlike plants that can use inorganic sulfur to synthesize sulfur-containing amino

acids, humans and monogastric animals must obtain essential sulfur-amino acids from the diet. Plants, therefore, are an important source of sulfur for humans and most animals. In addition, sulfur-containing amino acids, such as Met and Cys, are more abundant in animal and cereal proteins than in legume proteins (Atmaca, 2004). Glutathione, a cysteine-containing tripeptide, is also a source of dietary sulfur. Approximately 50% of dietary GSH is contributed by fruits and vegetables, while 25% is from meat (Atmaca, 2004).

- **Metabolism and *De Novo* Synthesis**

The microflora in ruminants are able to synthesize sulfur-containing amino acids from inorganic sulfate in the diet and these mechanisms have been well documented (Block et al., 1951; Emery et al., 1957). In swine and other monogastric animals, however, the capability to utilize inorganic S to synthesize organic S is still unclear. However, the pathways of Met metabolism that lead to *de novo* synthesis of Cys and taurine (**Figure 1-17**) are well understood. In particular, Met metabolism begins with its activation to S-adenosylmethionine, which then donates its methyl group to an acceptor to produce S-adenosylhomocysteine, followed by hydrolysis to homocysteine. This sequence of reactions is referred to as transmethylation pathway that is ubiquitously present in cells (Brosnan and Brosnan, 2006). Homocysteine may be methylated back to Met by the ubiquitously distributed methionine synthase in the liver as well as the kidney of some species. Therefore, the Met cycle does not lead to Met degradation. The transsulfuration pathway, which converts homocysteine to Cys, results in catabolism of Met. The transsulfuration pathway has a very limited tissue distribution, and is restricted

to the liver, kidney, intestine, and pancreas (Brosnan and Brosnan, 2006). The conversion of Met to Cys is an irreversible process, which explains the well-known nutritional principle that Cys is not a dietary essential amino acid as long as adequate Met is present, but Met is a dietary essential amino acid, regardless of Cys availability. Cysteine serves as a precursor for many compounds, and the catabolism of Cys results in the synthesis of taurine and GSH.

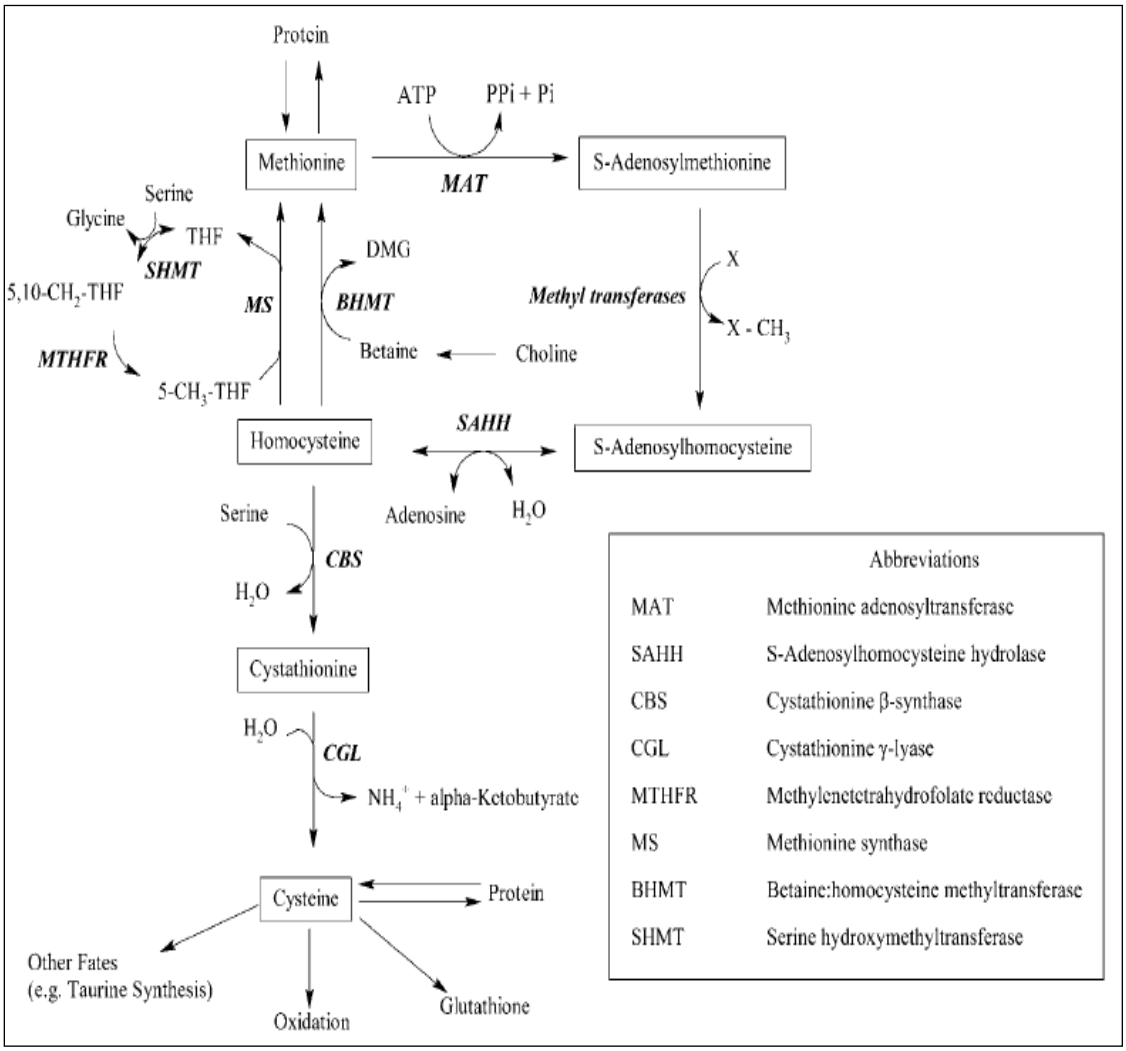


Figure 1-17. Major pathways of sulfur-containing amino acid metabolism (Brosnan and Brosnan, 2006)

Several cellular and *in vivo* studies have confirmed the existence of these

transmethylation and transsulfuration pathways. Glazenburg et al. (1983) reported that reducing dietary Met level decreased taurine concentration in serum and GSH content in liver of rats. 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 Sakehisekaran, 1997) expressed increased GPX activity in blood. The supplementation of SAA appears to be an effective method of restoring GSH status, because sulfur-containing amino acids play a role in determining the flux of cysteine between cysteine catabolism and GSH synthesis (Atmaca, 2004). However, the detailed mechanism of this response has not been determined.

- **Antioxidant Properties**

Biological S-containing compounds, including Met, Cys, taurine, GSH, and others (for example, N-acetylcysteine and lipoic acid) have been studied extensively for their antioxidant properties via mechanisms of radical scavenging, GPX activity, and metal-binding interactions (Parcell, 2002; Atmaca, 2004). In particular, Met can have a free radical scavenging effect by being oxidized to methionine sulfoxide in many animal species (Levine et al., 2000; Atmaca, 2004). Cysteine is required for GSH and protein synthesis. Cysteine also plays an important role in protein structure by forming disulfide crosslinks that stabilize protein conformation. Taurine can scavenge ROS 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) reported that feeding 5% taurine to rats increased their BW and decreased liver TBARS when diets

contained 3% oxidized fish oil, suggesting that taurine may protect animals against dietary oxidized lipids. Moreover, glutathione is the major cellular antioxidant in pigs. Glutathione acts as a detoxifying agent for endogenous radical species and as an essential co-factor for GPX, although glutathione and other sulfur-containing compounds do not have GPX activity (Battin and Brumaghim, 2009). Further studies evaluating the protective effects of these sulfur-containing antioxidants under a dietary oxidative challenge will further our understanding of these compounds, and facilitate the development of a nutritional strategy to overcome the negative effects of lipid peroxidation.

VI. Summary

Dried distillers grains with solubles has been used widely as a feed ingredient in swine production. However, DDGS often results in inconsistent growth performance when high dietary levels of DDGS (20-30%) are fed to growing-finishing pigs. Results from previous studies suggest that the reduction in growth performance could be due to several factors, such as poor amino acid balance and digestibility, low net energy level, antinutritional factors, or toxins in DDGS. DDGS contains approximately 10% corn oil, which is high in PUFA. The lipid fraction in corn DDGS may be easily oxidized when exposed to air or during the production process under high temperature and moisture conditions. Lipid peroxidation in animal feed can negatively affect pig health and growth performance. Therefore, one may reasonably speculate that the oxidized lipids in DDGS may be one of the causes for reduced growth performance observed in some studies. Furthermore, the toxic aldehydes formed from peroxidation of PUFA in meat products

could be a health concern if consumed by humans. However, no studies have been conducted to determine the effects of feeding DDGS containing oxidized lipids on meat quality and shelf-life stability.

Vitamin E is always supplemented in swine diets and functions as an important antioxidant *in vivo*. Vitamin E can protect against lipid peroxidation and increase pork shelf-life stability, especially when diets contain oxidized lipids (Jensen et al., 1998). Therefore, it appears that feeding DDGS containing oxidized corn oil to pigs may require supplementation of higher levels of vitamin E than currently being fed as suggested by NRC (1998). No studies have been conducted to determine if supplementation of vitamin E at NRC level is adequate when feeding oxidized DDGS to pigs from weaning to harvest.

By characterizing the level of lipid peroxidation in DDGS and understand the effects of lipid peroxidation on pig health, performance, nutrient digestibility and pork quality, we will determine if any of the negative effects of feeding high dietary levels of DDGS can be overcome by the using higher levels of vitamin E than currently recommended by NRC. Results of these research projects will further our understanding of the impact of feeding DDGS containing a high level of oxidized lipid to growing-finishing pigs and evaluate the supplementation of vitamin E as a potential nutritional intervention strategy to overcome any negative effects that may be observed. As a result, it may be possible for more pork producers to use high dietary inclusion rates of DDGS in growing-finishing diets without concerns about pig performance and pork product quality.

CHAPTER II. Technical note: Evaluation of lipid peroxidation level in corn dried distillers grains with solubles (DDGS)

Lipid peroxidation in feed can negatively affect animal health, growth performance, and meat quality. The objective of this study was to evaluate the lipid peroxidation level in corn dried distillers grains with solubles (**DDGS**) samples from 31 ethanol plants in the U.S. An unaltered corn sample was obtained from a corn processing plant to use as a reference. Oils were extracted with hexane and analyzed for peroxide value (**PV**) and thiobarbituric acid reactive substances (**TBARS**). Peroxide values of DDGS samples ranged from 4.2 to 84.1 meq/kg oil. The highest PV among DDGS samples was 27 times greater than that of the reference corn sample (3.1 meq/kg oil). The TBARS values for DDGS samples ranged from 1.0 to 5.2 ng MDA equivalents/mg oil. The DDGS sample with the highest TBARS value was 25 times greater than that of the reference corn sample (0.2 ng MDA equivalents/mg oil). Color of DDGS samples was measured by Minolta L*, a* and b* corresponding to the degree of lightness, redness and yellowness, respectively. Correlations between PV, TBARS, and color were determined. Values of PV and TBARS were correlated positively ($r = 0.81$; $P < 0.001$). Both TBARS and PV were correlated negatively with L* ($r = -0.73$; $P < 0.001$, and $r = -0.63$; $P < 0.001$, respectively) and b* ($r = -0.67$; $P < 0.001$, and $r = -0.57$; $P < 0.001$, respectively), which means that darker and less yellow colored DDGS was more likely to have a higher lipid peroxidation level, as measured by TBARS and PV, compared to lighter colored DDGS samples. However, a* was not correlated with either PV ($P = 0.97$) or TBARS ($P = 0.66$). These results indicate that color can be a preliminary indicator of lipid peroxidation level

in DDGS, but the exact level of peroxidation is more accurately measured by PV and TBARS.

Keywords: color, dried distillers grains with solubles, lipid peroxidation, peroxide value, thiobarbituric acid reactive substances

INTRODUCTION

With expansion of the ethanol industry, utilization of corn co-products, such as dried distillers grains with solubles (**DDGS**), in swine feeds has increased dramatically due to increased availability and cost competitiveness compared with corn and soybean meal. However, limits on dietary inclusion rates of DDGS occur because of inconsistent growth performance when high dietary levels of DDGS (20 to 30%) are fed to growing-finishing pigs (Whitney et al., 2006; Linneen et al., 2008; Stein and Shurson, 2009). Although several potential risk factors (i.e., low net energy levels, poor AA balance and digestibility, and excess dietary crude protein) have been suggested as potential contributors to inconsistent growth responses (Stein and Shurson, 2009; Xu et al., 2010), the presence of oxidized lipids in DDGS may also be a contributing factor.

Lipid peroxidation is a free-radical chain reaction that produces oxidized lipids and a series of toxic aldehydes (Blokhina et al., 2003). Because the lipid fraction of corn DDGS is made up largely of the PUFA, particularly linoleic acid (NRC, 1998), corn DDGS may be prone to lipid peroxidation. In addition, DDGS are heated at relatively high temperatures during the drying process that may accelerate lipid peroxidation by oxidizing unsaturated lipids. Oxidized lipids in animal feed can negatively affect animal health, growth performance, and meat quality (Miller and Brzezlnska-Slebodzlnska, 1993; Pfalzgraf et al., 1995). However, no studies have been published regarding lipid

peroxidation levels in corn DDGS. Therefore, the objective of this study was to evaluate the lipid peroxidation level in DDGS samples from 31 ethanol plants in the U.S. by measuring peroxide value (**PV**) and thiobarbituric acid reactive substances (**TBARS**), which are two commonly used indicators of lipid peroxidation (Shahidi and Zhong, 2005), and to determine the correlations between these indicators of lipid peroxidation with color in DDGS.

MATERIALS AND METHODS

Corn, DDGS samples, and chemicals

Fresh DDGS samples were obtained from 31 corn ethanol plants located in the Midwest U.S. In addition to the 31 sources of DDGS, an unaltered corn sample was obtained from a corn processing plant and used as a reference standard. All samples were stored at -20º C upon receipt.

Chemical reagents, including n-hexane, glacial acetic, chloroform, potassium iodide, and sodium thiosulfate ($Na_2S_2O_3$), were obtained from Fisher Scientific (Pittsburgh, PA). Reagents including malondialdehyde (**MDA**), trichloroacetic acid (**TCA**), 2-thiobarbituric acid (**TBA**), and hydrochloric acid (**HCl**) were purchased from Sigma-Aldrich (St. Louis, MO).

Oil extraction from corn and DDGS

Lipids from the corn and 31 DDGS samples were extracted with n-hexane following a modified procedure as describe by Seppanen and Csallany (2004). Specifically, approximately 20 g of corn and each DDGS sample were weighed and ground for 3 min to fine powder, and transferred to a 250 mL Erlenmeyer flask

containing 100 mL n-hexane. The corn and DDGS samples were mixed with n-hexane at room temperature for 15 min by stirring, and the mixture was filtered via Whatman #1 filter paper (Piscataway, NJ) to collect the hexane solution containing oils into a 250 mL round-bottom flask. This step was repeated two times by adding 50 ml n-hexane each time. Therefore, by mixing and filtering three times, lipids from corn and each DDGS samples were extracted into 200 mL of hexane solution. Lipids were obtained after the hexane solution was evaporated by mildly heating at 40 ºC for approximately 5 min. All lipid samples extracted from corn and 31 DDGS sources were stored at -20ºC until further analyses of lipid peroxidation was conducted.

Measurement of lipid peroxidation level in oils extracted from corn and DDGS

To determine the lipid peroxidation level, peroxide value (**PV**) was measured in lipids extracted from corn and 31 DDGS samples following the official method described by Association of Official Analytical Chemistry (**AOAC**; Method 965.33). In general, approximately 160 mg lipid was weighed into a 125 mL Erlenmeyer flask. A PV solution (15 mL) containing 9 mL glacial acetic and 6 mL chloroform, 1 mL saturated aqueous potassium iodide solution, and 15 mL deionized water were added into the flask, followed by mixing for 1 min. A 4×10^{-4} N $Na_2S_2O_3$ solution was used to titrate until yellow color disappeared. The PV of the lipid was calculated using the following equation: PV = [volume of $Na_2S_2O_3$ (mL) × (4×10^{-4}) x 1,000] / weight of lipid (g). Since PV measures the concentration of hydroperoxides and peroxides, it was expressed as milliequivalents of peroxide (**meq**) / kg oil. All samples and standards were conducted in duplicate in one batch, with the intra-assay CV of 4.2%.

Thiobarbituric acid reactive substances (**TBARS**) were measured in lipids extracted

from corn and 31 DDGS samples following the method described by Buege and Aust (1978). Briefly, 200 μL of lipid samples and standards of MDA were mixed with TBARS solution including 15% w/v TCA, 0.375% w/v TBA, and 0.25 N HCL. The mixtures were heated in a boiling water bath for 15 min followed by centrifugation at 3,000 × *g* for 15 min at 4ºC. The supernatant was removed and read at 532 nm using a spectrophotometer (SpectraMax 250, Molecular Device, Sunnyvale, CA). The result of TBARS was expressed as ng MDA equivalents/mg oil. This assay was conducted in 4 batches with triplicate samples and standards. The intra-assay CV was 5.8% and the inter-assay CV was 4.9%.

Measurement of color in corn and DDGS

Color (CIE scale of L*, a*, and b*) of corn and 31 DDGS samples was measured using a MiniScan XE Plus portable colorimeter (Model 45/O-S, Hunter Associates Laboratory, Reston, VA). Each sample was measured in triplicate, and the intra-assay CV for L*, a* and b* was 1.7, 6.8, and 3.3%, respectively.

Statistical analysis

Data were analyzed using the Corr procedures of SAS Inst. Inc. (Cary, NC) to determine the correlation between PV, TBARS and color of DDGS. Pearson's Correlation Coefficient (r) with a *P* value was reported to indicate the correlation between these parameters. The significance level chosen was $\alpha = 0.05$. Correlations were considered significant if $P < 0.05$, whereas values between $0.05 \leq P \leq 0.10$ were considered statistical trends.

RESULTS

Peroxide values and TBARS values varied among DDGS sources (**Table 2-1**). The

PV of DDGS samples ranged from 4.2 to 84.1 meq/kg oil with a CV of 97.5%, and the TBARS values of DDGS samples ranged from 1.0 to 5.2 ng MDA equivalents/mg oil with a CV of 43.6%. The highest PV and TBARS among DDGS samples were 27 and 25 times greater than the PV (3.1 meq/kg oil) and TBARS (0.2 ng MDA equivalents/mg oil) of the corn reference sample, respectively. Color of corn and DDGS samples was measured by Minolta L*, a* and b* corresponding to the degree of lightness, redness and yellowness, respectively (**Table 2-1**). Compared to corn (L* = 83.9; a* = 2.6; b* = 20.0), DDGS color is darker (mean value of L* = 54.1), more reddish (mean value of a* = 10.9), and more yellowish (mean value of b* = 37.3). Furthermore, the variation of color was smaller than that of lipid peroxidation among DDGS sources (CV of L*, a* and b* = 4.6, 7.2, 8.8 %, respectively).

Values of PV and TBARS were positively correlated (r = 0.81; $P < 0.001$; **Figure 2-1**). Both TBARS and PV were correlated negatively with L* (r = -0.73; $P < 0.001$, and r = -0.63; $P < 0.001$, respectively) and b*(r = -0.67; $P < 0.001$, and r = -0.57; $P < 0.001$, respectively, **Figure 2-2**). However, a* was not correlated with either PV ($P = 0.97$) or TBARS ($P = 0.66$).

DISCUSSION

A variety of measurements have been developed to determine peroxidation level in lipids (Shahidi and Zhong, 2005). However, none of them has been used to evaluate lipid peroxidation level in DDGS. Peroxide value is one of the most widely used indicators of lipid peroxidation in the oil industry because it is a relatively simple procedure with low cost (Shahidi and Zhong, 2005). Peroxide value measures the concentrations of

peroxides and hydroperoxides formed during the initial stages of lipid peroxidation, which is a dynamic process occurring at fast speed (Palmquist and Jenkins, 2003). Hydroperoxides generated by lipid peroxidation begin to decompose as soon as they are formed (Seppanen, 2005). As a result, the change in PV with increasing lipid peroxidation level is not linear, but follows a bell shaped curve. A high PV indicates a high lipid peroxidation status, whereas a moderate value may be due to low lipid peroxidation level, or the depletion of hydroperoxides after reaching high concentrations. Therefore, using PV as the only indicator of lipid peroxidation may not be accurate or sufficient.

The TBARS measures saturated aldehydes (i.e., MDA), 2-enals, and 2-dienals produced in the termination phase of lipid peroxidation (Palmquist and Jenkins, 2003). Thiobarbituric acid reactive substances is one of the most frequently employed methods for determining lipid peroxidation because of its simplicity and relative speed. However, limitations of this method also exist. For example, similar to PV, a low TBARS value could be the result of aldehydes that have not yet been produced or volatile aldehydes have been already been lost during processing and storage of the lipid. Furthermore, the TBARS value obtained from the color reaction should not be interpreted as absolute levels of rancidity. In addition to MDA and other aldehydes, a number of non-peroxidation substances such as soluble proteins, peptides, amino acids, and pigments in food samples can interfere with the TBA reagent and give falsely high readings (Pegg, 2001). Compared to the TBARS assay used in the current study, a direct quantification of specific aldehydes using HPLC or liquid chromatography–mass spectrometry can be more accurate, but these methods are relatively complex and expensive, and may not be

economically feasible for commercial use. In the current study, the combination of PV and TBARS were chosen as indicators to evaluate lipid peroxidation level in DDGS. The strong positive relationship between these two indicators suggests that PV and TBARS can be used simultaneously when determining lipid peroxidation level in DDGS.

In addition to PV and TBARS of DDGS, color measurement with Minolta spectrophotometers may also indicate lipid peroxidation level in DDGS. Previously, color has been used to predict the digestible AA content in DDGS based on the theory that darker-colored DDGS may have undergone a greater degree of heat damage via Maillard reactions during processing, and thus have a lower concentration of digestible AA compared with lighter-colored DDGS (Cromwell et al., 1993; Fastinger and Mahan, 2006). According to the same theory, a darker color may indicate a greater lipid peroxidation level of DDGS because of the higher drying temperature used (Fastinger and Mahan, 2006). In the current study, Minolta L* and b* were both negatively correlated with PV and TBARS. Additionally, the DDGS source that had the lowest L* and b*, also had the highest PV and TBARS value among other DDGS sources. These results suggest that darker and less yellow colored DDGS is more likely to have a higher lipid peroxidation level, as measured by TBARS and PV, compared to lighter colored DDGS samples. Furthermore, our results were in agreement with results from a preliminary study that showed that a dark-colored DDGS sample contained 40% more total polar aldehydes and 12.5% more total nonpolar aldehydes than a light-colored DDGS sample (Shurson and Csallany; unpublished). However, although color measurement is relatively fast and simple, it cannot be used to quantify lipid peroxidation products, and thus may not be used as an accurate indicator of lipid peroxidation.

Corn DDGS contains approximately 10% corn oil (Stein and Shurson, 2009). Compared with other vegetable oils, corn oil contains relatively high levels of PUFA (particularly linoleic acid; NRC, 1998) that are vulnerable to lipid peroxidation (Seppanen, 2005). In addition, increased drying time and temperature during the production process may accelerate lipid peroxidation by oxidizing unsaturated lipids in DDGS. In our survey of 31 ethanol plants, the drying temperature for producing DDGS varied substantially (371 to 593 ºC inside temperature of the dryer), suggesting that the degree of lipid peroxidation may be different during processing and drying among different DDGS sources. Our PV and TBARS values, which show a large range, confirm that lipid peroxidation levels vary greatly among DDGS sources.

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 high amounts of oxidized lipids 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, may impair animal health and growth performance (Logani and Davies, 1979; Comporti, 1993). Therefore, one may speculate that oxidized lipids in DDGS can be one of the contributing factors for reduced growth performance of growing-finishing pigs consuming high levels of DDGS (Whitney et al., 2006; Linneen et al., 2008). In another study conducted by our laboratory (Song et al., 2012a), the DDGS source with the highest TBARS and PV that was identified by the current study, resulted in reduced ADG and G:F of wean-finish pigs when included at 30% of the diet. Furthermore, feeding DDGS containing high levels of peroxidized lipids

to pigs may require supplementation of higher levels of antioxidants than currently being fed. Dietary supplementation of a blend of synthetic antioxidants improved growth performance in pigs consuming 20% DDGS or oxidized corn oil (Harrell et al., 2010). In contrast, other studies did not observe beneficial effects of vitamin E on growth performance when feeding 30% highly oxidized DDGS to nursery (Song et al., 2012b) or wean-finish pigs (Song et al., 2012a). Further studies comparing the effects of feeding a highly oxidized DDGS source vs. a low oxidized DDGS source on pig health and performance will better evaluate the impact of oxidized lipids in DDGS on swine production and the need for increasing dietary antioxidant.

Table 2-1. Lipid peroxidation and color in corn dried distillers grains with solubles (DDGS)

Item	Corn	DDGS[1]			
		Average	Minimum value	Maximum value	CV, %
PV, meq/kg oil	3.1	13.9	4.2	84.1	97.5
TBARS, ng MDA eq/mg oil	0.2	1.9	1.0	5.2	43.6
Color					
L*	83.9	54.1	45.2	58.1	4.6
a*	2.6	10.9	9.3	12.4	7.2
b*	20.0	37.3	26.6	42.7	8.8

[1]Data from 31 DDGS sources.

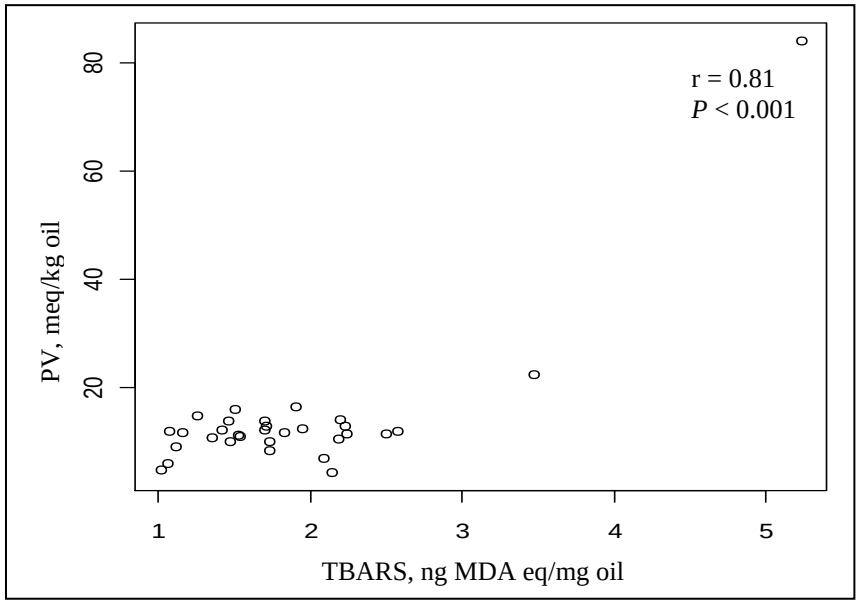


Figure 2-1. Correlation between peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) in corn dried distillers grains with solubles (DDGS)

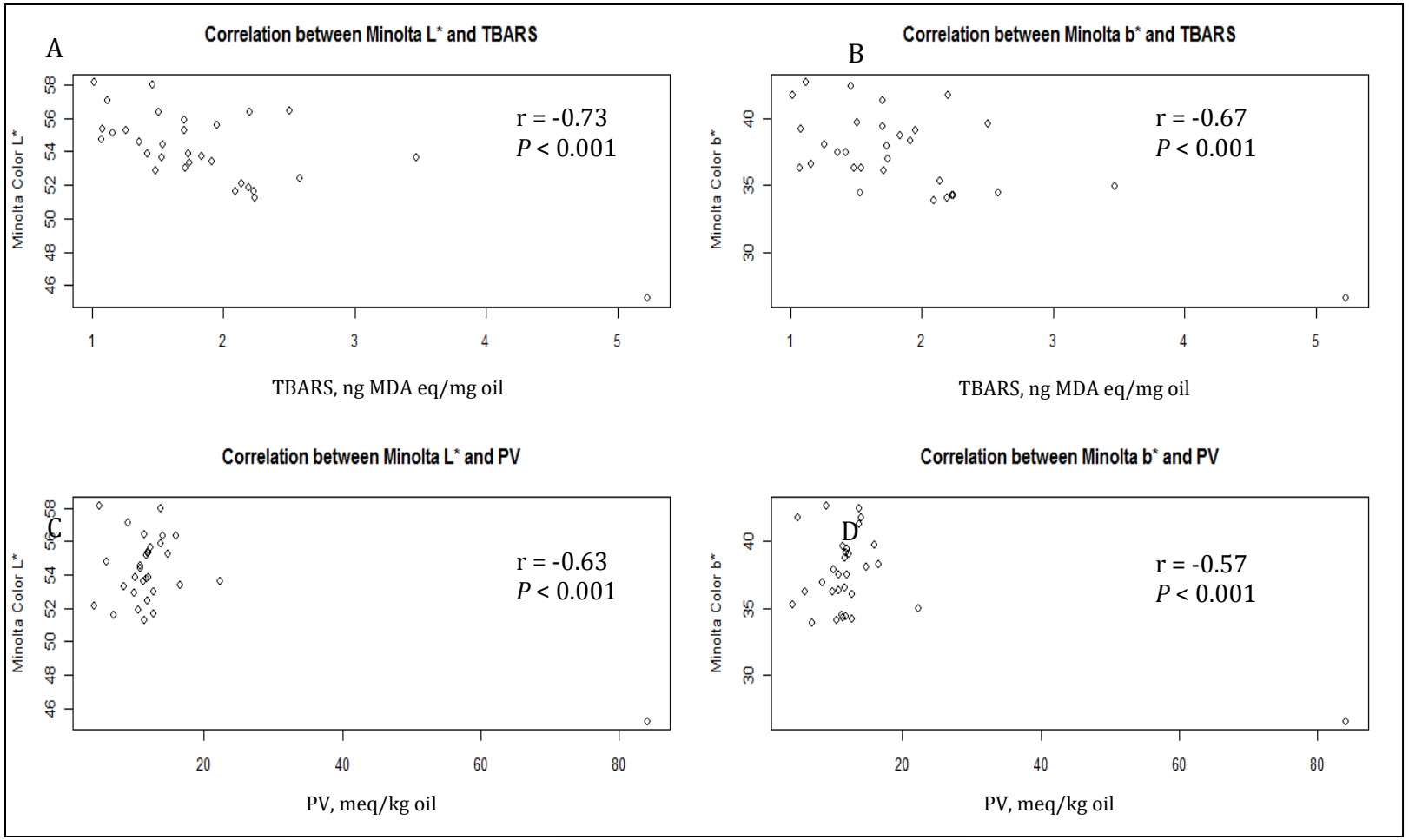


Figure 2-2. Correlation between thiobarbituric acid reactive substances (TBARS) and Minolta color (A and B), and peroxide value (PV) and Minolta color (C and D)_in corn dried distillers grains with solubles (DDGS)

CHAPTER III. High Sulfur Content in Corn Dried Distillers Grains with Solubles (DDGS) Protects against Oxidized Lipids by Increasing Sulfur-Containing Antioxidants in Nursery Pigs

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 one of three levels of supplemental vitamin E (dl-α-tocopheryl acetate): none, NRC (1998) 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 a high 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 to 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 concentration for serum levels 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 10X NRC level. Pigs fed DDGS diets had greater serum concentrations of S-containing AA, particularly methionine ($P < 0.001$) 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, respectively). 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 (methionine, taurine, and glutathione) in vivo may protect pigs against potential oxidative stress when feeding highly oxidized DDGS. Therefore, increasing levels of vitamin E in diets may not be necessary to protect pigs against metabolic oxidative stress when feeding high S and high oxidized DDGS.

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

INTRODUCTION

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

total S content in corn DDGS can exceed 1% due to 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 amino acids, proteins, enzymes and micronutrients (Atmaca, 2004). However, very little is known about the impact of feeding DDGS containing a high concentration of S on pig health and performance.

It is hypothesized that feeding DDGS containing oxidized lipids to pigs may require supplementation of greater levels 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 containing 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 level of vitamin E.

MATERIALS AND METHODS

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

Animals and Housing

Weanling terminal cross barrows (n = 54; initial BW = 7.0 ± 0.3 kg) of sows (Landrace ×

Yorkshire, Genetically Advanced Pigs, Winnipeg, Manitoba, 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 two 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 one 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 one of 3 levels of supplemental vitamin E (dl-α-tocopheryl acetate): none (No-E), 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 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 and were monitored for health daily. Individual pig BW and pen feed disappearance were measured initially, and when dietary phases were changed at the end of wk 2, wk 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, followed by 1-d collection of fasted urine samples, and collection of blood samples on the last day. After the collection of these samples, 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 their feed throughout the entire adaptation and collection periods. 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 DDGS Source

Diet composition and nutrient concentrations of experimental diets for Phase 1 to 3 are presented in **Tables 3-1 to 3-3**. All diets were fed in meal form and were formulated on a standardized ileal digestible AA and available P basis with similar concentrations of ME and Ca. Crystalline lysine (L-lysine) was added at 0.20 and 0.28% in Phase 2 and Phase 3 DDGS diets to provide comparable SID lysine 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/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 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. Thus, these levels and 10X of these levels were used as target concentrations of dietary vitamin E when formulating the 1X-E and 10X-E diets, respectively. The actual level of α-tocopherol in each experiment 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). This DDGS source 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) 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 h 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 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 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.

After the last feeding on the third day of feces and urine collection, pigs were fasted for 24 h with free access to water only. Fasted urine sample from each pig was collected into an empty plastic bucket over a period of 6 h after pigs were fasted for 24 h. Fasted urine sample was filtered through a 4 layers of cheesecloth into a small plastic container and stored at -20°C until analysis of TBARS. Pigs were fed their respective diets after urine samples were collected.

Blood (approximately 8 mL) was collected 1 h after feeding at 0700 h on the last day 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**).

Liver, spleen and kidney samples (approximately 50 g of each) were collected and weighed after pigs were euthanized using a captive bolt pistol on the last day of the study. Percentage of organ weight was calculated using the following equation: organ weight, % = organ weight, kg / live BW, kg × 100. Liver samples were frozen immediately on dry ice and stored at -80°C for glutathione (**GSH**) analysis.

TBARS Assay in Serum and Fasted Urine Samples

To evaluate the metabolic oxidative status in vivo, serum and urine TBARS were performed using the method described by Animal Models of Diabetic Complications Consortium (AMDCC, Version 1). In general, 100 μL serum or urine 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 of supernatant were 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 cooled to room temperature and read at 532 nm using a spectrophotometer (SpectraMax 250, Molecular Device, Sunnyvale, CA). All samples and standards were conducted in duplicate. The TBARS assay in serum was conducted in 4 batches with intra-assay CV of 6.7% and inter-assay CV of 5.2%. The TBARS assay in urine was conducted in 2 batches with intra-assay CV of 7.6% and inter-assay CV of

6.5%.

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 (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

Hepatic glutathione. Glutathione concentration in liver was analyzed using a commercial GSH Assay kit (Sigma-Aldrich, St. Louis, MO., Catalog number CS0260). Fifty mg 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 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 one 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 (Cayman Chemical, Catalog number: 703102, Ann Arbor, MI). Briefly, indirect GPX activity was measured by a coupled reaction with glutathione reductase. The reaction was initiated after cumene hydroperoxide addition. Oxidation of NADPH to NADP was measured colorimetrically using a spectrophotometer (SpectraMax 250, Molecular Device, Sunnyvale, CA) at 340

nm for at least 5 min. Glutathione peroxidase activity was expressed as μmol/min/mg of protein and compared to a bovine erythrocyte GPX standard curve over time. Each sample and standard were analyzed in duplicate. This assay was conducted in one batch with the intra-assay CV of 5.9%.

Serum sulfur-containing amino acids (SAA). Serum levels of methionine (**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 were mixed with 40 μL Na_2CO_3 (10 mM, pH:11) and 100 μL Dansyl chloride (3 mg/ml in acetone). The mixture was incubated in a water bath at 60º C for 10 min, followed by centrifugation at 10,000 × *g* 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.

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 for analysis of total S content and 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, % × S in Met, % + Cys in feed, % × S in Cys, % + taurine in feed, % × S in taurine, %, where S in Met, % = $\frac{32\ (atomic\ weight\ of\ S)}{149\ (molecular\ weight\ of\ Met)}$ × 100 = 21%; S in Cys, % = $\frac{32\ (atomic\ weight\ of\ S)}{121\ (molecular\ weight\ of\ Cys)}$ × 100 = 26%; S in taurine, % = $\frac{32\ (atomic\ weight\ of\ S)}{125\ (molecular\ weight\ of\ taurine)}$ × 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 the following equation: ATTD, % = [(St − Sf)/St] × 100%, where St = the total consumption of S (g) in 3 consecutive d, and Sf = the total fecal excretion of S (g) in 3 consecutive d.

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS Institute, 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, as well as random effect of block. Group was used as a covariate for all these responses if it was significant ($P < 0.05$). 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 between DDGS and vitamin E were observed for any of the

growth performance responses (**Table 3-4**). 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.

Metabolic Oxidation Status and Organ Weight

There were no effects of DDGS, vitamin E supplementation level, or DDGS × vitamin E concentration on TBARS values in serum (**Table 3-5**). Results of urine TBARS were inconsistent with serum TBARS. Pigs fed DDGS had a higher concentration of urine TBARS compared with pigs fed CON (0.47 vs. 4.82, $P = 0.03$). An interaction between 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, respectively). However, when vitamin E supplementation was increased to 10X NRC level, serum α-tocopherol concentration was similar for pigs fed DDGS and CON diets. On the other hand, as expected, 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, respectively), but similar for pigs fed 1X-E and No-E diets. These also reflect the interaction observed between DDGS × dietary vitamin E concentration.

There were no effects of DDGS, vitamin E supplementation level, or DDGS ×

vitamin E concentration on percentage of organ weight (**Table 3-5**).

Sulfur-containing Antioxidants

Sulfur-containing antioxidants, including Met, Cys, taurine, GSH, and GPX activity, were evaluated *in vivo* in this study (**Table 3-6**). The Cys concentration in the serum was unable to detect due to the low level or poor derivation by dansyl chloride regents 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 μM, respectively). No effect of vitamin E supplementation level was observed for serum SAA concentrations. Liver GSH concentration was greater in pigs fed DDGS than CON (56.3 vs. 41.8 nmol/g, respectively, $P < 0.001$). 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, respectively, $P < 0.001$). Serum GPX activity increased when supplemental vitamin E levels increased ($P = 0.03$).

Sulfur Content and Digestibility in Experimental Diets

Total S content in DDGS containing diets was 2 times greater than that in CON (0.39 vs. 0.19%, respectively, **Table 3-7**), which was largely contributed by higher inorganic S content in DDGS diets than CON (0.23 vs. 0.16%, respectively), while the organic S content was similar between DDGS and CON diets (0.15 vs. 0.13%, respectively). As a result of higher 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, respectively, $P < 0.001$, **Table 3-7**). 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 to the CON (86.8 vs. 84.6 %, respectively, $P < 0.001$). 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 (**Table 3-7**). It should be noted that 1 feed sample in No-E/CON treatment, 2 feed samples in 10X-E/CON treatment and 1 feed sample in No-E/DDGS treatment were missing during storage. Results presented in Table 7 were obtained by analyzing all available data.

DISCUSSION

The utilization of corn co-products, such as DDGS, in swine feeds has increased dramatically in recent years due to increased availability and cost competitiveness compared with corn and soybean meal. Limits on dietary inclusion rates of DDGS often occur because pig performance and pork quality decline when high dietary levels of DDGS (30-40%) are fed to growing-finishing pigs (Xu et al., 2010). The reduction in growth performance sometimes observed when feeding high dietary levels of DDGS may be potentially caused by antinutritional factors, toxins, low net energy level, poor amino acid digestibility, and/or oxidized lipid (Stein and Shurson, 2009). 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 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 a previous study (see Chapter II), two 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 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, which suggests 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 corn reference sample (0.2 ng MDA/mg oil and 3.1 meq/kg oil, respectively). The DDGS source with the highest 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 corn-soybean meal diet (CON) used in the current study was used as a standard reference point for typical U.S. swine diets, and thus, all the comparisons are relative to responses when feeding a corn-soybean meal diet.

Growth suppression from oxidized lipids has been documented in several different animal species (Dibner et al., 1996; DeRouchey et al., 2004; Harrell et al., 2010). The presence of high amounts of oxidized lipids 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 (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/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 x 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 the following 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 level 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, acids esters, hydrocarbons, and aromatic compounds (Gray, 1978). Therefore, a low PV could be due to either minimal oxidation or decomposition of hydroperoxides that have already begun. Furthermore, it should be also noted that although we observed reduced final BW in pigs fed DDGS, without the comparisons to 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 net energy content or overestimation of lysine digestibility.

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. 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, serum and urine TBARS were determined in this study to evaluate the metabolic oxidation status of pigs. Compared with the urine TBARS assay, serum TBARS may be a more reliable and accurate measurement in pigs.

Serum samples that were relatively clean compared to many urine samples that were contaminated with particles of feces and feed that dropped into the collection containers. These particles may interfere the reaction between aldehyde and TBA or lead to inaccurate optical density (**OD**) results. Moreover, unlike serum samples that were almost colorless, some urine samples collected in this study were yellow or orange. Since the TBARS assay measures the absorption of pink color under 532 nm, the yellowish or orange color that existed in some urine samples may have resulted in a greater OD reading. Finally, water concentration in urine could vary substantially based on different water consumption and excretion rates in individual pigs, and thus concentration of TBARS in urine could be largely affected by the water content in urine. As a result, we consider serum TBARS, rather than urine TBARS, as a better indicator of metabolic oxidation status in the current study.

Pigs fed DDGS exhibited a greater level of serum α-tocopherol than those fed CON, while the serum 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 across dietary treatments (data not presented). 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 suggest 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 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 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 suggests that taurine may protect against lipid peroxidation. Glutathione is the major cellular antioxidant in pigs, and it is a co-factor of 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 suggests 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 (**Table 3-7**) and Met (**Table 3-1 to 3-3**), and improved S digestibility (**Table 3-7**) in DDGS diets compared with CON.

Corn DDGS contains a relatively high S content (0.33 to 1.04%, DM basis, Kim et al., 2012) compared to other feed ingredients due to 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 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. 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; Uwituze et al., 2011b). 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 level of inorganic S content in DDGS diets than CON, while the organic S content was similar between DDGS and CON diets (**Table 3-7**). 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 monogastric 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 one amino acid in the blood is usually in agreement with the relative concentration of that amino acid in the diet, and that dietary supplementation of AA increases blood-borne levels of corresponding AA. This statement was further confirmed by the study from Puchal et al. (1962), 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. 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, elevated 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, since Met can be converted rapidly to cysteine via the transsulfurylation pathway, and in turn, cysteine serves as a precursor for the synthesis of taurine and GSH (Atmaca, 2004; Bauchart-Thevret et al., 2009). In fact, dietary Met level (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 (r) of 0.81 (data not presented). This observation is consistent with previous findings in rats where reducing dietary Met level 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 Sakehisekaran, 1997) expressed increased GPX activity in blood. The supplementation of SAA appears to be an effective method of restoring GSH status, since SAA play a role in determining the flux of cysteine between cysteine 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.

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. However, it remains unclear which source(s) of S in DDGS (inorganic S or organic S or both) resulted in this protective mechanism. It appears that higher Met concentration in DDGS diets may be an important factor, and thus diets formulated with more crystalline lysine and containing a lower concentration of Met may have less S-containing antioxidants *in vivo* and less of this

benefit. 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. (1997b) and Ebrahim and Sakehisekaran (1997), it may not be necessary to increase the levels of vitamin E greater than those recommended by NRC (1998) to protect pigs against oxidative stress when feeding high S and highly oxidized DDGS.

Table 3-1. Composition and nutrient analysis of Phase 1 diets (7 - 11 kg BW, 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
Calculated nutrient composition						
ME,[6] kcal/kg	3,283	3,282	3,273	3,297	3,295	3,286
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, %	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
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,[7] %	2.54	–	–	3.91	–	–
Crude fiber,[7] %	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,[7] %	1.72	–	–	1.40	–	–
P,[7] %	0.99	–	–	0.89	–	–
α-tocopherol, IU/kg	<10	13	151	<10	14	127

[1]CON = corn-soybean meal based control diet.
[2]DDGS = 30% inclusion of dried distillers grains with solubles

[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 = 9 kg; 10X-E = 10X NRC (1998) level of vitamin E supplied as *dl*-α-tocopheryl acetate, 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 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 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^{TM}); 55.11 mg of iron as ferrous sulfate (SQM^{TM}); 5.51 mg of copper as copper sulfate (SQM^{TM}); and 17.64 mg of manganese as manganese oxide (SQM^{TM}).

[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 3416 kcal/kg for DDGS, which was obtained as the average value of 10 DDGS samples analyzed by Pedersen et al. (2007).

[7]Nutrient concentrations were only analyzed in No-E/CON and No-E/DDGS treatments. Values should be very similar to those in corresponding 1X-E and 10X-E treatments.

Table 3-2. Composition and nutrient analysis of Phase 2 diets (11 - 25 kg BW, 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%)	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
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, %	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
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,[7] %	2.68	–	–	3.70	–	–
Crude fiber, [7] %	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, [7] %	0.77	–	–	0.98	–	–
P, [7] %	0.64	–	–	0.66	–	–
α-tocopherol, IU/kg	<10	14	124	<10	13	118

[1]CON = corn-soybean meal based control diet.
[2]DDGS = 30% inclusion of dried distillers grains with solubles
[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 = 18 kg; 10X-E = 10X NRC (1998) level of vitamin E supplied as *dl*-α-tocopheryl acetate, which is 110 IU/kg for BW = 18 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 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 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^{TM}); 55.11 mg of iron as ferrous sulfate (SQM^{TM}); 5.51 mg of copper as copper sulfate (SQM^{TM}); and 17.64 mg of manganese as manganese oxide (SQM^{TM}).

[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 3416 kcal/kg for DDGS, which was obtained as the average value of 10 DDGS samples analyzed by Pedersen et al. (2007).

[7]Nutrients concentrations were only analyzed in No-E/CON and No-E/DDGS treatments. Values should be very similar to those in corresponding 1X-E and 10X-E treatments.

Table 3-3. Composition and nutrient analysis of Phase 3 diets (25 - 50 kg BW, 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%)	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
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, %	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
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,[7] %	2.23	–	–	3.71	–	–
Crude fiber,[7] %	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,[7] %	0.76	–	–	0.76	–	–
P,[7] %	0.62	–	–	0.56	–	–
α-tocopherol, IU/kg	<10	13	117	<10	14	105

[1]CON = corn-soybean meal based control diet.
[2]DDGS = 30% inclusion of dried distillers grains with solubles
[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 = 37 kg; 10X-E = 10X NRC (1998) level of vitamin E supplied as *dl*-α-tocopheryl acetate, which is 110 IU/kg for BW = 37 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 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 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^{TM}); 55.11 mg of iron as ferrous sulfate (SQM^{TM}); 5.51 mg of copper as copper sulfate (SQM^{TM}); and 17.64 mg of manganese as manganese oxide (SQM^{TM}).
[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 3416 kcal/kg for DDGS, which was obtained as the average value of 10 DDGS samples analyzed by Pedersen et al. (2007).
[7]Nutrients concentrations were only analyzed in No-E/CON and No-E/DDGS treatments. Values should be very similar to those in corresponding 1X-E and 10X-E treatments.

Table 3-4. 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 8 wks after weaning, 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-soybean 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; 10X-E = 10X NRC (1998) level of vitamin E. Vitamin E was supplied as *dl*-α-tocopheryl acetate.

Table 3-5. Influence of DDGS and vitamin E supplementation on metabolic oxidation status and organ weight[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
Metabolic oxidation status										
Serum TBARS, μM	3.69	3.54	3.68	3.72	3.63	3.56	0.08	0.23	0.95	0.27
Urine TBARS, μM	5.94	4.56	3.96	5.72	6.87	8.63	1.25	0.94	0.03	0.14
Serum α-tocopherol, μg/mL	0.42	0.95	3.32	1.60	1.61	3.54	0.11	<0.001	<0.001	<0.001
Organ weight, %										
Liver	2.92	2.73	2.75	2.87	2.99	2.82	0.13	0.68	0.40	0.49
Spleen	0.22	0.21	0.22	0.22	0.24	0.20	0.10	0.68	0.97	0.44
Kidney	0.51	0.48	0.48	0.54	0.47	0.48	0.13	0.25	0.88	0.80

[1]Values are least square means of nine replicate pigs per dietary treatment.
[2]CON = corn-soybean 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; 10X-E = 10X NRC (1998) level of vitamin E. Vitamin E was supplied as *dl*-α-tocopheryl acetate.

Table 3-6. 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, 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
Liver glutathione, nmol/g	35.1	44.6	45.7	50.2	56.4	62.3	3.5	0.01	<0.001	0.78
Serum glutathione peroxidase 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-soybean meal 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; 10X-E = 10X NRC (1998) level of vitamin E. Vitamin E was supplied as *dl*-α-tocopheryl acetate.
[5]One unit of activity equals 1 μmol NADPH oxidized per minute/mL serum.

Table 3-7. Sulfur content, daily S balance and apparent total tract digestibility (ATTD) in experimental diets (as-fed basis)

Item	CON[1]			DDGS[2]			SE	*P*-value		
	No-E[3]	1X-E[3]	10X-E[3]	No-E	1X-E	10X-E		Vit E	DDGS	Vit E × DDGS
Dietary S content[4]										
Inorganic S, %	0.13	0.13	0.12	0.15	0.15	0.14	–	–	–	–
Organic 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[5]										
No. of pigs	8	9	7	8	9	9				
S intake, g	3.11	2.90	3.15	5.55	5.63	5.94	0.20	0.36	<0.001	0.67
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	0.65	0.40	0.09	1.97	1.95	1.88	0.24	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, %	84.3	84.0	85.4	87.3	85.9	87.3	0.70	0.14	<0.001	0.68

[1]CON = corn-soybean meal based control diet.
[2]DDGS = 30% dietary inclusion of dried distillers grains with solubles
[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]Values are from Phase 3 experimental diets.
[5]Values are least square means.

CHAPTER IV. Impact of Feeding Highly Oxidized Dried Distillers Grains With Solubles at Increasing Dietary Vitamin E Levels to Growing Pigs on Energy and Nutrient Digestibility and Metabolic Oxidation Status

A study was conducted to evaluate the effects of feeding diets containing highly oxidized corn dried distillers grains with solubles (**DDGS**) with 3 levels of vitamin E on metabolic oxidation status and apparent total tract digestibility (**ATTD**) of energy, N, C and S in diets for growing pigs. The DDGS source used in this study contained the highest thiobarbituric acid reactive substances 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 (mean values = 1.8 ng/mg oil and 11.5 meq/kg oil, respectively). Growing barrows (n = 54) were assigned randomly to one of 6 dietary treatments in a 2 × 3 factorial design. Pigs were fed a corn-soybean meal (**CON**) diet or diets containing 30% DDGS with 3 levels of vitamin E (α-tocopheryl acetate): none supplemented, NRC (11 IU/kg), or 10X NRC (110 IU/kg). Pigs were housed individually in metabolism cages for a 5-d adaptation period followed by a 3-d total collection of feces and urine. Feed, feces and urine were analyzed for GE, N, C and S to calculate the corresponding ATTD, daily balance, as well as dietary DE and ME content. Although pigs were fed highly oxidized DDGS, serum TBARS were similar between DDGS and CON treatments. Serum α-tocopherol concentrations were higher ($P < 0.001$) in pigs fed DDGS diets compared with those fed CON when α-tocopheryl acetate was not provided or at NRC level. Feeding DDGS tended to improve intestinal barrier function because lactulose to mannitol ratio tended to be lower ($P = 0.07$) in pigs fed DDGS diets compared with those fed CON. The DE and

ME content of DDGS diets were lower ($P < 0.05$) than CON. Carbon retained was lower in DDGS diets than CON (487.3 vs. 533.3 g/d, $P = 0.01$). Intake, fecal and urinary excretion of S, and S absorbed and retained were higher ($P < 0.001$) in DDGS diets than CON. Dietary inclusion of 30% DDGS reduced dietary ATTD of GE (84.3 vs. 89.2%, respectively, $P < 0.001$), N (84.1 vs. 88.7%, respectively, $P < 0.001$) and C (85.0 vs. 90.0%, respectively, $P < 0.001$), but improved ATTD of S (86.8 vs. 84.6%, respectively, $P < 0.001$) compared to CON. There was no significant interaction between DDGS and vitamin E level or effects of vitamin E on nutrient digestibility. In conclusion, feeding diets containing 30% highly oxidized DDGS, reduced the DE and ME content of diets, and decreased the ATTD of GE, N and C, but improved the ATTD of S in diets. Supplementation of vitamin E in the diet did not counteract these negative effects of DDGS on nutrient digestibility.

Key words: apparent total tract digestibility, corn dried distillers grains with solubles, lipid peroxidation, growing pig, vitamin E

INTRODUCTION

In the body of an animal, there is a balance between the formation of free radicals and the oxidation defense system (Vázquez-Añón and Jenkins, 2007). However, under certain conditions, the levels of free radicals can exceed the antioxidant capacity of the animal, leading to oxidative stress (Miller and Brezeinska-Slebodizinska, 1993). Dietary fatty acids, especially PUFA, may play an important role in oxidative stress since they are easily oxidized via free radical-mediated lipid peroxidation (Shiota et al., 1999).

Lipid peroxidation may occur during the production of corn dried distillers grains with solubles (**DDGS**) because DDGS contains high levels of PUFA (NRC, 1998). In addition, drying time and temperature used by some ethanol plants may also accelerate lipid peroxidation in DDGS. Therefore, feeding DDGS containing oxidized lipids may induce oxidative stress and thus, negatively affect animal growth performance (Song et al., 2012a). Furthermore, feeding oxidized lipids can reduce the digestibility of energy and protein in broilers (Engberg et al., 1996) and weanling pigs (Yuan et al., 2007). However, there is no information regarding the impact of lipid peroxidation in DDGS on energy and nutrient digestibility in swine.

Feeding DDGS containing oxidized lipids to pigs may require a higher dietary level of antioxidants (e.g. vitamin E) than are currently being recommended. Results with laying hens (Seven, 2008) and Japanese quail (Sahin and Kucuk, 2001) in which additional vitamin C and vitamin E were included in the diets showed improved digestibility of DM, CP and ether extract when these animals were kept under a heat stress-induced oxidative challenge. Therefore, the objective of this study was to evaluate the effects of feeding a highly oxidized DDGS source to growing pigs on energy and nutrient digestibility, and to determine whether any of the negative effects, if there were, could be overcome by increasing the dietary level of vitamin E (α-tocopheryl acetate).

MATERIALS AND METHODS

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

Animals and Housing

Growing terminal cross barrows (n = 54; initial BW = 38.1 ± 4.3 kg) produced from sows (Landrace × Yorkshire, TOPIGS, Winnipeg, Manitoba, Canada) mated to Duroc boars (Compart's Boar Store, Nicollet, MN) were housed individually in metabolism cages at the University of Minnesota, Southern Research and Outreach Center (Waseca, MN). The experiment was conducted in two replicated groups with 24 barrows used in the first group and 30 pigs used in the second group. Pigs were assigned randomly to one of 6 dietary treatments in a 2 × 3 factorial arrangement, resulting in 9 pigs per treatment. Pigs were fed corn-soybean meal (**CON**) or corn-soybean meal-30% DDGS diets containing one of 3 levels of vitamin E (α-tocopheryl acetate): none supplemented (**No-E**), NRC (1998) recommended concentration of vitamin E (**1X-E**), or 10X NRC (**10X-E**). Pigs were allowed a 5-d adaptation period followed by a 3-d total collection of feces and urine, followed by collection of blood samples, and determination of intestinal permeability on the last day. Pigs were fed a daily amount of their respective diets equivalent to 4% of their initial 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. A constant daily feed intake was achieved in all pigs during the 5-d adaptation period before the 3-d 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.

DDGS Source and Diet Composition

The highly oxidized DDGS source used in this study was selected from a collection of 31 corn DDGS sources produced by U.S. ethanol plants (Song et al., 2011;

Table 4-1). The DDGS source used in this experiment 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). Diet composition and nutrient concentrations of experimental diets are presented in **Table 4-2**. All diets were fed in meal form and were formulated on a standardized ileal digestible AA and available P basis, and nutrient concentrations of the diets met or exceeded NRC (1998) nutrient requirements for pigs with 350 g of fat-free lean gain/d, except for vitamin E concentration in the No-E diets. Vitamin E was supplemented in the form of *dl*-*α*-tocopheryl acetate in 1X-E and 10X-E diets.

Sample Collection

Feces and urine from each pig were collected twice (0700 h and 1900 h) daily for 3 consecutive d and stored at -20° C. Fecal samples from each pig were pooled, weighed, and dried in a forced-draft oven at 60°C, and subsamples were obtained for analysis of GE, N, C, and 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. The collection containers were emptied after the morning and afternoon feedings. Thirty mL of 6N HCl were added to the containers every time they were emptied to limit microbial growth and to reduce loss of ammonia. At each collection time, urine volume was measured and a subsample of approximately 20% of the urine excreted from each pig was stored at -20°C. Urine samples collected from the same pig over the 3 consecutive d were pooled and used for subsequent chemical analysis.

After the 3-d collection of feces and urine samples, 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 (SST* brand, BD, Franklin Lakes, NJ). Blood samples in tubes were placed on ice immediately after collection and were centrifuged at 2,000 × *g* for 20 min at room temperature approximately 1 h after the first blood sample was collected. Serum was then removed and stored at -20° C until analysis of TBARS and α-tocopherol.

TBARS Assay in Serum

To evaluate metabolic oxidative status *in vivo*, serum TBARS was analyzed using the method described by Animal Models of Diabetic Complications Consortium (AMDCC, Version 1). Specifically, 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 of supernatant were 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 cooled to room temperature and read at 532 nm using a spectrophotometer (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 inter-assay CV was 5.2%.

Analysis of α-Tocopherol Concentration in Serum

Analysis of α-tocopherol concentration in serum was conducted by the 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 a chromatographic mobile phase and analyzed by high-performance liquid chromatography (HPLC, Separation Module 2690, Waters, Milford, MA) followed by the procedure described by Katsanidis and Addis (1999).

Measurement of Intestinal Permeability

Intestinal permeability was measured using lactulose and mannitol as previously described by Kansagra et al. (2003). One day after blood samples were collected, pigs were administrated an oral dose of 10 g lactulose (MP Biomedicals, LLC, Solon, OH) and 2 g of mannitol (Sigma-Aldrich, St. Louis, MO) in 500 g feed at 0700 h. Urine was collected into a container with 2 mL chlorhexidine to prevent microbial contamination for a period of 6 h following this feeding. Urine samples were then stored at -20°C until analysis of lactulose and mannitol by HPLC were performed as described by Kansagra et al. (2003).

Chemical Analysis and Calculations

All analyses were conducted in duplicate. Samples of experimental diets, feces and urine were analyzed for GE via bomb calorimetry (Model 1261, Parr Instruments, Moline, IL; Miller and Payen, 1959), and N via a combustion method (LECO FP428, LECO Corporation, St. Joseph, MI; Stump and Frazer, 1973). Carbon and S concentrations in diets, feces and urine were determined using the methods described by Amundson et al. (1988) and Greweling et al. (1972), respectively.

Following chemical analysis, the DE and ME content in each diet were calculated by subtracting the GE content excreted in feces and urine from the GE content of the diets consumed (Adeola, 2001). Nitrogen, C, and S balance in each diet were also calculated (Adeola, 2001). The apparent total tract digestibility (**ATTD**) of energy, N, C and S in each diet were calculated according to the following equation: ATTD, % = [(Ni - Nf) / Ni] x 100%, where Ni = the total consumption of energy (kcal), N (g), C (g) and S (g) in 3 consecutive d, and Nf = the total fecal output of energy (kcal), N (g), C (g) and S (g) in 3 consecutive d.

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS Inst. Inc. (Cary, NC). Analysis of variance was conducted for this complete 2 × 3 factorial arrangement. The statistical model included the fixed effects of DDGS, vitamin E concentration, and DDGS × vitamin E interactions. Group was used as a covariate if it was significant ($P < 0.05$). All results are reported as least squares means. Multiple comparisons among treatments were performed using the Tukey adjustment option of SAS. Pig was used as the experimental unit. 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

Metabolic Oxidation Status

There were no effect of DDGS, vitamin E supplementation level, or their interaction on serum TBARS values (**Table 4-3**). An interaction between DDGS ×

vitamin E concentration was detected ($P < 0.001$) for serum α-tocopherol. 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 (1.60 and 1.61 vs. 0.42 and 0.95 μg/mL, respectively). However, when vitamin E supplementation was increased to 10X NRC level, serum α-tocopherol concentration was similar for pigs fed DDGS and CON diets (3.54 vs. 3.32 μg/mL, respectively). 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, respectively), but similar for pigs fed 1X-E and No-E diets.

Intestinal Permeability

There was no effect of vitamin E supplementation or an interaction between DDGS and vitamin E on urinary lactulose and mannitol, as well as lactulose to mannitol ratio (**L/M**) in this study (**Table 4-4**). Concentration of urinary mannitol was similar between pigs fed DDGS vs. CON diets. However, pigs fed DDGS diets excreted less lactulose in urine (0.08 vs. 0.13 mg/mL; $P = 0.02$), and tended to have a lower L/M (0.08 vs. 0.11; $P = 0.07$) than those fed CON, which suggests a tendency of reduced intestinal permeability and improved intestinal barrier function in pigs consuming DDGS diets.

Energy and N Balance

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 energy, N, C and 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 4-5 to 4-7. No interaction between DDGS and vitamin E were observed for any energy or N balance measurements (**Table 4-5**). There were no differences in GE intake among dietary treatments. Pigs fed DDGS diets excreted more ($P < 0.001$) GE in the feces compared with those fed CON, but the GE excretion in urine was similar. As a result, the DE ($P = 0.02$) and ME ($P = 0.01$) content in DDGS containing diets (3,285 and 3,180 kcal/kg, as-fed basis, respectively) were lower than DE and ME content in CON (3,347, and 3,247 kcal/kg, as-fed basis, respectively). Increasing dietary level of vitamin E did not affect energy balance.

Nitrogen intake was similar among dietary treatments, but pigs fed DDGS diets excreted more ($P < 0.001$) N in the feces than those fed CON. As a result, N absorption tended to be lower for DDGS containing diets (36.7 vs. 39.2 g/d, respectively, $P = 0.08$). However, N retained was not affected by dietary treatments. Increasing dietary vitamin E levels tended to increase ($P = 0.10$) N absorption, but had no effect on N retention.

Carbon and S Balance

No interaction between DDGS and vitamin E, or the main effect of dietary vitamin E level were observed for any response of C and S balance (**Table 4-6**). Carbon intake and urinary excretion were similar among dietary treatments. However, C excretion in feces was 45% greater ($P < 0.001$) in pigs fed DDGS diets than those fed CON. As a result, C absorbed (499.5 vs. 545.3 g/d, respectively, $P = 0.01$) and retained (487.3 vs. 533.3 g/d, respectively, $P = 0.01$) were lower in DDGS containing diets than CON. Sulfur intake, and fecal and urinary excretion were higher ($P < 0.001$ for all of these responses) in DDGS diets than CON. Additionally, feeding 30% DDGS increased

S absorbed (4.96 vs. 2.59 d/g, respectively, $P < 0.001$) and retained (2.94 vs. 2.07, respectively, $P = 0.001$) compared with feeding CON to growing pigs.

Apparent Total Tract Digestibility

No interaction between DDGS and vitamin E was detected for ATTD of energy, N, C, and S (**Table 4-7**). Feeding 30% oxidized DDGS reduced ATTD of GE (84.3 vs. 89.2%, respectively, $P < 0.001$), N (84.1 vs. 88.7%, respectively, $P < 0.001$), and C (85.0 vs. 90.0 %, respectively, $P < 0.001$) by 5.8, 5.4 and 5.9%, respectively compared to CON. However, ATTD of S was improved by 2.7% in pigs fed DDGS diets compared with those fed CON (86.8 vs. 84.6%, respectively, $P < 0.001$). There was no effect of dietary vitamin E on ATTD of these nutrients.

DISCUSSION

With expansion of the ethanol industry, the availability and use of corn co-products, such as DDGS, in swine diets has increased dramatically. Currently, the long-term sustainability of feeding high levels of DDGS (up to 40%) in grower-finisher swine diets is in jeopardy because of concerns related to the negative effects of DDGS on pork fat quality, and the potential negative effects of oxidized lipids in DDGS on growth performance, health, and shelf-life stability of pork products. The lipid fraction of corn DDGS is made up largely of PUFA, particularly linoleic acid (NRC, 1998), which is prone to lipid peroxidation. Lipid peroxidation is a free-radical chain reaction that produces oxidized lipids and a series of toxic aldehydes (Blokhina et al., 2003), which can negatively affect growth and health status in several animal species (Dibner et al., 1996; DeRouchey et al., 2004; Harrell et al., 2010). Additionally, during the drying

process, DDGS are heated at relatively high temperatures that likely accelerate lipid peroxidation by oxidizing unsaturated lipids. In the current study, a DDGS source with the highest amount of peroxidized lipids was selected according to a recent study conducted in our laboratory (Song et al., 2011), and used to evaluate effects of feeding a highly oxidized DDGS source on nutrient digestibility and utilization in growing pigs. In this study, we did not compare this highly oxidized DDGS source to a low oxidized DDGS source. However, the corn-soybean meal diet (CON) used in the current study was used as a standard reference point for typical U.S. swine diets, and thus, all the comparisons are relative to responses when feeding a corn-soybean meal diet.

Serum concentrations of α-tocopherol and TBARS were determined in this study to evaluate the metabolic oxidation status of pigs. Interestingly, feeding DDGS to pigs increased concentration of serum α-tocopherol by 44%, while the TBARS value was not different in pigs fed highly oxidized DDGS or CON diets. The lack of an effect of DDGS on serum TBARS, as a systemic indicator of oxidative stress, agrees with results from studies evaluating the impact of DDGS on pork quality and oxidation status (Leick et al., 2010; Xu et al., 2010). These studies showed that fresh muscle samples from pigs fed DDGS did not differ in their TBARS from pigs fed corn-soybean based diets. However, our results are in contrast with other experiments in poultry and swine where 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 suggest that either feeding DDGS may not induce an oxidative stress to the extent of 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.

There seems to be a threshold for rancidity above which oxidative stress is induced. DeRouchey et al. (2004) suggested that a PV greater than 40 meq/kg for oxidized choice white grease (6% dietary inclusion rate), which is approximately equal to a PV of the diet greater than 2.4 meq/kg (2.4 meq/kg = 40 meq/kg x 6%), might be necessary to result in oxidative stress and decreased growth performance in nursery pigs. In the current study, the highly oxidized DDGS source contained 9.66% crude fat and a PV of 84.1 meq/kg oil. Therefore, by including 30% DDGS in the diet, the calculated PV of the diet was 2.4 meq/kg (PV of the diet, meq/kg = 84.1 meq/kg oil × 9.66% crude fat × 30% inclusion rate), which is just at the threshold level suggested by DeRouchey et al. (2004). However, PV may not accurately reflect degree of lipid peroxidation since PV is a measurement of hydroperoxides which begin to decompose as soon as they are formed. As a result, a low PV could be due to either minimal oxidation or decomposition of hydroperoxides that have already begun. In addition, the increased serum α-tocopherol concentration in pigs fed DDGS diets suggests a vitamin E-sparing effect that may be caused by increases in other antioxidants or improved overall antioxidant status which might alleviate the oxidative challenge. This assumption is supported by a recent study conducted in our laboratory (Song et al., 2012b) that used pigs from the same genetic source and were fed the same source of DDGS. The authors reported that pigs fed DDGS showed increased endogenous S-containing antioxidants (glutathione, Met, taurine and glutathione peroxidase) originating from the high S content in DDGS. Whether due to insufficient oxidative challenge, and (or) improved oxidation defense system, the

unchanged serum TBARS and elevated α-tocopherol concentration in the current study indicate that pigs fed the high S and highly oxidized DDGS source did not experience significant metabolic oxidative stress.

Dibner and Knight (2008) stated that oxidative stress in the gastrointestinal system, regardless of its cause, is associated with loss of barrier function due to the denaturation of the protein component of enterocyte junctional complexes (Musch et al., 2006). This denaturation results in failure of both cell to cell adhesion and apical membrane integrity (Blikslager et al., 2007). There is little information regarding the effects of feeding oxidized lipids on intestinal permeability in the literature. Dibner et al. (1996) reported that feeding oxidized poultry fat to broilers resulted in structural injury to the intestine due to decreased half-life of enterocytes. Assimakopoulos et al. (2004) suggested that a decrease in villous density and total mucosal thickness were important indicators of physical intestinal injury leading to intestinal oxidative stress. Ringseis et al. (2007) showed that feeding thermally-oxidized sunflower oil to growing pigs increased markers of oxidative stress in the small intestine. In a recent study, Liu et al. (2012) evaluated the effects of feeding oxidized lipids (corn oil, canola oil, beef tallow, and poultry fat) to nursery pigs on intestinal barrier function. They found no effect of oxidized lipids on intestinal barrier function as measured by the lactulose-mannitol procedure.

In the current study, intestinal barrier function was measured using mannitol and lactulose. This dual sugar test was first introduced by Menzies (1974). The principle of the test is based on the fact that orally administered mannitol, a monosaccharide, will be passively absorbed through the intestinal epithelium, while lactulose, a disaccharide,

normally does not pass the intestinal epithelium through the paracellular route unless the barrier function is compromised. However, there are several pre-mucosal (e.g., bacterial degradation) and post-mucosal (e.g., completeness of urine collection) factors (Bjarnason et al., 1995) that affect the test results, and this test assumes that both probes are affected by these factors to a similar degree. Therefore, the L/M ratio, instead of the urinary concentration of these compounds, is a more accurate indicator to determine the intestinal barrier function (Bjarnason et al., 1995). For example, an increased L/M ratio indicates an increased intestinal permeability and an impaired intestinal barrier function, whereas a decreased L/M ratio indicates a decreased intestinal permeability and an improved intestinal barrier function.

In the current study, pigs fed the highly oxidized DDGS source tended to have a better intestinal barrier function, as indicated by a 28% lower L/M ratio, compared with those fed CON. Whitney et al. (2006b) reported that feeding a diet containing 10% DDGS to growing pigs may provide some protection and aid the pig in resisting an ileitis challenge by reducing the severity of lesions in ileum and colon. These findings indicate that feeding DDGS may be beneficial to the gut barrier function because a common consequence of ileitis is a breakdown in the intestinal barrier function that normally protects the animal against invasion by commensal and pathogenic gut microbiota (Dibner and Knight, 2008). However, the mechanisms of this beneficial effect are still unknown. Regardless of the reasons, feeding DDGS containing oxidized lipids did not compromise the intestinal barrier function in the current study, which indicates again that pigs consuming DDGS diets did not experience metabolic or intestinal oxidative stress.

Previous studies found that feeding oxidized lipids may negatively affect nutrient digestibility and utilization in animals due to the localized oxidative damage to the intestinal epithelial cells. For example, Ringseis et al. (2007) reported that feeding pigs oxidized sunflower oil, which contains high levels of linoleic acid similar to corn oil, increased TBARS in intestinal epithelial cells, which indicates an increased localized oxidative stress within the intestine. Additionally, Dibner et al. (1996) observed increased intestinal epithelial cell turnover and decreased lymphocyte proliferation in follicles of the lamina propia in broilers fed oxidized poultry fat, indicating that nutrient absorption and digestion may be compromised due to the impairment of intestinal cells. Moreover, the negative effects of feeding oxidized lipids on energy and nutrient digestibility have been shown in several different animal species. For instance, decreased digestibility of DM, CP, fat, and ME were reported in nursery pigs fed oxidized fish oil (Yuan et al., 2007). Similarly, reduced digestibility of GE and fat were reported in mink fed oxidized fish oil (Borsting et al., 1994). Lastly, decreased retention of fat and energy were reported in broilers fed oxidized vegetable oil (Engberg et al., 1996). However, conflicting results were also obtained in another experiment where digestibility of GE, DM, N, fat and/or fatty acids were not affected by feeding thermally-oxidized choice white grease to nursery pigs (DeRouchey et al., 2004). These contradictory findings may be due to differences concerning the fat quality and lipid peroxidation status in these studies.

In the current study, the lack of response on systemic indicators of oxidative stress suggests that the reduced digestibility of energy and N from feeding highly oxidized DDGS were not likely due to increased localized oxidative stress within the intestine.

The reduced digestibility was more likely due to other factors, such as high fiber content of DDGS, excessive heat damage of AA during DDGS production. The reduced digestibility of C may be due to a combination of reduced GE and N digestibility. Stein and Shurson (2009) concluded that the high fiber content (35% insoluble and 6% soluble dietary fiber) and low ATTD of dietary fiber (43.7%) in DDGS results in a reduced digestibility of DM, and is also the reason for reduced digestibility of energy in DDGS compared with many other feed ingredients. In addition, excessive heat during the drying process not only darkens the color of DDGS (Parsons et al., 1992), but also impairs digestibility and availability of AA, especially Lys, through the Maillard reactions between the amino residues and carbohydrate moieties (Fastinger and Mahan, 2006). The bound and converted Lys is apparently not available for use by the animal, which leads to reduced digestibility of Lys in DDGS. Indeed, the digestibility of most AA in DDGS is approximately 10 percentage units less than in corn (Stein and Shurson, 2009). Color measurement with Minolta or HunterLab spectrophotometers may sometimes be used to predict the digestibility of Lys in DDGS based on the theory that darker-colored DDGS may have undergone a greater degree of Maillard reactions during processing, and thus have a lower AA digestibility compared with lighter-colored DDGS (Cromwell et al., 1993; Fastinger and Mahan, 2006). In the present study, we did not determine AA digestibility of DDGS. However, as previously reported by Song et al. (2011), this highly oxidized DDGS source happened to have the darkest (L* = 45.2) and least yellow color (b* = 26.6) among other 30 DDGS sources sampled (mean values of L* and b* = 54.4 and 37.6, respectively), suggesting a reduced digestibility and availability of AA, especially Lys, in this highly oxidized DDGS source. Therefore, it appears that reduced

N digestibility observed in the current study may have been due to reduced Lys and other AA digestibility.

Although the digestibility of GE, N, and C were reduced in DDGS containing diets in this study, S absorbed, S retained, and ATTD of S were improved in DDGS diets by 92%, 42% and 2.7%, respectively, compared with CON. Daily S balance and digestibility in DDGS containing diets have previously been studied in ruminants that have the ability to convert inorganic sulfate to biologically available forms, such as sulfur-containing AA (Block et al., 1951; Emery et al., 1957). In a study utilizing lambs, S intake, S excretion in feces and urine, and S retention increased with increasing DDGS inclusion level from 0 to 60% (Neville et al., 2011). However, ATTD of S was not affected in that study. Corn DDGS contains a relatively high S content (0.33 to 1.04%, DM basis; Kim et al., 2012) compared to other feed ingredients, which is mainly due to the addition of sulfuric acid for pH adjustment and cleaning of fermenters used in the dry-grind ethanol production process. The high S concentration in DDGS has been shown to negatively affect health and performance in cattle (Sarturi et al., 2011; Uwituze et al., 2011a; Uwituze et al., 2011b), whereas very little is known about S utilization in pigs. Kerr et al. (2011) reported that growing pigs can tolerate relatively high amounts of dietary inorganic S (1.21% of diet), but high dietary S content alters inflammatory mediators and intestinal bacteria. In a recent study, Kim et al. (2012) concluded that high S content in DDGS-containing diets did not affect growth performance of weanling or growing-finishing pigs, nor is it the cause for reduced growth performance observed in some previous experiments (Whitney et al., 2006a; Linneen et al., 2008). In fact, the high S content and improved S digestibility in DDGS diets may enhance the capability of

pigs to cope with the oxidative challenge when feeding highly oxidized DDGS or other types of oxidized fats/oil by increasing some S-containing antioxidants (Song et al., 2012b).

In addition to evaluating the effect of feeding DDGS containing oxidized lipids on nutrient digestibility in pigs, the other objective of the current study was to determine if any of the negative effects from feeding oxidized DDGS could be overcome by increasing the dietary level of vitamin E (α-tocopheryl acetate). The antioxidant properties of vitamin E or α-tocopherol have been well documented (Burton and Traber, 1990). Vitamin E is considered the major chain-breaking antioxidant that protects cells and tissues from oxidative damage induced by free radicals (Burton and Traber, 1990). However, there is little information regarding the effect of vitamin E, or other antioxidants on nutrient digestibility in swine, and the results from other animal species have been controversial. In the current study, increasing vitamin E level to 110 IU/kg of diet did not affect digestibility of GE, N, C, and S. These data are in agreement with results reported by Goñi et al. (2007) and Açıkgöz et al. (2011), in which digestibility of CP, AA and ether extract were not affected by providing 200 IU/kg of diet vitamin E (α-tocopheryl acetate) to broilers. However, in some other studies, dietary supplementation of vitamin E to Japanese quail (Sahin and Kucuk, 2001), and vitamin C to laying hens (Seven, 2008) at 250 IU/kg of diet improved digestibility of DM, CP and ether extract when those animals were under heat stress-induced oxidative challenge. These contradictory results may not only reflect species differences with respect to the effect of antioxidants on nutrient digestibility, but also suggest a dose-dependent and stress-dependent response. It appears that supplementation of a higher level of an antioxidant,

such as vitamin E, to animals in stressed conditions where vitamin E may be depleted, is more likely to improve the nutrient digestibility compared with providing a lower dose of an antioxidant to animals under less stressful conditions. However, the mechanism(s) of response to vitamin E (or other antioxidants) on nutrient digestibility is poorly understood. The lack of response of vitamin E to energy and nutrient digestibility in the current study suggest that it may not be necessary to increase the levels of vitamin E higher than those recommended by NRC (1998) to benefit pigs when feeding highly oxidized DDGS.

Table 4-1. Analyzed composition of dried distillers grains with solubles (DDGS) source used in this experiment (as-fed basis)

Item, %	DDGS source
DM	91.3
CP	26.6
Crude fat	6.3
Crude fiber	7.7
ADF	12.5
NDF	25.0
Ash	5.3
P	0.97
Ca	0.03
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

Table 4-2. Composition and nutrient analysis of experimental diets (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%)	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
NaCl	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
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, %	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
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,[7] %	2.23	–	–	3.71	–	–
Crude fiber,[7] %	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,[7] %	0.76	–	–	0.76	–	–
P,[7] %	0.62	–	–	0.56	–	–
α-tocopherol, IU/kg	<10	13	117	<10	14	105

[1]CON = corn-soybean meal based control diet.
[2]DDGS = 30% inclusion of dried distillers grains with solubles
[3]No-E = No supplemental vitamin E; 1X-E = NRC (1998) level of vitamin E supplied as α-tocopheryl acetate, which is 11.0 IU/kg; 10X-E = 10X NRC (1998) level of vitamin E supplied as α-tocopheryl acetate, which is 110 IU/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 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 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^{TM}); 55.11 mg of iron as ferrous sulfate (SQM^{TM}); 5.51 mg of copper as copper sulfate (SQM^{TM}); and 17.64 mg of manganese as manganese oxide (SQM^{TM}).

[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 3416 kcal/kg for DDGS, which was obtained as the average value of 10 DDGS samples analyzed by Pedersen et al. (2007).

[7]Nutrients concentrations were only analyzed in No-E/CON and No-E/DDGS treatments. Values should be very similar to those in corresponding 1X-E and 10X-E treatments.

Table 4-3. Influence of DDGS and vitamin E supplementation on serum thiobarbituric acid-reactive substance (TBARS) and α-tocopherol concentration[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-soybean based control diet.
[3]DDGS = 30% dietary inclusion of dried distillers grains with solubles
[4]No-E = No supplemental vitamin E; 1X-E = NRC (1998) level of vitamin E; 10X-E = 10X NRC (1998) level of vitamin E. Vitamin E was supplied as α-tocopheryl acetate.

Table 4-4. Influence of DDGS and vitamin E supplementation on intestinal permeability[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
Urinary lactulose, mg/mL	0.16	0.12	0.11	0.06	0.10	0.08	0.03	0.70	0.02	0.13
Urinary mannitol, mg/mL	1.02	1.44	0.89	0.84	1.30	0.98	0.31	0.13	0.71	0.80
L/M[5]	0.15	0.08	0.11	0.08	0.08	0.09	0.03	0.31	0.07	0.19

[1]Values are least square means of nine replicate pigs per dietary treatment.
[2]CON = corn-soybean based control diet.
[3]DDGS = 30% dietary inclusion of dried distillers grains with solubles
[4]No-E = No supplemental vitamin E; 1X-E = NRC (1998) level of vitamin E; 10X-E = 10X NRC (1998) level of vitamin E. Vitamin E was supplied as α- tocopheryl acetate.
[5]L/M = lactulose to mannitol ratio

Table 4-5. Energy and N balance for pigs fed 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
No. of pigs	8	9	7	8	9	9				
GE intake, kcal/d	5,915	5,674	6,042	5,738	5,777	5,783	234	0.72	0.57	0.71
GE in feces, kcal/d	671	603	649	878	939	907	50	0.99	<0.001	0.41
GE in urine, kcal/d	176	152	140	156	148	163	21	0.68	0.99	0.57
DE, kcal/kg	3,319	3,317	3,406	3,307	3,263	3,284	32	0.21	0.02	0.22
ME, kcal/kg	3,207	3,218	3,315	3,203	3,162	3,173	32	0.21	0.01	0.11
N intake, g/d	44.0	42.2	45.9	41.0	43.4	46.2	1.9	0.15	0.75	0.52
N in feces, g/d	5.3	4.8	5.0	6.5	7.2	7.1	0.4	0.93	<0.001	0.40
N in urine, g/d	13.3	11.4	11.1	10.0	9.7	11.1	1.5	0.77	0.19	0.59
N absorbed, g/d	39.1	37.4	41.3	34.7	36.3	39.1	1.7	0.10	0.08	0.63
N retained, g/d	26.1	26.0	30.6	25.0	26.7	28.1	2.1	0.19	0.57	0.75

[1]Values are least square means.
[2]CON = corn-soybean meal based control diet.
[3]DDGS = 30% dietary inclusion of dried distillers grains with solubles
[4]No-E = No supplemental vitamin E; 1X-E = NRC (1998) level of vitamin E; 10X-E = 10X NRC (1998) level of vitamin E. Vitamin E was supplied as α-tocopheryl acetate.

Table 4-6. Daily C and S balance for pigs fed 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
No. of pigs	8	9	7	8	9	9				
C intake, g	606.6	586.7	619.4	583.9	587.4	589.1	25.8	0.78	0.39	0.79
C in feces, g	63.8	57.6	62.3	85.7	91.8	88.1	4.9	0.99	<0.001	0.42
C in urine, g	14.8	12.5	11.6	12.7	12.0	13.2	1.7	0.63	0.82	0.59
C absorbed, g	545.6	529.7	560.7	500.5	496.3	501.7	21.1	0.69	0.01	0.83
C retained, g	531.9	517.5	550.6	488.7	484.6	488.8	20.6	0.67	0.01	0.78
S intake, g	3.11	2.90	3.15	5.55	5.63	5.94	0.20	0.36	<0.001	0.67
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	0.65	0.40	0.09	1.97	1.95	1.88	0.24	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

[1]Values are least square means.
[2]CON = corn-soybean meal based control diet.
[3]DDGS = 30% dietary inclusion of dried distillers grains with solubles
[4]No-E = No supplemental vitamin E; 1X-E = NRC (1998) level of vitamin E; 10X-E = 10X NRC (1998) level of vitamin E. Vitamin E was supplied as α-tocopheryl acetate.

Table 4-7. Apparent total tract digestibility (ATTD) of GE, N, C and S of experimental diets[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
GE, %	88.75	89.39	89.39	84.80	83.71	84.41	0.63	0.85	<0.001	0.37
N, %	88.21	88.58	89.33	84.20	83.52	84.70	0.77	0.42	<0.001	0.78
C, %	89.60	90.21	90.10	85.43	84.36	85.16	0.60	0.84	<0.001	0.36
S, %	84.30	84.01	85.41	87.29	85.91	87.32	0.70	0.14	<0.001	0.68

[1]Values are least square means of nine replicate pigs per dietary treatment.
[2]CON = corn-soybean meal based control diet.
[3]DDGS = 30% dietary inclusion of dried distillers grains with solubles
[4]No-E = No supplemental vitamin E; 1X-E = NRC (1998) level of vitamin E; 10X-E = 10X NRC (1998) level of vitamin E. Vitamin E was supplied as α-tocopheryl acetate.

CHAPTER V. Effects of Feeding Diets Containing Highly Oxidized Dried Distillers Grains with Solubles and Increasing Vitamin E Levels to Wean-Finish Pigs on Growth Performance, Carcass Characteristics, and Pork Fat Composition

Lipid peroxidation in animal feed can negatively affect growth performance and meat quality. Weanling pigs (n = 432; BW = 6.6 ± 0.4 kg) were used to evaluate the effects of feeding a peroxidized dried distillers grains with solubles (**DDGS**) source with 3 levels of vitamin E (α-tocopheryl acetate) on growth performance, carcass composition, fatty acid composition of pork fat, and lipid peroxidation in LM. 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. Pens within blocks were assigned randomly to one of 6 dietary treatments in a 2 × 3 factorial design. Pigs were fed a corn-soybean meal (**CON**) or 30% DDGS diets with 3 levels of vitamin E: none supplemented (**No-E**), NRC (**1X-E**), or 10X NRC (**10X-E**). Compared to CON, inclusion of 30% DDGS in diets reduced ($P < 0.001$) final BW (110 vs. 107 kg, respectively), overall ADG (0.76 vs. 0.74 kg/d, respectively) and G:F (0.39 vs. 0.37, respectively). Increasing dietary vitamin E level increased overall G:F ($P = 0.03$). Hot carcass weight, dressing percentage, backfat depth, and LM area were reduced ($P < 0.01$) in pigs fed DDGS compared to CON, but percentage of fat-free carcass lean was not affected. Feeding DDGS increased ($P < 0.001$) PUFA concentration, particularly linoleic acid ($P < 0.001$),

and iodine value ($P < 0.001$) in belly fat and backfat compared to pigs fed CON. Dietary vitamin E levels did not significantly affect fatty acid profiles in belly or back fat. Although pigs were fed a peroxidized DDGS source in this study, TBARS in LM were similar between DDGS and CON treatments. There was no interaction between DDGS and dietary vitamin E concentration in LM TBARS. Alpha-tocopherol concentration in LM was higher ($P < 0.001$) in 10X-E than No-E or 1X-E dietary treatments. Compared to CON, feeding DDGS increased α-tocopherol concentration in LM of pigs fed No-E (1.0 vs. 3.1 mg/kg, respectively, $P = 0.005$), but not in those fed 1X-E or 10X-E. These results indicate that feeding highly oxidized, 30% DDGS diets to wean-finish pigs may negatively affect growth performance, and supplementation of additional vitamin E in the diet did not counteract these effects, but did improve G:F and α-tocopherol level in LM at the 10X NRC level.

Key words: corn dried distillers grains with solubles, growth performance, lipid peroxidation, wean-finish pigs, vitamin E

INTRODUCTION

The use of dried distillers grains with solubles (**DDGS**) in swine grower-finisher diets has increased dramatically in recent years due to increased supply and its cost competitiveness compared with corn and soybean meal (Stein and Shurson, 2009). Currently, the long-term sustainability of using high levels of DDGS (up to 40%) in grower-finisher swine diet is in jeopardy because of concerns related to the negative

effects of lipids in DDGS on pork fat quality. The lipid fraction of corn DDGS is made up largely of PUFA, particularly linoleic acid (NRC, 1998), which is prone to lipid peroxidation. Lipid peroxidation is a free-radical chain reaction that produces oxidized lipids and a series of toxic aldehydes (Blokhina et al., 2003). Additionally, during the drying process, DDGS are heated at relatively high temperatures that likely accelerate lipid peroxidation by oxidizing unsaturated lipids.

Oxidative damage in feed ingredients represents a significant economic loss. Lipid peroxidation in animal feed can negatively affect animal health and growth performance (Miller and Brzezlnska-Slebodzlnska, 1993; Pfalzgraf et al., 1995). Furthermore, changes in body composition, pork quality, and reduced shelf-life stability of fresh and ground pork may occur, leading to a decrease in overall acceptance of pork from animals fed under these dietary conditions. Additionally, toxic secondary lipid peroxidation products may be present in meat products from pigs consuming diets containing high amounts of peroxidized lipids which may cause concern for human health.

Vitamin E, supplemented in diets, can be deposited in fat associated with muscle tissue as α-tocopherol. Vitamin E is the most important natural antioxidant to protect against lipid peroxidation and increase pork shelf-life stability (Jensen et al., 1998). Thus, the objective of the study was to evaluate the effects of feeding highly oxidized DDGS with increasing levels of vitamin E on pig growth performance, carcass characteristics, fatty acid composition and LM oxidation in pigs from weaning to harvest.

MATERIALS AND METHODS

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

Animals and Housing

Weanling, mixed-sex pigs (n = 432; initial BW = 6.6 ± 0.4 kg) produced from sows (Landrace × Yorkshire, TOPIGS, Winnipeg, Manitoba, Canada) mated to Duroc boars (Compart's Boar Store, Nicollet, MN) were used in this experiment conducted at the University of Minnesota, West Central Research and Outreach Center (Morris, MN). Pigs were blocked by initial BW, and 9 pigs (either 5 gilts and 4 barrows, or 4 gilts and 5 barrows) within block were grouped together in a pen. Pens within block were assigned randomly to one of 6 dietary treatments in a 2 × 3 factorial arrangement resulting in 8 pens per treatment. Each pen (1.6 × 4.5 m) was equipped with 1 nipple drinker, one 4-space self-feeder, and had totally slatted floors. Pigs were fed corn-soybean meal (**CON**) or corn-soybean meal-30% DDGS diets containing one of 3 levels of vitamin E (α-tocopheryl acetate): none supplemented (**No-E**), NRC (1998) recommended concentration of vitamin E (**1X-E**), or 10X NRC (**10X-E**). Pigs were offered diets in a 7-phase feeding program throughout the 136 d wean-to-finish feeding period, with targeted BW for each diet phase of 6 to 9 kg, 9 to 16 kg, 16 to 27 kg, 27 to 45 kg, 45 to 68 kg, 68 to 91 kg, and 91 to 113 kg, respectively. Pigs had *ad libitum* access to their assigned dietary treatments and water throughout the experiment.

Diet Composition and DDGS Source

Diet composition and nutrient concentration of experimental diets for Phase 1 to 7 are presented in **Tables 5-1 to 5-7**. All diets were fed in meal form and were formulated on a standardized ileal digestible AA and available P basis. Nutrient concentration of all diets met or exceeded NRC (1998) recommended 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 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). This DDGS source 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).

Growth Performance

Pigs were weighed individually on the day dietary treatments were imposed and every 2 wk or every week if a diet-phase change was needed during the experiment. Individual BW of the pigs within pens was used to calculate ADG on a pig and pen basis. On each weigh day, feed disappearance was measured to calculate ADFI of pigs on a pen basis. Pen ADG and ADFI were used to calculate G:F.

Ultrasound and Carcass Measurements

Ultrasound was used to determine 10th rib backfat thickness and LM area 2 d before harvest when the average BW of all pigs reached 113 kg. Scanning was accomplished by a trained technician using an ALOKA 500V (Corometrics Medical Systems, Wallingford, CT) real-time ultrasonic machine fitted with a 12.5-cm long, 3.5-MHz linear array transducer. Ultrasound measurements were taken along the dorsal midline at the 10th rib. The transducer was aligned perpendicular to the spine at the 10th rib. Digitized images were processed using Quality Evaluation and Prediction (Iowa State University, Ames, IA) specifically developed to measure linear distance and area of digitized images and matriculate to a data file. Tenth rib backfat depth was measured at a point ¾ of the distance of the LM, curvilinear from the spine, and perpendicular to the LM surface.

All pigs were harvested on the same day by Hormel Foods Corporation (Austin, MN). Pigs were weighed individually 1 d before harvest to obtain a final live BW. Hot carcass weight was measured on carcasses immediately after harvest. Live BW of pigs along with HCW were used to calculate dressing percentage using the following equation: dressing, % = (HCW / live BW) × 100. Percentage of fat-free carcass lean was calculated using the following equation according to National Pork Producers Council (2000): fat-free carcass lean, % = [2.620 + (0.401 × HCW, kg) - (3.358 × ultrasound 10th rib backfat depth, cm) + (0.306 × ultrasound 10th rib LM area, cm^2) + (0.456 × sex of pig) (barrow=1, gilt=2)] / [HCW, kg] × 100.

Pork Fat Composition of Belly and Backfat

Forty-eight gilts (1 from each pen) weighing closest to the mean BW of their pen were selected to determine pork fat composition. Twenty-four hours after carcasses were chilled at 1.7 to 4.4°C, 2.54-cm cores from the belly and backfat were collected. The belly tissue cores were collected at the midline opposite the last rib, and the backfat cores were collected at the 10th-rib location on the right side of the carcass. Core samples of belly and backfat were stored at -20°C after collection and sent to University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO) for analysis of fatty acid profile using gas chromatography according to AOCS (1998) method Ce 1-62. The iodine value (**IV**) of fat was calculated using the following equation (AOCS, 1998): IV = (C16:1 × 0.95) + (C18:1 × 0.86) + (C18:2 × 1.732) + (C18:3 × 2.616) + (C20:1 × 0.785) + (C22:1 × 0.723).

Lipid Peroxidation in LM

To evaluate the lipid peroxidation level and α-tocopherol concentration in LM, LM samples from the 48 selected carcasses were collected at the 10^{th} rib from the right side 24 h after chilling. Each LM was separated into 2 equal halves, and stored at -80 °C immediately. One section of LM was sent to Michigan State University Diagnostic Center for Population & Animal Health (Lansing, MI) for analysis of α-tocopherol concentration using ethanol and hexane extraction followed by quantification via HPLC (Separation Module 2690, Waters, Milford, MA; Katsanidis and Addis, 1999). Another section of LM was used to perform a tissue TBARS assay following the method described by Animal Models of Diabetic Complications Consortium (AMDCC, Version

1). Specifically, 50 mg of LM sample was weighed into a 2 mL flat-bottom centrifuge tube containing 500 μL PBS and 1% Triton X-100 (Sigma-Aldrich, St. Louis, MO). The mixture was homogenized on ice for 15 seconds, followed by centrifugation at 10,000 × *g* for 5 min at 4 °C to collect the supernatant. One hundred μL supernatant 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 of supernatant were 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 cooled to room temperature and read at 532 nm using a spectrophotometer (SpectraMax 250, Molecular Device, Sunnyvale, CA). This assay was conducted in 4 batches with duplicate samples and a standard. The intra-assay CV was 7.4% and the inter-assay CV was 4.6%.

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS Inst. Inc. (Cary, NC). Pen was used as the experimental unit for all responses. The statistical model included the fixed effects of DDGS, vitamin E concentration, and DDGS × vitamin E interactions, and random effect of block. Live BW before harvest was used as the covariate in analysis of carcass characteristics if it was significant ($P < 0.05$). Repeated measures in time were used to analyze growth performance data in each diet phase. The unstructured option was used to fit a variance-covariance matrix in the model for repeated measures in

time. The slice option of SAS was used to separate main effects within different phases. All results are reported as least squares means. Multiple comparisons among treatments were performed using PDIFF and adjusted by Tukey 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

During the experiment, 11 pigs (CON/No-E = 1, CON/10X-E = 4, DDGS/No-E = 1, DDGS/1X-E = 3, and DDGS/10X-E = 2) were removed for reasons unrelated to dietary treatments ($\chi^2 = 4.17$, df = 2; $P = 0.12$). Fifty-nine out of 421 pigs (CON/No-E = 3, CON/1X-E = 9, CON/10X-E = 6, DDGS/No-E = 17, DDGS/1X-E = 13, and DDGS/10X-E = 11; $\chi^2 = 90.42$, df = 2; $P < 0.001$) were excluded from collection of carcass data because they were too light to fit specifications of the processor and could not be slaughtered on the same day as contemporaries.

Growth Performance

No interaction between dietary DDGS and vitamin E supplementation level was observed for overall growth performance responses (**Table 5-8**). There were time effects on ADG ($P < 0.001$), ADFI ($P < 0.001$) and G:F ($P < 0.001$) across phases, but no time × DDGS × vitamin E interactions. For the overall period, initial BW at weaning was similar among dietary treatments. However, final BW at harvest was lower in pigs consuming highly oxidized DDGS (106.6 vs. 110.2 kg, respectively, $P < 0.001$), which

was largely due to the reduction in ADG (0.74 vs. 0.76 kg, respectively, $P < 0.001$), compared with pigs consuming CON, respectively. There were no effects of feeding DDGS on ADFI, but G:F was lower (0.37 vs. 0.39, respectively, $P < 0.001$) in pigs fed DDGS compared with those fed CON, respectively, because of the reduced ADG. Increasing dietary vitamin E concentrations had no effect on BW and ADG, but tended to decrease ADFI ($P = 0.10$), resulting in an improved G:F ($P = 0.03$) of pigs fed 10X-E and 1X-E vs. No-E diets (0.39 and 0.39 vs. 0.38, respectively).

Ultrasound and Carcass Characteristics

No effects of vitamin E supplementation or interaction between dietary DDGS and vitamin E were detected for any of the carcass measurements (**Table 5-9**). Because of reduced growth rate, pigs fed DDGS had a lower live BW (113.3 vs. 115.2 kg, $P = 0.04$) and HCW (87.9 vs. 89.2 kg, $P < 0.001$) compared with those fed CON, respectively. Dressing % was also decreased in pigs consuming DDGS diets (76.9 vs. 78.2 %, $P < 0.001$) compared with those fed CON, respectively. Additionally, ultrasound 10^{th} rib backfat depth was less in pigs fed DDGS than those fed CON (2.3 vs. 2.4 cm, respectively; $P = 0.008$). Ultrasound LM area was also smaller when comparing pigs fed DDGS with those fed CON (36.1 vs. 28.7 cm^2, respectively; $P < 0.001$). However, the fat-free carcass lean calculated as the percentage of HCW was unaffected by dietary treatments.

Fatty Acid Composition and IV of Belly and Backfat

Changes in the fatty acid composition of belly fat and backfat resulting from feeding highly oxidized DDGS and increasing levels of vitamin E are presented in **Table 5-10**. Compared to pigs fed CON, feeding DDGS reduced SFA content of belly fat (39.04 vs. 35.19 %, respectively; $P < 0.001$), including palmitic acid (**C16:0**, $P < 0.001$), and stearic acid (**C18:0**, $P < 0.001$), and MUFA content (51.45 vs. 47.22 %, respectively; $P < 0.001$), including palmitoleic acid (**C16:1**, $P = 0.003$), and oleic acid (**C18:1**, $P < 0.001$), by 10% and 8%, respectively. Conversely, pigs fed DDGS diets compared to CON had higher PUFA content (16.20 vs. 8.36 %, respectively; $P < 0.001$), including linoleic acid (**C18:2**, 15.22 vs. 7.64 %, respectively; $P < 0.001$), linolenic acid (**C18:3**, 0.58 vs. 0.42 %, respectively; $P < 0.001$), eicosatrienoic acid (**C20:3**, $P < 0.001$), and arachidonic acid (**C20:4**, $P < 0.001$). Because of the increase in the concentration of PUFA, the IV of belly fat was increased when comparing DDGS vs. CON treatments (68.2 vs. 58.4, respectively; $P < 0.001$). There were no effects of vitamin E supplementation level on fatty acid profile of belly fat. However, a significant DDGS × vitamin E interaction was observed for SFA ($P = 0.04$) and MUFA ($P = 0.05$). In pigs fed 30% DDGS diets, there were no effects of vitamin E on SFA or MUFA concentration in belly fat. However, in pigs fed CON, supplementing vitamin E at the NRC level reduced SFA concentration but increased MUFA concentration compared with supplementing at 10X NRC level (37.92 vs. 40.18 %, and 52.88 vs. 50.78 %, respectively). These interactions indicate that the magnitude of change in fatty acid

composition resulting from feeding different levels of vitamin E varied between pigs fed DDGS and CON diets.

Similar to belly fat, backfat samples from pigs fed DDGS had approximately 11% less SFA content compared to samples from pigs fed CON (37.36 vs. 41.73 %, respectively; P < 0.001), including myristic acid (**C14:0**, *P* = 0.01), C16:0 (*P* < 0.001), C18:0 (*P* < 0.001) and arachidic acid (**C20:0**, *P* = 0.01), and had a 9% reduction in MUFA (42.66 vs. 46.77 %, respectively; *P* < 0.001), including C16:1 (*P* < 0.001) and C18:1 (*P* < 0.001). In contrast, feeding DDGS increased PUFA content of back fat compared to CON by approximately 82% (18.13 vs. 9.95 %, respectively; *P* < 0.001) , including C18:2, C18:3, C20:3 and C20:4 (*P* < 0.001). In addition, IV of backfat was increased when comparing DDGS with CON treatments (68.1 vs. 57.6, respectively; *P* < 0.001). Again, the effects of dietary vitamin E level on fatty acid composition of backfat were not significant. However, there were a significant interaction between DDGS and vitamin E level detected for SFA (*P* = 0.02) and MUFA (*P* = 0.03). Compared with supplementing vitamin E at 10X NRC level, providing vitamin E at 1X NRC level reduced SFA (43.94 vs. 39.71 %) and increased MUFA (45.96 vs. 48.32 %) concentrations in pigs fed CON, but increased SFA (36.50 vs. 38.12 %) and reduced MUFA (43.31 vs. 41.69 %) concentrations in pigs fed DDGS. These interactions suggest that the magnitude of response to different dietary levels of vitamin E on fatty acid profile varied in DDGS and CON treatments.

Lipid Peroxidation in LM

There were no effects of DDGS, vitamin E supplementation level, or their interaction on TBARS values in fresh LM (**Table 5-11**). An interaction between DDGS × vitamin E concentration was detected ($P = 0.02$) for α-tocopherol concentration in LM (**Table 5-11**). Specifically, pigs fed DDGS without vitamin E supplementation (No-E) had a higher concentration of serum α-tocopherol concentration compared with those fed CON with No-E (3.10 vs. 1.03 mg/kg, respectively; $P = 0.005$). However, when vitamin E was supplemented at NRC or 10X NRC levels, LM α-tocopherol concentration was similar for pigs fed DDGS vs. CON diets. Additionally, LM α-tocopherol concentration was higher when dietary vitamin E was provided at 10X NRC level than 1X-E and No-E in both DDGS and CON treatments (8.43 vs. 2.25 and 2.06 mg/kg, respectively; $P < 0.001$). However, no differences were observed between No-E and 1X-E treatments for LM α-tocopherol concentration.

DISCUSSION

With expansion of the ethanol industry, the utilization of corn co-products, such as DDGS, in swine feeds has increased dramatically due to increased availability and cost competitiveness compared with corn and soybean meal. However, limits on dietary DDGS inclusion rates may occur because of reduced growth performance and pork fat quality when high dietary levels of DDGS (> 20%) are fed to growing-finishing pigs. Results from some experiments showed that feeding diets containing 20 or 30% DDGS results in similar growth performance (Cook et al., 2005; DeDecker et al., 2005; Gaines

et al., 2007), whereas other experiments (Whitney et al., 2006a; Linneen et al., 2008) showed reduced ADG and ADFI in pigs fed diets containing DDGS compared with those fed standard corn-soybean meal diets. The reduction in growth performance could be caused by several factors, such as poor amino acid balance and digestibility, low net energy level, antinutritional factors, toxins, and/or oxidized lipids in DDGS (Stein and Shurson, 2009).

The lipid content in corn DDGS is approximately 10% and consists of PUFA, particularly C18:2 (NRC, 1998) that are vulnerable to lipid peroxidation. Lipid peroxidation is a free-radical chain reaction that produces oxidized lipids and a series of toxic aldehydes (Blokhina et al., 2003). These peroxidation products can negatively affect animal health and growth performance in several species (Dibner et al., 1996; DeRouchey et al., 2004; Harrell et al., 2010). Additionally, 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 current study, a DDGS source containing a high amount of oxidized lipids was selected according to a recent study conducted in our laboratory (Song et al., 2011). This DDGS source with the highest amount of oxidized lipids also contained highest S concentration (0.95%; Song et al., 2012) was used in the current study.

The presence of high amounts of oxidized lipids 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). Growth performance has been previously evaluated in swine and poultry when feeding different kinds of oxidized lipids, but the response was inconsistent. For example, reduced ADG was 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). In contrast, some other studies reported no differences in growth rate and feed intake when diets contained oxidized lipids for swine and poultry (Sheehy et al., 1994; Mitchaothai et al., 2007; Fernández-Dueñas et al., 2008). The lack of negative effects on animal performance may be due to insufficient dietary oxidative challenge. Peroxide value, which measures hydroperoxides, is a commonly used indicator of lipid peroxidation. However, one should note that using PV as the only indicator of lipid peroxidation may not be accurate or sufficient because the hydroperoxides generated by lipid peroxidation begin to decompose as soon as they are formed (Gray, 1978). Therefore, a low PV could be due to either minimal peroxidation or decomposition of hydroperoxides that have already begun. DeRouchey et al. (2004) suggested that there appears to be a threshold for rancidity above which growth performance is decreased. The authors reported that a PV for oxidized lipids (6% dietary inclusion rate) of 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 x 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 was 2.4 meq/kg (84.1 meq/kg oil × 9.66% crude fat × 30% inclusion rate), which is at the threshold level suggested by DeRouchey et al. (2004). To our knowledge, no study has been conducted to evaluate the influence of feeding oxidized lipids in DDGS on pig growth performance. In the current study, compared with pigs fed CON, those fed DDGS containing a high amount of oxidized lipids showed a 3% reduction in overall ADG, 3% reduction in final BW and 5% reduction in overall G:F, which were in agreement with the negative effects reported from feeding oxidized oil on pig growth performance by Fernández-Dueñas (2009) and Harrell et al. (2010). However, since we did not compare performance relative to a low oxidized DDGS source in the present study, it is difficult to determine if the depressed growth performance was due to the oxidized lipids in DDGS and/or due to some other factors, such as overestimation of lysine digestibility in the DDGS source used, reduced net energy content, or other factors. Further studies are needed to investigate the actual causes for reduced growth performance that sometimes occur when feeding high levels (20-30%) of DDGS to pigs.

The effects of supplementing antioxidants to animals under dietary peroxidative challenge on growth performance have been inconsistent. Harrell et al. (2010) reported that supplementing a blend of antioxidants (AGRADO PLUS, Novus International Inc., St Charles, MO) to diets containing 5% oxidized corn oil (PV = 7.5 meq/kg of diet)

improved ADG, ADFI, BW, but not G:F in nursery pigs. In contrast, other studies in finishing pigs fed 5% oxidized corn oil (PV = 9 meq/kg of diet; Fernández-Dueñas, 2009) with 10 or 132 ppm of ethoxyquin, in broilers fed 3% oxidized soybean oil (PV = 1.5 meq/kg of diet; Anjum et al., 2002) with 125 or 175 ppm ethoxyquin , and in rainbow trout fed 7.5% fish oil (PV = 9 meq/kg of diet; Hung et al., 1981) with 85 ppm ethoxyquin or α-tocopheryl acetate, showed no benefit effects of supplemental antioxidants on growth performance when these animals were under dietary oxidative challenge. The reasons for the inconsistent response are unclear, but may be due to different types and levels of antioxidants used in these studies, or differences in metabolic oxidation status of the animals. In the present study, compared to pigs fed diets without supplementation of vitamin E, feeding pigs α-tocopheryl acetate at either NRC (11 mg/kg of feed) or 10X NRC (110 mg/kg of feed) levels from weaning until harvest improved G:F, but had no effects on ADG, ADFI and final BW. Additionally, no differences were observed between 1X-E and 10X-E treatments for G:F. Therefore, increasing the dietary vitamin E levels above than those recommended by NRC when feeding a highly oxidized DDGS source may not be necessary.

In addition to growth performance, feeding high levels of DDGS has been studied extensively relative to pig carcass characteristics. Our results are in agreement with those reported by Whitney et al. (2006a) and Fu et al. (2004), who reported reduced HCW when feeding 30% DDGS to growing-finishing pigs. Additionally, dressing percentage was also decreased in pigs fed 30% DDGS diets in the current study, which is consistent

with other reports (Cook et al., 2005; Whitney et al., 2006a; Xu et al., 2010) where pigs fed diets containing 0 to 30% DDGS had linearly reduced dressing percentage. The reduction in HCW and dressing percentage could be due to the high levels of NDF in DDGS, which can potentially increase the gut fill (Pond et al., 1988). Fu et al. (2004) observed an increased fecal mass when feeding increased dietary DDGS levels, which was attributed to reduced HCW at the same harvest BW in pig fed DDGS containing diets. However, this does not explain the inconsistent responses for no change or a decrease in dressing percentage among studies where up to 30% DDGS was included in grower-finisher pig diets (Stein and Shurson, 2009). Oxidized lipids in DDGS may be another contributing factor for reduced HCW and dressing percentage observed in the current study. Fernández-Dueñas (2009) showed reduced HCW and yield in pigs fed 5% oxidized corn oil compared with those fed fresh corn oil, but the reason(s) still remains unclear. Again, without a comparison to a low oxidized DDGS source, the reason for these reductions remains unclear.

Our observation that dietary DDGS reduced 10^{th} rib backfat depth disagree with some previous studies (Fu et al., 2004; Cook et al., 2005; Whitney et al., 2006a), in which backfat depth was unaffected when feeding 30% DDGS to pigs. However, in a more recent study conducted by Xu et al. (2010) using pigs from the same genetic lines, last-rib backfat depth was reduced linearly with increasing dietary DDGS from 0 to 30%. The authors explained that the reduction in backfat depth might be due to the reduction in ADFI, which resulted in a reduction in totally daily energy intake, and thus, leaving less

energy available for fat deposition. In the current study, however, ADFI was not affected by dietary DDGS, suggesting that there might be other reasons for the reduction of backfat. Feeding thermally-oxidized oil/fat to rats (Koch et al., 2007; Sulzle et al., 2004) and pigs (Luci et al., 2007; Liu et al., 2012) alters *in vivo* lipid metabolism by activating the peroxisome proliferator-activated receptor α (PPARα) via up-regulation of some target genes in PPARα, such as acyl CoA oxidase, catalase, and carnitine palmitoyltransferase-1. The transcription factor PPARα controls the expression of fatty acid oxidative metabolism (Cabrero et al., 2001), and PPARα activation stimulates catabolic pathways of fatty acids (Desvergne and Wahli, 1999). Therefore, up-regulation of the target genes in PPARα involved in the oxidation of fatty acids is expected to enhance β-oxidation capacity in peroxisomes and reduce concentrations of lipids in liver and plasma (Sulzle et al., 2004). Considering these biological relationships, one may reasonably speculate that feeding the oxidized oil in DDGS source may have increased fatty acid β-oxidation through the activation of PPARα and thus, reducing the amount of fatty acids available for deposition in adipose tissue. Indeed, Fernández-Dueñas (2009) reported reduction in both10th-rib and last-rib backfat depth in pigs fed 5% oxidized corn oil, suggesting that the oxidized lipid or its components in DDGS, may lead to reduced backfat depth observed in the current study. Further studies focusing on PPARα gene expressions in liver and adipose tissue of pigs fed diets containing DDGS are necessary to better determine if this is a contributing factor to reduced backfat thickness observed in some studies. Regardless of the effect of oxidized lipids in DDGS on carcass

characteristics, our results are in agreement with Asghar et al. (1991), Cannon et al. (1996), and Waylan et al. (2002), who reported that vitamin E supplementation did not affect carcass characteristics in pigs.

Fatty acid composition in animal tissues depends on (1) *de novo* fatty acid synthesis and (2) fatty acid composition in the diet (Wiseman and Agunbiade, 1998). Particularly in pigs, *de novo* fatty acid synthesis, and direct deposition of synthesized fatty acids in adipose tissue, is effectively inhibited by dietary fat, especially PUFA (Farnworth and Kramer, 1987; Chilliard, 1993). Thus, the fatty acid composition of adipose tissue triglycerides, particularly essential PUFA such as C18:2 and C18:3, which cannot be synthesized by animals, is significantly influenced by the composition of dietary fat (Gatlin et al., 2002). Corn DDGS contains approximately 10% corn oil, which is high in unsaturated fatty acids (81% of total) and C18:2 (54% of total; Xu et al., 2010). In the current study, including 30% DDGS in the diet increased the concentration of PUFA of belly fat and backfat by 94 and 82%, respectively, and increased the concentration of C18:2 of belly fat and backfat by 99 and 87%, respectively, compared with CON. Our results support previous findings that fatty acid composition could be manipulated by the dietary fat consumed. In addition, our results are consistent with results from studies reported by Gatlin et al. (2003) and Xu et al. (2010), where the magnitude of the change in PUFA and C18:2 content was similar in belly fat and backfat, suggesting similar lipogenic activity in these adipose tissue sites.

Iodine value is a measure of the degree of unsaturation of fatty acids in fat and is defined as the number of grams of iodine absorbed by 100 g of fat. Since the concentration of PUFA in belly fat and backfat increased when 30% DDGS was included in the diets, the IV of belly fat and backfat increased by approximately 17 and 18%, respectively. Similarly, Xu et al. (2010) reported an increase of approximately 14 and 11%, respectively, of IV in belly fat and backfat of pigs fed diets containing 0 to 30% DDGS. Although belly firmness was not measured in the present study, the increased IV in pork fat, together with increased concentration of PUFA, clearly suggest that pigs fed DDGS had reduced belly firmness.

No effects of dietary vitamin E level on fatty acid composition or IV of backfat or belly fat were observed in the current study, which is consistent with results reported by Fernández-Dueñas (2009). These authors also reported no effects of dietary antioxidants on fatty acid profile of belly and backfat in pigs fed diets containing oxidized corn oil. In another study, however, the PUFA concentration in the fresh LM was higher in rabbits fed 200 vs. 50 IU/kg vitamin E, due to the antioxidant properties of vitamin E that protect against the lipid peroxidation of PUFA (Bosco et al., 2003).

Concentrations of α-tocopherol and TBARS in LM were determined in this study to evaluate the effect of feeding diets containing 30% DDGS containing a high level of lipid oxidation level on LM. Lipids in muscle tissues undergo autoxidation through the free-radical chain reaction after harvest, and the susceptibility of fatty acids to peroxidation increases with their degree of unsaturation (Shahidi, 1998; Skidsted et al.,

1998). The susceptibly of PUFA to peroxidation is the main reason for reduced meat shelf-life stability, increased rancidity flavor, and meat discoloration during storage, as observed in several studies when feeding pigs with vegetable oil (Leszczynski et al., 1992; Ahn et al., 1996; Leskanich et al., 1997) or DDGS (Leick et al., 2010) that contain high levels of PUFA. Additionally, dietary oxidized lipids may also promote lipid peroxidation and discoloration of loin under commercial storage conditions (Fernández-Dueñas, 2009). In the present study, the TBARS value in fresh LM was not different in pigs fed DDGS vs. CON diets, and pigs fed DDGS even exhibited approximately 2 times higher levels of α-tocopherol in the loin than those fed CON when no additional vitamin E was added to the diets. These results are consistent with results from a recent study conducted by Song et al.(2012b), in which serum TBARS values were not different, and serum concentrations of α-tocopherol were higher in pigs of the same genetic source and fed the same source of highly oxidized DDGS compared with those fed the same CON dietary treatments. Song et al. (2012b) discovered that the DDGS source used in that study as well as the current study not only contained the highest amount of oxidized lipids, but also contained a high total S concentration (0.95%). They explained that the unchanged TBARS and increased α-tocopherol concentration could be due to the increase in sulfur-containing antioxidants (glutathione, Met, taurine and glutathione peroxidase) from feeding this high S DDGS source which protected against the lipid peroxidation of PUFA and led to a vitamin E-sparing effect. As expected, increasing dietary vitamin E to 10X-NRC increased the α-tocopherol concentration in LM, which is in agreement with

results from several studies (Mahan et al., 1990; Leskanich et al., 1997; Boler, 2008). However, since increasing dietary vitamin E levels did not overcome the negative effects of feeding highly oxidized DDGS on pig growth performance and carcass characteristics, and it did not change the fatty acid composition in pork fat, it may not be necessary to increase the vitamin E levels higher than those recommended by NRC when feeding diets containing a high level of oxidized DDGS to pigs from weaning to harvest.

Table 5-1. Composition and nutrient analysis of Phase 1 diets (6 - 9 kg BW, 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	43.85	43.81	43.53	23.41	23.37	23.09
Soybean meal (46.5%)	23.13	23.13	23.13	13.75	13.75	13.75
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	0.89	0.89	0.89	1.27	1.27	1.27
Monocalcium phosphate	0.70	0.70	0.70	-	-	-
NaCl	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine HCl	-	-	-	0.17	0.17	0.17
DL-Methionine	0.05	0.05	0.05	-	-	-
L-Tryptophan	-	-	-	0.03	0.03	0.03
L-Threonine	0.01	0.01	0.01	-	-	-
Vitamin/ trace mineral premix[4]	0.50	0.50	0.50	0.50	0.50	0.50
Denegard[5]	0.18	0.18	0.18	0.18	0.18	0.18
Chlortetracycline	0.40	0.40	0.40	0.40	0.40	0.40
Zinc oxide	0.05	0.05	0.05	0.05	0.05	0.05
Vitamin E[6]	-	0.032	0.32	-	0.032	0.32
Calculated nutrient composition:						
ME,[7] kcal/kg	3,276	3,275	3,265	3,288	3,287	3,277
CP, %	23.10	23.10	23.08	25.13	25.13	25.10
P, %	0.87	0.87	0.87	0.90	0.90	0.89
Available P, %	0.62	0.62	0.62	0.62	0.62	0.62
Ca, %	1.22	1.22	1.22	1.22	1.22	1.22
SID Lys, %	1.31	1.31	1.31	1.31	1.31	1.31
SID Met + Cys, %	0.75	0.75	0.75	0.76	0.76	0.76
SID Thr, %	0.82	0.82	0.82	0.81	0.81	0.81
SID Trp, %	0.24	0.24	0.24	0.24	0.24	0.24
α-tocopherol, IU/kg	0.00	14.1	141.1	0.00	14.1	141.1
Analyzed nutrient composition[8]:						
CP, %	22.9	–	–	24.7	–	–
Crude fat, %	2.48	–	–	3.78	–	–
Crude fiber, %	2.0	–	–	3.0	–	–
Lys, %	1.54	–	–	1.52	–	–
Met, %	0.38	–	–	0.42	–	–
Thr, %	0.95	–	–	1.00	–	–
Trp, %	0.27	–	–	0.28	–	–

Ca, %	1.32	–	–	1.35	–	–
P, %	0.92	–	–	0.91	–	–
α-tocopherol, IU/kg	<10	13	137	<10	19	127

[1]CON = corn-soybean meal based control diet.
[2]DDGS = 30% inclusion of dried distillers grains with solubles
[3]No-E = No supplemental vitamin E; 1X-E = NRC (1998) level of vitamin E supplied as α-tocopheryl acetate, which is 14.0 IU/kg for BW = 7.5 kg; 10X-E = 10X NRC (1998) level of vitamin E supplied as α-tocopheryl acetate, which is 140 IU/kg for BW = 7.5 kg.
[4]Vitamin-trace mineral premix (without vitamin E) provided the following nutrients per kilogram of diet: 17,637 IU of vitamin A as retinyl acetate; 3,307 IU of vitamin D_3 as cholecalciferol; 6.16 mg of vitamin K as menadione dimethylpyrimidinol bisulfite; 11.02 mg of riboflavin; 66.14 mg of niacin; 44.09 mg of pantothenic acid as D-calcium pantothenate; 0.07 mg of vitamin B_{12}; 0.60 mg of iodine as ethylenediamine dihydroiodide; 0.60 mg of selenium as sodium selenite; 110.23 mg of zinc as zinc oxide (SQM™); 66.14 mg of iron as ferrous sulfate (SQM™); 7.72 mg of copper as copper sulfate (SQM™); and 11.02 mg of manganese as manganese oxide (SQM™).
[5]Denagard® 10 Medicated Premix from Novartis Animal Health US, Inc (Greensboro, NC), containing Tiamulin (as hydrogen fumarate) of 10 g/lb of product.
[6]Vitamin E was supplied as α-tocopheryl acetate with concentration of 44,092 IU/kg.
[7]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).
[8]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 5-2. Composition and nutrient analysis of Phase 2 diets (9 - 16 kg BW, 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	59.16	59.13	58.87	38.95	38.92	38.66
Soybean meal (46.5%)	37.64	37.64	37.64	28.00	28.00	28.00
DDGS	–	–	–	30.00	30.00	30.00
Limestone	0.95	0.95	0.95	1.34	1.34	1.34
Monocalcium phosphate	1.31	1.31	1.31	0.61	0.61	0.61
NaCl	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine HCl	0.07	0.07	0.07	0.25	0.25	0.25
DL-Methionine	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]	-	0.029	0.29	-	0.029	0.29
Calculated nutrient composition:						
ME,[6] kcal/kg	3,282	3,281	3,272	3,293	3,292	3,283
CP, %	22.49	22.48	22.46	24.43	24.43	24.41
P, %	0.69	0.69	0.69	0.72	0.72	0.71
Available P, %	0.36	0.36	0.36	0.36	0.36	0.36
Ca, %	0.73	0.73	0.73	0.73	0.73	0.73
SID Lys, %	1.17	1.17	1.17	1.17	1.17	1.17
SID Met + Cys, %	0.66	0.66	0.66	0.70	0.70	0.70
SID Thr, %	0.73	0.73	0.73	0.73	0.73	0.73
SID Trp, %	0.24	0.24	0.24	0.21	0.21	0.21
α-tocopherol, IU/kg	0.00	12.8	127.9	0.00	12.8	127.9
Analyzed nutrient composition[7]:						
CP, %	21.7	–	–	23.3	–	–
Crude fat, %	2.20	–	–	3.67	–	–
Crude fiber, %	2.7	–	–	3.6	–	–
Lys, %	1.37	–	–	1.35	–	–
Met, %	0.33	–	–	0.39	–	–
Thr, %	0.84	–	–	0.89	–	–
Trp, %	0.27	–	–	0.25	–	–
Ca, %	0.96	–	–	0.97	–	–
P, %	0.70	–	–	0.72	–	–
α-tocopherol, IU/kg	<10	12	117	<10	13	124

[1]CON = corn-soybean meal based control diet.
[2]DDGS = 30% inclusion of dried distillers grains with solubles
[3]No-E = No supplemental vitamin E; 1X-E = NRC (1998) level of vitamin E supplied as α-tocopheryl acetate, which is 12.8 IU/kg for BW = 12.5 kg; 10X-E = 10X NRC (1998)

level of vitamin E supplied as α-tocopheryl acetate, which is 128 IU/kg for BW = 12.5 kg.

[4]Vitamin-trace mineral premix (without vitamin E) provided the following nutrients per kilogram of diet: 17,637 IU of vitamin A as retinyl acetate; 3,307 IU of vitamin D_3 as cholecalciferol; 6.16 mg of vitamin K as menadione dimethylpyrimidinol bisulfite; 11.02 mg of riboflavin; 66.14 mg of niacin; 44.09 mg of pantothenic acid as D-calcium pantothenate; 0.07 mg of vitamin B_{12}; 0.60 mg of iodine as ethylenediamine dihydroiodide; 0.60 mg of selenium as sodium selenite; 110.23 mg of zinc as zinc oxide (SQM™); 66.14 mg of iron as ferrous sulfate (SQM™); 7.72 mg of copper as copper sulfate (SQM™); and 11.02 mg of manganese as manganese oxide (SQM™).

[5]Vitamin E was supplied as 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]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 5-3. Composition and nutrient analysis of Phase 3 diets (16 - 27 kg BW, 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	60.12	60.09	59.86	40.49	40.46	40.23
Soybean meal (46.5%)	37.08	37.08	37.08	26.82	26.82	26.82
DDGS	–	–	–	30.00	30.00	30.00
Limestone	0.91	0.91	0.91	1.30	1.30	1.30
Monocalcium phosphate	1.01	1.01	1.01	0.31	0.31	0.31
NaCl	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine HCl	0.03	0.03	0.03	0.23	0.23	0.23
Vitamin/ trace mineral premix[4]	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin E[5]	-	0.026	0.26	-	0.026	0.26
Calculated nutrient composition:						
ME[6], kcal/kg	3,296	3,295	3,287	3,306	3,306	3,298
CP, %	22.26	22.25	22.23	23.99	23.99	23.97
P, %	0.63	0.63	0.63	0.65	0.65	0.65
Available P, %	0.29	0.29	0.29	0.29	0.29	0.29
Ca, %	0.66	0.66	0.66	0.66	0.66	0.66
SID Lys, %	1.12	1.12	1.12	1.12	1.12	1.12
SID Met + Cys, %	0.64	0.64	0.64	0.69	0.69	0.69
SID Thr, %	0.72	0.72	0.72	0.71	0.71	0.71
SID Trp, %	0.24	0.24	0.24	0.21	0.21	0.21
α-tocopherol, IU/kg	0.00	11.46	114.64	0.00	11.46	114.64
Analyzed nutrient composition[7]:						
CP, %	20.6	–	–	23.0	–	–
Crude fat, %	2.41	–	–	3.82	–	–
Crude fiber, %	2.5	–	–	3.4	–	–
Lys, %	1.32	–	–	1.30	–	–
Met, %	0.32	–	–	0.38	–	–
Thr, %	0.83	–	–	0.88	–	–
Trp, %	0.27	–	–	0.24	–	–
Ca, %	0.71	–	–	0.81	–	–
P, %	0.62	–	–	0.64	–	–
α-tocopherol, IU.kg	<10	16	129	<10	16	118

[1]CON = corn-soybean meal based control diet.
[2]DDGS = 30% inclusion of dried distillers grains with solubles
[3]No-E = No supplemental vitamin E; 1X-E = NRC (1998) level of vitamin E supplied as α-tocopheryl acetate, which is 11.5 IU/kg for BW = 21.5 kg; 10X-E = 10X NRC (1998)

level of vitamin E supplied as α-tocopheryl acetate, which is 115 IU/kg for BW = 21.5 kg.

[4]Vitamin-trace mineral premix (without vitamin E) provided the following nutrients per kilogram of diet: 17,637 IU of vitamin A as retinyl acetate; 3,307 IU of vitamin D_3 as cholecalciferol; 6.16 mg of vitamin K as menadione dimethylpyrimidinol bisulfite; 11.02 mg of riboflavin; 66.14 mg of niacin; 44.09 mg of pantothenic acid as D-calcium pantothenate; 0.07 mg of vitamin B_{12}; 0.60 mg of iodine as ethylenediamine dihydroiodide; 0.60 mg of selenium as sodium selenite; 110.23 mg of zinc as zinc oxide (SQM™); 66.14 mg of iron as ferrous sulfate (SQM™); 7.72 mg of copper as copper sulfate (SQM™); and 11.02 mg of manganese as manganese oxide (SQM™).

[5]Vitamin E was supplied as 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]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 5-4. Composition and nutrient analysis of Phase 4 diets (27 - 45 kg BW, 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	69.16	69.14	68.91	47.41	47.39	47.16
Soybean meal (46.5%)	28.47	28.47	28.47	20.42	20.42	20.42
DDGS	–	–	–	30.00	30.00	30.00
Limestone	0.86	0.86	0.86	1.24	1.24	1.24
Monocalcium phosphate	0.81	0.81	0.81	0.10	0.10	0.10
NaCl	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine HCl	0.10	0.10	0.10	0.23	0.23	0.23
Vitamin/ trace mineral premix[4]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin E[5]	-	0.025	0.25	-	0.025	0.25
Calculated nutrient composition:						
ME[6], kcal/kg	3,317	3,317	3,309	3,329	3,328	3,321
CP, %	19.06	19.05	19.04	21.59	21.59	21.57
P, %	0.56	0.56	0.55	0.58	0.58	0.58
Available P, %	0.24	0.24	0.24	0.24	0.24	0.24
Ca, %	0.58	0.58	0.58	0.58	0.58	0.58
SID Lys, %	0.97	0.97	0.97	0.97	0.97	0.97
SID Met + Cys, %	0.56	0.56	0.56	0.63	0.63	0.63
SID Thr, %	0.61	0.61	0.61	0.63	0.63	0.63
SID Trp, %	0.20	0.20	0.20	0.18	0.18	0.18
α-tocopherol, IU/kg	0.00	11.02	110.20	0.00	11.02	110.20
Analyzed nutrient composition[7]:						
CP, %	16.5	–	–	19.3	–	–
Crude fat, %	2.61	–	–	3.79	–	–
Crude fiber, %	2.2	–	–	3.3	–	–
Lys, %	1.14	–	–	1.12	–	–
Met, %	0.28	–	–	0.35	–	–
Thr, %	0.70	–	–	0.77	–	–
Trp, %	0.22	–	–	0.20	–	–
Ca, %	0.72	–	–	0.72	–	–
P, %	0.55	–	–	0.54	–	–
α-tocopherol, IU/kg	<10	12	105	<10	15	124

[1]CON = corn-soybean meal based control diet.
[2]DDGS = 30% inclusion of dried distillers grains with solubles
[3]No-E = No supplemental vitamin E; 1X-E = NRC (1998) level of vitamin E supplied as α-tocopheryl acetate, which is 11.0 IU/kg for BW = 36.3 kg; 10X-E = 10X NRC (1998)

level of vitamin E supplied as α-tocopheryl acetate, which is 110 IU/kg for BW = 36.3 kg.

[4]Vitamin-trace mineral premix (without vitamin E) provided the following nutrients per kilogram of diet: 8,818.34 IU of vitamin A as retinyl acetate; 1,653.44 IU of vitamin D_3 as cholecalciferol; 3.31 mg of vitamin K as menadione dimethylpyrimidinol bisulfite; 5.51 mg of riboflavin; 33.07 mg of niacin; 22.05 mg of pantothenic acid as D-calcium pantothenate; 0.03 mg of vitamin B_{12}; 0.30 mg of iodine as ethylenediamine dihydroiodide; 0.30 mg of selenium as sodium selenite; 55.11 mg of zinc as zinc oxide (SQM^{TM}); 33.07 mg of iron as ferrous sulfate (SQM^{TM}); 3.86 mg of copper as copper sulfate (SQM^{TM}); and 5.51 mg of manganese as manganese oxide (SQM^{TM}).

[5]Vitamin E was supplied as 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]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 5-5. Composition and nutrient analysis of Phase 5 diets (45 - 68 kg BW, 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	75.20	75.18	74.95	51.91	51.89	51.66
Soybean meal (46.5%)	22.59	22.59	22.59	16.15	16.15	16.15
DDGS	–	–	–	30.00	30.00	30.00
Limestone	0.79	0.79	0.79	1.16	1.16	1.16
Monocalcium phosphate	0.72	0.72	0.72	-	-	-
NaCl	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine HCl	0.10	0.10	0.10	0.18	0.18	0.18
Vitamin/ trace mineral premix[4]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin E[5]	-	0.025	0.25	-	0.025	0.250
Calculated nutrient composition:						
ME[6], kcal/kg	3,327	3,326	3,319	3,340	3,339	3,332
CP, %	16.82	16.82	16.80	19.94	19.94	19.92
P, %	0.51	0.51	0.51	0.54	0.54	0.54
Available P, %	0.22	0.22	0.22	0.22	0.22	0.22
Ca, %	0.52	0.52	0.52	0.52	0.52	0.52
SID Lys, %	0.83	0.83	0.83	0.83	0.83	0.83
SID Met + Cys, %	0.51	0.51	0.51	0.59	0.59	0.59
SID Thr, %	0.53	0.53	0.53	0.57	0.57	0.57
SID Trp, %	0.17	0.17	0.17	0.15	0.15	0.15
α-tocopherol, IU/kg	0.00	11.02	110.20	0.00	11.02	110.20
Analyzed nutrient composition[7]:						
CP, %	12.1	–	–	17.9	–	–
Crude fat, %	2.84	–	–	3.91	–	–
Crude fiber, %	2.0	–	–	3.6	–	–
Lys, %	0.97	–	–	0.96	–	–
Met, %	0.25	–	–	0.33	–	–
Thr, %	0.61	–	–	0.71	–	–
Trp, %	0.18	–	–	0.18	–	–
Ca, %	0.61	–	–	0.65	–	–
P, %	0.52	–	–	0.56	–	–
α-tocopherol, IU/kg	<10	12	126	<10	12	106

[1]CON = corn-soybean meal based control diet.
[2]DDGS = 30% inclusion of dried distillers grains with solubles
[3]No-E = No supplemental vitamin E; 1X-E = NRC (1998) level of vitamin E supplied as α-tocopheryl acetate, which is 11.0 IU/kg for BW = 56.7 kg; 10X-E = 10X NRC (1998)

level of vitamin E supplied as α-tocopheryl acetate, which is 110 IU/kg for BW = 56.7 kg.

[4]Vitamin-trace mineral premix (without vitamin E) provided the following nutrients per kilogram of diet: 8,818.34 IU of vitamin A as retinyl acetate; 1,653.44 IU of vitamin D_3 as cholecalciferol; 3.31 mg of vitamin K as menadione dimethylpyrimidinol bisulfite; 5.51 mg of riboflavin; 33.07 mg of niacin; 22.05 mg of pantothenic acid as D-calcium pantothenate; 0.03 mg of vitamin B_{12}; 0.30 mg of iodine as ethylenediamine dihydroiodide; 0.30 mg of selenium as sodium selenite; 55.11 mg of zinc as zinc oxide (SQM^{TM}); 33.07 mg of iron as ferrous sulfate (SQM^{TM}); 3.86 mg of copper as copper sulfate (SQM^{TM}); and 5.51 mg of manganese as manganese oxide (SQM^{TM}).

[5]Vitamin E was supplied as 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]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 5-6. Composition and nutrient analysis of Phase 6 diets (68 - 91 kg BW, 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	80.20	80.18	79.95	55.98	55.96	55.73
Soybean meal (46.5%)	17.59	17.59	17.59	12.11	12.11	12.11
DDGS	–	–	–	30.00	30.00	30.00
Limestone	0.79	0.79	0.79	1.16	1.16	1.16
Monocalcium phosphate	0.72	0.72	0.72	-	-	-
NaCl	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine HCl	0.10	0.10	0.10	0.15	0.15	0.15
Vitamin/ trace mineral premix[4]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin E[5]	-	0.025	0.25	-	0.025	0.250
Calculated nutrient composition:						
ME[6], kcal/kg	3,331	3,330	3,323	3,344	3,343	3,336
CP, %	14.91	14.91	14.89	18.38	18.38	18.36
P, %	0.49	0.49	0.49	0.53	0.53	0.53
Available P, %	0.21	0.21	0.21	0.21	0.21	0.21
Ca, %	0.51	0.51	0.51	0.51	0.51	0.51
SID Lys, %	0.70	0.70	0.70	0.70	0.70	0.70
SID Met + Cys, %	0.47	0.47	0.47	0.56	0.56	0.56
SID Thr, %	0.46	0.46	0.46	0.52	0.52	0.52
SID Trp, %	0.14	0.14	0.14	0.13	0.13	0.13
α-tocopherol, IU/kg	0.00	11.02	110.20	0.00	11.02	110.20
Analyzed nutrient composition[7]:						
CP, %	12.1	–	–	16.6	–	–
Crude fat, %	2.51	–	–	4.22	–	–
Crude fiber, %	2.0	–	–	3.2	–	–
Lys, %	0.83	–	–	0.82	–	–
Met, %	0.23	–	–	0.31	–	–
Thr, %	0.54	–	–	0.64	–	–
Trp, %	0.15	–	–	0.15	–	–
Ca, %	0.61	–	–	0.56	–	–
P, %	0.53	–	–	0.51	–	–
α-tocopherol, IU/kg	<10	12	107	<10	16	112

[1]CON = corn-soybean meal based control diet.
[2]DDGS = 30% inclusion of dried distillers grains with solubles
[3]No-E = No supplemental vitamin E; 1X-E = NRC (1998) level of vitamin E supplied as α-tocopheryl acetate, which is 11.0 IU/kg for BW = 79.4 kg; 10X-E = 10X NRC (1998)

level of vitamin E supplied as α-tocopheryl acetate, which is 110 IU/kg for BW = 79.4 kg.

[4]Vitamin-trace mineral premix (without vitamin E) provided the following nutrients per kilogram of diet: 8,818.34 IU of vitamin A as retinyl acetate; 1,653.44 IU of vitamin D_3 as cholecalciferol; 3.31 mg of vitamin K as menadione dimethylpyrimidinol bisulfite; 5.51 mg of riboflavin; 33.07 mg of niacin; 22.05 mg of pantothenic acid as D-calcium pantothenate; 0.03 mg of vitamin B_{12}; 0.30 mg of iodine as ethylenediamine dihydroiodide; 0.30 mg of selenium as sodium selenite; 55.11 mg of zinc as zinc oxide (SQM^{TM}); 33.07 mg of iron as ferrous sulfate (SQM^{TM}); 3.86 mg of copper as copper sulfate (SQM^{TM}); and 5.51 mg of manganese as manganese oxide (SQM^{TM}).

[5]Vitamin E was supplied as 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]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 5-7. Composition and nutrient analysis of Phase 7 diets (91 - 113 kg BW, 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	85.35	85.33	85.10	60.21	60.19	59.96
Soybean meal (46.5%)	12.43	12.43	12.43	7.91	7.91	7.91
DDGS	–	–	–	30.00	30.00	30.00
Limestone	0.77	0.77	0.77	1.14	1.14	1.14
Monocalcium phosphate	0.73	0.73	0.73	–	–	–
NaCl	0.35	0.35	0.35	0.35	0.35	0.35
L-Lysine HCl	0.12	0.12	0.12	0.14	0.14	0.14
Vitamin/ trace mineral premix[4]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin E[5]	–	0.025	0.25	–	0.025	0.250
Analyzed nutrient composition:						
ME[6], kcal/kg	3,335	3,334	3,326	3,348	3,348	3,340
CP, %	12.96	12.96	12.94	16.77	16.77	16.75
P, %	0.48	0.48	0.48	0.51	0.51	0.51
Available P, %	0.21	0.21	0.21	0.21	0.21	0.21
Ca, %	0.49	0.49	0.49	0.49	0.49	0.49
SID Lys, %	0.59	0.59	0.59	0.59	0.59	0.59
SID Met + Cys, %	0.42	0.42	0.42	0.52	0.52	0.52
SID Thr, %	0.40	0.40	0.40	0.46	0.46	0.46
SID Trp, %	0.11	0.11	0.11	0.11	0.11	0.11
α-tocopherol, IU/kg	0.00	11.02	110.20	0.00	11.02	110.20
Analyzed nutrient composition[7]:						
CP, %	11.2	–	–	15.5	–	–
Crude fat, %	2.48	–	–	4.20	–	–
Crude fiber, %	2.2	–	–	3.0	–	–
Lys, %	0.70	–	–	0.69	–	–
Met, %	0.21	–	–	0.29	–	–
Thr, %	0.46	–	–	0.57	–	–
Trp, %	0.13	–	–	0.13	–	–
Ca, %	0.55	–	–	0.54	–	–
P, %	0.50	–	–	0.50	–	–
α-tocopherol, IU/kg	<10	14	102	<10	11	109

[1]CON = corn-soybean meal based control diet.
[2]DDGS = 30% inclusion of dried distillers grains with solubles
[3]No-E = No supplemental vitamin E; 1X-E = NRC (1998) level of vitamin E supplied as α-tocopheryl acetate, which is 11.0 IU/kg for BW = 102.1 kg; 10X-E = 10X NRC (1998)

level of vitamin E supplied as α-tocopheryl acetate, which is 110 IU/kg for BW = 102.1 kg.

[4]Vitamin-trace mineral premix (without vitamin E) provided the following nutrients per kilogram of diet: 8,818.34 IU of vitamin A as retinyl acetate; 1,653.44 IU of vitamin D_3 as cholecalciferol; 3.31 mg of vitamin K as menadione dimethylpyrimidinol bisulfite; 5.51 mg of riboflavin; 33.07 mg of niacin; 22.05 mg of pantothenic acid as D-calcium pantothenate; 0.03 mg of vitamin B_{12}; 0.30 mg of iodine as ethylenediamine dihydroiodide; 0.30 mg of selenium as sodium selenite; 55.11 mg of zinc as zinc oxide (SQM^{TM}); 33.07 mg of iron as ferrous sulfate (SQM^{TM}); 3.86 mg of copper as copper sulfate (SQM^{TM}); and 5.51 mg of manganese as manganese oxide (SQM^{TM}).

[5]Vitamin E was supplied as 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]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 5-8. Effects of dietary dried distillers grains with solubles (DDGS) and increasing levels of vitamin E on growth performance[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
Phase 1:										
ADG, kg	0.13	0.12	0.12	0.11	0.12	0.11	0.02	0.98	0.55	0.94
ADFI, kg	0.19	0.17	0.18	0.17	0.18	0.18	0.05	1.00	0.94	1.00
G:F	0.70	0.69	0.68	0.63	0.68	0.63	0.01	0.05	<0.001	<0.001
Phase 2:										
ADG, kg	0.49	0.47	0.48	0.47	0.49	0.48	0.02	0.98	0.90	0.91
ADFI, kg	0.76	0.69	0.72	0.72	0.71	0.73	0.05	0.77	0.88	0.96
G:F	0.65	0.68	0.66	0.66	0.69	0.67	0.01	0.04	0.47	0.23
Phase 3:										
ADG, kg	0.70	0.66	0.69	0.65	0.65	0.64	0.02	0.61	0.01	0.14
ADFI, kg	1.23	1.16	1.20	1.19	1.24	1.23	0.05	0.98	0.57	0.89
G:F	0.57	0.57	0.57	0.55	0.52	0.52	0.01	0.67	<0.001	0.005
Phase 4:										
ADG, kg	0.94	0.91	0.92	0.86	0.87	0.87	0.02	0.85	<0.001	0.01
ADFI, kg	2.06	1.87	1.95	1.99	1.90	1.91	0.05	0.02	0.54	0.13
G:F	0.46	0.49	0.47	0.44	0.46	0.46	0.01	0.09	0.05	0.12
Phase 5:										
ADG, kg	1.00	1.01	1.00	0.94	0.95	0.97	0.02	0.73	<0.001	0.02
ADFI, kg	2.57	2.52	2.50	2.69	2.64	2.49	0.05	0.04	0.08	0.04
G:F	0.39	0.40	0.40	0.35	0.36	0.39	0.01	0.18	0.006	0.03
Phase 6:										
ADG, kg	0.96	0.95	0.93	0.90	0.94	0.93	0.02	0.62	0.06	0.15
ADFI, kg	2.84	2.75	2.71	2.85	2.90	2.80	0.05	0.22	0.05	0.12
G:F	0.34	0.35	0.34	0.32	0.33	0.33	0.01	0.78	0.08	0.57
Phase 7:										
ADG, kg	0.78	0.80	0.80	0.80	0.80	0.80	0.02	0.90	0.59	0.97
ADFI, kg	3.00	2.91	2.86	3.01	3.00	3.04	0.05	0.46	0.03	0.11

G:F	0.26	0.27	0.28	0.27	0.27	0.26	0.01	0.80	0.57	0.90
Overall:										
Initial BW, kg	6.6	6.6	6.6	6.6	6.6	6.6	0.35	0.69	1.00	0.33
Final BW, kg	111.4	109.5	109.8	105.5	107.2	106.9	1.71	1.00	<0.001	0.21
ADG, kg	0.77	0.76	0.76	0.73	0.74	0.74	0.01	1.00	<0.001	0.21
ADFI, kg	1.99	1.90	1.91	1.99	1.96	1.91	0.04	0.10	0.23	0.66
G:F	0.39	0.40	0.40	0.37	0.38	0.38	0.13	0.03	<0.001	0.99

[1]Values are least square means of 8 replicate pens per dietary treatment.
[2]CON = corn-soybean meal 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; 10X-E = 10X NRC (1998) level of vitamin E. Vitamin E was supplied as α-tocopheryl acetate.

Table 5-9. Effects of dietary dried distillers grains with solubles (DDGS) and increasing levels of vitamin E on carcass and ultrasound measurements[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
No. of pens	8	8	8	8	8	8				
No. of pigs	71	72	68	71	69	70				
Live BW, kg	116.4	114.3	114.8	113.0	114.0	112.8	1.4	0.71	0.04	0.37
No. of pens	8	8	8	8	8	8				
No. of pigs	68	63	62	54	56	59				
HCW[5], kg	89.2	89.3	89.2	87.4	87.9	88.3	0.4	0.43	<0.001	0.36
Dressing, %	78.2	78.1	78.1	76.4	76.9	77.2	0.003	0.47	<0.001	0.31
Ultrasound backfat depth[5], cm	2.37	2.45	2.45	2.28	2.27	2.27	0.09	0.85	0.008	0.72
Ultrasound LM area[5], cm^2	39.4	38.5	38.1	36.4	36.1	35.8	0.6	0.26	<0.001	0.81
Fat-free carcass lean, %	48.5	47.6	47.5	47.9	47.8	47.7	0.004	0.26	0.83	0.49

[1]Values are least square means of 8 replicates per dietary treatment.
[2]CON = corn-soybean meal 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; 10X-E = 10X NRC (1998) level of vitamin E. Vitamin E was supplied as α-tocopheryl acetate.
[5]Values were adjusted by live BW as a covariate because live BW in the model was significant ($P < 0.05$). P-values did not change between significant ($P < 0.05$) and tend ($P < 0.10$) if not adjusted by live BW.

Table 5-10. Effects of dietary dried distillers grains with solubles (DDGS) and increasing levels of vitamin E on fatty acid profile of belly fat and backfat samples[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
Belly fat sample										
Fatty acid, % of total fat										
C14:0	1.44	1.39	1.39	1.35	1.38	1.35	0.04	0.77	0.11	0.51
C16:0	25.13	24.74	25.13	22.74	22.97	22.67	0.33	0.97	<0.001	0.51
C16:1	3.12	3.23	2.66	2.60	2.53	2.70	0.15	0.36	0.003	0.05
C18:0	11.88	11.21	13.10	10.40	10.53	10.25	0.37	0.08	<0.001	0.02
C18:1	46.32	48.37	46.80	43.29	43.28	43.27	0.46	0.08	<0.001	0.08
C18:2	8.48	7.25	7.20	15.27	15.03	15.36	0.63	0.46	<0.001	0.52
C18:3	0.44	0.41	0.41	0.59	0.57	0.57	0.02	0.48	<0.001	0.95
C20:0	0.20	0.18	0.22	0.18	0.20	0.19	0.01	0.23	0.18	0.03
C20:1	0.82	0.80	0.91	0.83	0.85	0.85	0.04	0.32	0.98	0.37
C20:3	0.04	0.05	0.06	0.08	0.08	0.08	0.01	0.31	<0.001	0.65
C20:4	0.22	0.20	0.20	0.28	0.27	0.29	0.02	0.35	<0.001	0.58
SFA	39.02	37.92	40.18	35.15	35.51	34.92	0.57	0.31	<0.001	0.04
MUFA	50.69	52.88	50.78	47.22	47.13	47.31	0.52	0.11	<0.001	0.05
PUFA	9.23	7.94	7.90	16.26	15.99	16.34	0.66	0.45	<0.001	0.54
IV[5]	59.27	58.90	57.02	68.31	67.82	68.52	0.95	0.53	<0.001	0.28
Backfat sample										
Fatty acid, % of total fat										
C14:0	1.38	1.35	1.35	1.26	1.29	1.27	0.04	0.98	0.01	0.72
C16:0	25.58	25.04	26.09	23.32	23.52	23.01	0.50	0.84	<0.001	0.25
C16:1	2.44	2.74	2.13	2.05	1.89	2.16	0.14	0.48	0.001	0.01
C18:0	13.92	12.64	15.83	12.13	12.56	11.44	0.64	0.24	<0.001	0.004
C18:1	42.33	44.28	42.54	39.62	38.55	39.83	0.69	0.80	<0.001	0.04
C18:2	9.98	9.36	8.12	16.87	17.42	16.93	1.05	0.36	<0.001	0.38
C18:3	0.52	0.50	0.45	0.64	0.64	0.66	0.03	0.61	<0.001	0.20

C20:0	0.23	0.20	0.27	0.20	0.22	0.20	0.01	0.19	0.01	0.003
C20:1	0.83	0.79	0.92	0.82	0.81	0.80	0.04	0.27	0.25	0.21
C20:3	0.07	0.07	0.08	0.08	0.08	0.09	0.00	0.08	<0.001	0.83
C20:4	0.20	0.19	0.18	0.27	0.26	0.28	0.02	0.87	<0.001	0.40
SFA	41.55	39.71	43.94	37.46	38.12	36.50	1.07	0.41	<0.001	0.02
MUFA	46.03	48.32	45.96	42.97	41.69	43.31	0.79	0.80	<0.001	0.03
PUFA	10.83	10.16	8.86	17.91	18.44	18.03	1.09	0.37	<0.001	0.37
IV[5]	58.58	59.65	54.66	67.69	67.79	68.93	1.68	0.44	<0.001	0.11

[1]Values are least square means of 8 replicates per dietary treatment.
[2]CON = corn-soybean meal 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; 10X-E = 10X NRC (1998) level of vitamin E. Vitamin E was supplied as α-tocopheryl acetate.
[5]IV = iodine value.

Table 5-11. Influence of dietary dried distillers grains with solubles (DDGS) and vitamin E supplementation on thiobarbituric acid-reactive substance (TBARS) and α-tocopherol concentration in LM[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, mg/kg	0.44	0.43	0.44	0.45	0.44	0.43	0.01	0.27	0.93	0.31
α-tocopherol, mg/kg	1.03	2.03	7.99	3.10	2.46	8.87	0.89	<0.001	0.005	0.02

[1]Values are least square means of 8 replicates per dietary treatment.
[2]CON = corn-soybean meal 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; 10X-E = 10X NRC (1998) level of vitamin E. Vitamin E was supplied as α-tocopheryl acetate.

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