EFFECTS OF DIETARY PEROXIDIZED LIPIDS ON THE GROWTH PERFORMANCE AND METABOLIC OXIDATIVE STATUS OF NURSERY PIGS

A DISSERTATION
SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF THE UNIVERSITY OF MINNESOTA
BY

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

Gerald C. Shurson, Adviser

June 2014

ACKNOWLEDGEMENTS

My Ph.D. research program has truly been a collaborative effort which would not have been possible without support and assistance from many dedicated people.

Firstly, I would like to thank my adviser, Dr. Jerry Shurson for his guidance, support, and encouragement. Without you, I would not have had the opportunity to study at Minnesota. Your unwavering confidence in me empowered my pursuit of a wide variety of successful endeavors. To the other members of my examining committee, Drs. Lee Johnston, Chi Chen, Sally Noll, and Dan Gallaher, thank you for all you have done to help me learn and grow, and develop as a student, a scientist, and a person.

Thank you to my graduate school colleagues and friends for the great conversations, challenges, and support on my experiments. I have learned so much from each and every one of you, and feel as though I have travelled around the world by learning about your diverse, multi-cultural backgrounds.

The experiments described in this dissertation are the results of the combined labor of numerous members of the swine group and beyond. I will do my best to list all who contributed, but I apologize if I have excluded anyone. Thanks to Erin Harris, Yijie He, Zhimin Huang, Zhaohui Luo, Hayford Manu, Woldedab Meried, Devi Pangeni, Ping Ren, Cassio Villela, Fangzhou Wu, Dr. Sakda Aunsiam, Dr. Sopon Wilaison, Dr. Feng Zhao, Dan Hedges, Xiaojing Li, Pai Liu, Christina Phillips, Jenna Davis, and Ran Song. I am eternally grateful for the advice, support, and guidance from Drs. Pedro Urriola and Mu Li.

Thanks to the support from the crew and staff and the Southern Research and Outreach Center and West Central Research and Outreach Center for helping care for the animals and prepare the diets used in my experiments. To Dr. Sam Baidoo, Dr. Brian Kerr, and Adrienne Hilbrands, thanks for your extensive time and efforts to help develop and implement my research.

Special appreciation goes to Drs. Jerry Torrison, Nubia Macedo, Christine Watson, and others at the Veterinary Diagnostic Laboratory for their advice and histological support on my experiments.

I would like to thank my friend, the consistently amazing statistical consultant, Nitipong Homowong, who constantly answered my questions and devoted countless hours to helping me understand the answers. To the others that patiently offered statistical guidance, including Drs. Roger Moon, Jon Anderson, William Thomas, Lee Johnston, and Josh Jendza, I express my upmost gratitude.

Thanks to Lei Wang, for patiently answering my questions, explaining laboratory procedures, and being willing to lend a hand when needed. I am also grateful to Dana Yao for her technical support.

Finally, thank you to all of my family. Without your constant love, support, and guidance, none of this would have been possible. To my partner, Shawn Mavis, I appreciate your endless companionship and willingness to listen.

FINANCIAL ACKNOWLEDGEMENTS

I would like to thank the following groups and individuals for their generous financial support and/or donation of product of this research:

- Minnesota Pork Board
- Minnesota Corn Growers Research and Promotion Council
- Agricultural Utilization Research Institute
- Kemin Industries, Des Moines Iowa
  - Dr. Fernando Valdez and other members of Kemin Animal Nutrition & Health group
- POET, LLC, Sioux Falls, SD
  - Mr. Matt Reiners
- CHS, Inc., Inver Grove Heights, MN
  - Mr. Todd Biedenfeld
- Novus Intl., St. Louis, MO
  - Ms. Vanessa Stewart and Dr. Brad Lawrence

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CHAPTER 1. LITERATURE REVIEW

INTRODUCTION

Energy is the most expensive component of swine diets. Corn or dried distillers grains with solubles (**DDGS**) commonly provide a substantial portion of dietary energy in U.S. swine diets, but sources of concentrated lipids (e.g. vegetable oils and animal fats) are used as well. Supplemental lipid sources have been readily available and used in the feed and animal industries for many years, and some sources like distiller's corn oil (**DCO**) and recycled restaurant grease may be purchased at discounted prices relative to other sources. Over 85% of U.S. ethanol plants currently extract DCO before manufacturing DDGS. In fact, more than 105,000 tonnes of DCO were produced by the ethanol industry in 2013 for use in the production of biodiesel and animal feeds (Renewable Fuels Association, 2014). Annual production of DCO may increase as ethanol companies adopt new oil extraction methods and technologies to improve oil yield.

Dietary lipids are more energy dense than carbohydrates (NRC, 2012). Therefore, lipids increase dietary energy density but they also provide several other benefits when added to animal feeds. For example, dietary lipids reduce feed intake due to increased caloric density, improve gain efficiency, improve palatability, reduce dust, and supply essential fatty acids and vitamins (Azain, 2001). However, lipids may become peroxidized depending on the extent and duration of exposure to heat, air, moisture, and pro-oxidant metals during processing and storage, but antioxidants can impede peroxidation (Belitz et al., 2009). Furthermore, the susceptibility of lipids to peroxidation increases with increasing degree of unsaturation (Belitz et al., 2009). For example, the

polyunsaturated fatty acids, linoleic and linolenic acid, are peroxidized at rates 12 and 25-times faster than the monounsaturated fatty acid, oleic acid (Belitz et al., 2009).

Swine diets in the United States commonly include sources of unsaturated lipids such as those found in corn, DDGS, and other concentrated lipid sources. The lipid content of corn is generally 3.5% (NRC, 2012). However, the crude fat content of DDGS varies from 3.6 to 10.4% (NRC, 2012). Linoleic acid (18:2) is the predominant fatty acid (53.5% of total lipid) present in corn oil, with linolenic acid (18:3) representing a minor proportion (1.2% of total lipid; NRC, 2012). Other vegetable oils such as soy and sunflower oil also contain relatively high (≥ 39%) concentrations of linoleic acid, but animal based lipid sources such as tallow, choice white grease, and poultry fat generally contain relatively low concentrations (≤ 20%; NRC, 2012). Therefore, vegetable oils are more susceptible to peroxidation than animal fats.

In addition, metals such as Fe, Cu, and Mn promote peroxidation of unsaturated fats (Flider and Orthoefer, 1981). These pro-oxidants may be present in feedstuffs, complete feeds, and vitamin-trace mineral premixes, or they may be introduced during storage and processing (NRC, 2012). In addition to metals and other contaminants, lipids may be exposed to other peroxidative conditions during storage and processing. For example, oil present in DDGS may be exposed to temperatures up to 500˚C during drying (Rosentrater et al., 2012). Animal fats are exposed to temperatures ranging from 115 to 145°C for 40 to 90 minutes during rendering (Meeker and Hamilton, 2006). Vegetable oils that are recycled from restaurants may be exposed to temperatures exceeding 180°C for variable durations (Belitz et al., 2009). Therefore, animal fats, vegetable oils, and other lipid rich

ingredients may be peroxidized to varying extents depending on the extent and duration of exposure to high temperatures (Dibner et al., 2011; Song and Shurson, 2013).

Peroxidation degrades fatty acids into numerous products (Spiteller et al., 2001; Seppanen and Csallany, 2002; Belitz et al., 2009), and degrades antioxidants such as vitamin E (Seppanen and Csallany, 2002; Liu, 2012). However, less is known about the degradation of other fat soluble antioxidants (e.g. vitamin A) during peroxidation. Several analytical assays exist to evaluate lipid peroxidation, but the practical applications of these peroxidation indicators on the productivity and health of swine is unclear.

In addition to dietary lipids, peroxidation occurs *in vivo*. At the metabolic level, lipids within cellular and subcellular membranes can peroxidize. Compounds produced during peroxidation can alter enzymes, protein, and DNA, potentially affecting functionality (Yu, 1994). Dietary inclusion of peroxidized lipids contributes to adverse health and metabolic conditions (Fitch Haumann, 1993) and affects the growth performance of livestock and poultry negatively (Robey and Shermer, 1994; Dibner et al., 2011). For example, feeding peroxidized lipids reduces gain efficiency (McGill et al., 2011a; McGill et al., 2011b; Tavárez et al., 2011), reduces growth rate (Boler et al., 2012; Liu, 2012), alters metabolic oxidative status (Boler et al., 2012; Liu, 2012), reduces energy digestibility (Inoue et al., 1984; Engberg et al., 1996), increases mortality (Takahashi and Akiba, 1999; Anjum et al., 2004), and reduces immune function (Dibner et al., 1996b) for swine and broilers. Therefore, understanding the peroxidation status of dietary lipids is of critical importance for animal nutritionists. Specific mechanism(s) underlying the negative effects of peroxidized lipids have not been elucidated. Furthermore, dietary thresholds of peroxidized lipids which elicit negative nutritional and physiological effects

have not been established. One of the primary challenges of understanding the connection between dietary peroxidized lipids and animal growth performance is the lack of a universally accepted standard assay that accurately characterizes the extent of peroxidation in all sources of lipid.

Numerous compounds act directly or indirectly to inhibit peroxidation in dietary or metabolic lipids. Vitamin A, carotenoids, flavonoids, phenolic acids, vitamin E, butylated hydroxytoluene (**BHT**), butlyated hydroxylanisole (**BHA**), ethoxyquin, propyl gallate, tertiary-butylhydroquinone (**TBHQ**), vitamin C, lignans, ferulic acid, citric acid, and numerous other additives or dietary components can function as antioxidants (Wanasundara and Shahidi, 2005). Additionally, nutrients such as Se, riboflavin, niacin, P, amino acids (e.g. Met, Cys, Tau, Glu, Gly, and Trp), Mn, Cu, Fe, and Zn have diverse, important roles in the metabolic antioxidant system. Ultimately, numerous compounds with antioxidant properties are present naturally in feed ingredients, are added as dietary supplements, or are synthesized endogenously.

Dietary antioxidants may ameliorate the negative effects of dietary peroxidized lipids on growth and efficiency of animals (Cabel et al., 1988; McGill et al., 2011b; Tavárez et al., 2011), but these effects have not been demonstrated consistently (Wang et al., 1997; McGill et al., 2011a; Boler et al., 2012). Therefore, the optimal strategy for antioxidant supplementation in diets containing peroxidized lipids has not been established.

The objectives of this dissertation are to evaluate the effects of dietary peroxidized lipids for swine on growth performance, health, and metabolic oxidative status of young pigs. Furthermore, the peroxidation of lipids in DDGS and corn oil were

assessed during heating and storage in the presence and absence of antioxidants. This information will provide feed formulators and animal nutritionists with practical guidelines for the use of peroxidized lipids and antioxidants in feed ingredients and diets for swine and poultry.

LIPID PEROXIDATION

Peroxidation Process

Lipid peroxidation (also known as oxidation or auto-oxidation) is a complex process which produces and degrades numerous compounds during 3 general phases, initiation, propagation, and termination (Belitz et al., 2009). First, free radical abstraction of hydrogen adjacent to a methylene group in a fatty acid forms water and a lipid free radical. Common initiators of peroxidation include the reactive oxygen species (ROS) listed in Table 1-1. Unstable lipid free radicals interact with divalent oxygen to form lipid peroxyl free radicals which can abstract hydrogen from reduced PUFA (Figure 1-1). This step generates lipid hydroperoxides and lipid free radicals which catalyze propagation of peroxidation (Figure 1-1). Subsequently, lipid hydroperoxides can decompose in the presence of Fe or Cu to generate hydroxyl radicals via Fenton and Haber-Weiss reactions (Figure 1-2), and thereby accelerate peroxidation (Gutteridge, 1995). Termination results when unstable products of propagation combine, and no longer contribute to the propagation step.

Lipid hydroperoxides are primary products of lipid peroxidation which subsequently react to form numerous products including aldehydes, ketones, acids, esters, hydrocarbons, epoxides, polymers, lactones, furans, and aromatic compounds (Belitz et al., 2009; Figure 1-3). At least 19 volatile compounds are formed by the peroxidation of

linoleic acid (Table 1-2), and each compound may decompose to numerous others (Belitz et al., 2009). For example, 2,4-decadienal (**DDE**) is an aldehyde produced during peroxidation, but upon exposure to air, DDE degrades to hexanal, 2-butenal, 5-oxodecanal, and 2-buten-1,4-dial (Spiteller et al., 2001). Furthermore, peroxidation products may combine to form cyclic dimers and polymers (Choe and Min, 2007).

In addition to peroxidation within ingredients or in complete diets, metabolic lipids may be peroxidized *in vivo*. Superoxides and peroxides are generated endogenously via the electron transport chain, immune system activation, phagocytosis, and some enzymatic reactions (Yu, 1994). Furthermore, dietary ROS can be absorbed by animals (Aw, 2005). Metabolically, endogenous and exogenous products of peroxidation may further promote peroxidative damage to physiological lipids within cellular and subcellular membranes, and can also modify enzymes, protein, and DNA (Yu, 1994). For example, aldehydes produced during peroxidation such as malondialdehyde (**MDA**) and hydroxynonenal (**HNE**) are cytotoxic and mutagenic (Esterbauer et al., 1991). Consequently, their presence *in vivo* is of importance. While HNE is more reactive than MDA, both compounds form adducts with glutathione (**GSH**), Cys, thiol containing proteins (e. g., albumin), and deoxyguanosine (Esterbauer et al., 1991).

Ultimately, understanding the levels of peroxidation in dietary lipids is of importance because peroxidation can have negative physiological implications for animals. However, variable concentrations of numerous products produced and degraded during lipid peroxidation create a challenge for quantifying and comparing the extent of peroxidation in dietary lipids **(**Figure 1-4).

Table 1-1. Reactive oxygen species (adapted from Jacob, 1995)

Species	Common name
HO•	hydroxyl radical
HO_2•	hydroperoxyl radical
O_2^-•	superoxide anion radical
$^1O_2$	singlet oxygen
RO•	alkoxyl radical
ROO•	peroxyl radical
NO•	nitric oxide radical

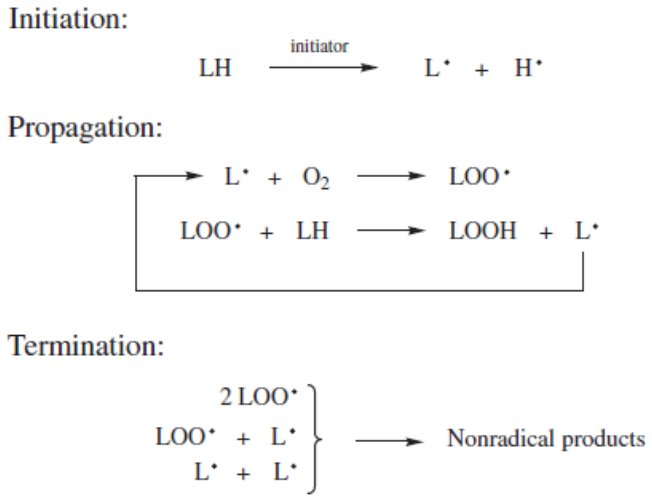

Figure 1-1. Free radical induced lipid peroxidation (adapted from Shahidi and Zhong, 2005).

Fenton: $H_2O_2 \xrightarrow{Fe^{2+}} HO\bullet + OH^- + Fe^{3+}$

Haber-Weiss: $O_2^-\bullet + H_2O_2 \xrightarrow{Fe^{2+}} HO\bullet + OH^- + O_2$

Figure 1-2. Metal catalyzed formation of free radicals (adapted from Yu, 1994).

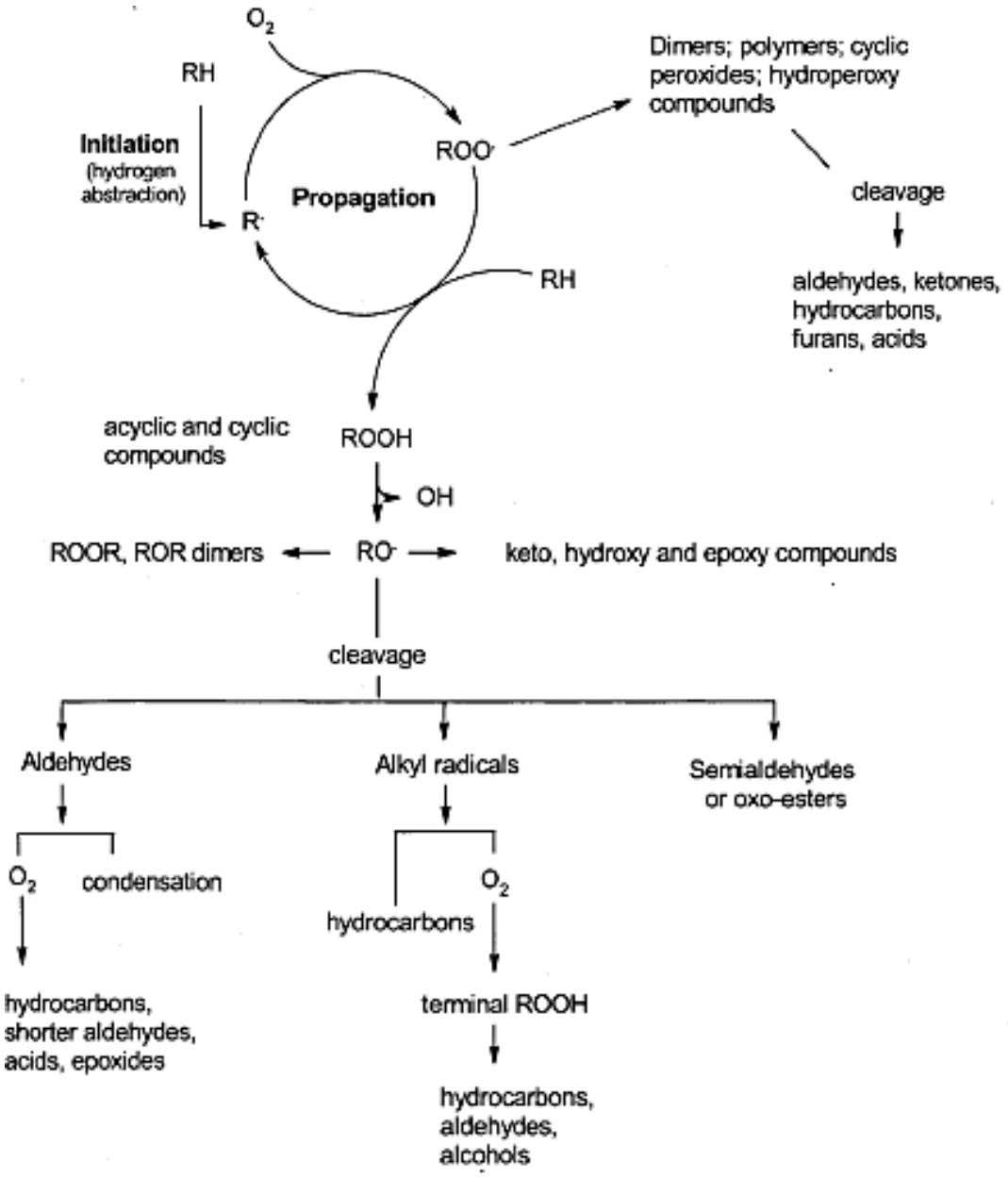

Figure 1-3. General schematic of lipid peroxidation (adapted from Seppanen, 2005).

Table 1-2. Volatile compounds formed by autoxidation of linoleic acid[a] (adapted from Belitz et al., 2009)

	µg/g
Pentane	[b,c]
Pentanal	55
Hexanal	5,100
Heptanal	50
2-trans-Heptenal	450
Octanal	45
1-Octen-3-one	2
1-Octen-3-hydroperoxide	[c]
2-cis-Octenal	990
2-trans-Octenal	420
3-cis-Nonenal	30
3-trans-Nonenal	30
2-cis-Nonenal	[c]
2-trans-Nonenal	30
2-cis-Decenal	20
2-trans, 4-trans-Nonadienal	30
2-trans, 4-cis-Decadienal	250
2-trans, 4-trans-Decadienal	150
Trans-4,5-Epoxy-2-trans-Decenal	[c]

[a]1 g linoleic acid was autoxidized at 20°C by an uptake of 0.5 mol oxygen/mol
[b]Major compound of autoxidation.
[c]Detected, but not quantified.

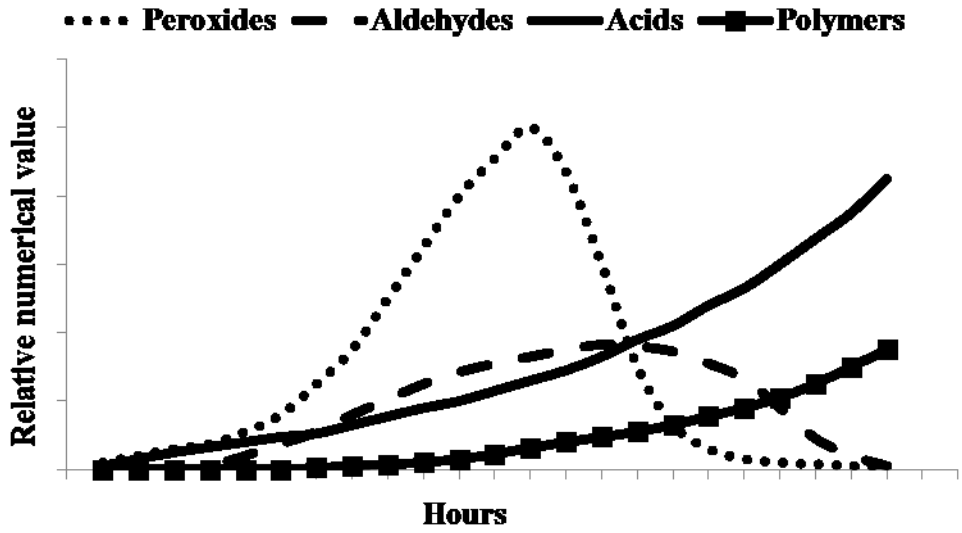

Figure 1-4. Theoretical relative production and degradation of several products of peroxidation (adapted from Fitch Haumann, 1993).

MEASUREMENT OF DIETARY LIPID PEROXIDATION

A universally acceptable, comprehensive measure of lipid peroxidation does not exist because of the fluctuating concentrations of numerous compounds produced throughout the peroxidation process. Furthermore, the amount and rate of production of various peroxidation products may depend on the unique fatty acid profiles of various dietary lipids. Therefore, multiple measures must be used to comprehensively describe the peroxidation status of a lipid (Shahidi and Zhong, 2005; NRC, 2012). Several analytical tests used routinely to assay lipid peroxidation are generally classified into two categories: indicative tests and predictive tests.

Indicative Tests

Products of peroxidation are measured at a specific point in time to indicate the extent of peroxidation that has occurred. Examples of commonly utilized indicators include: fatty acid profile, *p*-anisidine value, peroxide value, conjugated dienes,

thiobarbituric acid reactive substances, and hexanal value. Alternatively, specific products of peroxidation such as DDE and HNE can be measured.

Generally, peroxidation causes the unsaturated fatty acid content, as measured by iodine value (**IV**), to decline (Figure 1-5**;** Johnson and Kummerow, 1957) as PUFA are degraded, suggesting that IV can indicate extent of peroxidation. Furthermore, the weight of lipid samples increases during peroxidation as oxygen incorporates into lipid hydroperoxides (Wanasundara and Shahidi, 1994). Additionally, the concentration of free fatty acids increases in lipids after exposure to heat (Sánchez-Muniz et al., 1993; Liu, 2012). However, these methods have limited usefulness in practical situations because they require compositional knowledge of the original (unperoxidized) lipid to ascertain the magnitude of change elicited by peroxidation. Furthermore, these general measurements are not specific and require additional assays to identify specific products of peroxidation.

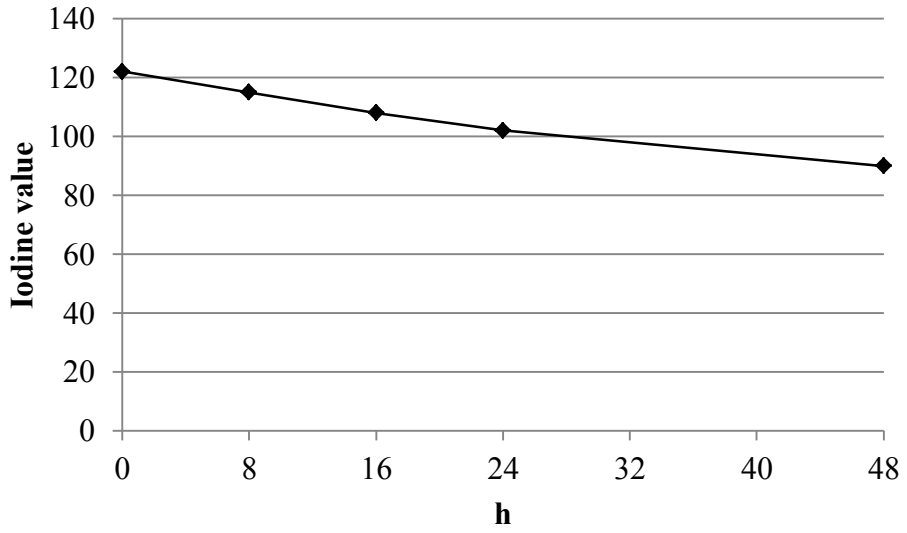

Figure 1-5. Iodine value declines over time in corn oil heated at 200^{o}C (adapted from Johnson and Kummerow, 1957).

Peroxide value (PV)

The PV quantifies the concentration of peroxides and hydroperoxides in a lipid using an iodometric procedure with titration of sodium thiosulfate (Cd 8-53; AOCS, 1992). This procedure has been criticized for being too subjective because the end point is determined by the analyst (Shermer and Giesen, 1997). Consequently, more objective PV assays using colorimetric procedures, high performance liquid chromatography, infrared spectroscopy, or gas chromatography have been developed (Shahidi and Wanasundara, 2008). Regardless of methodology, the utility of PV is limited when assessing the extent of lipid peroxidation because peroxides are simultaneously created and degraded during peroxidation. For example, DeRouchey et al. (2004) found that PV of choice white grease increased to 105 meq O_2/kg and later decreased to 1 meq O_2/kg

after exposure to 80°C for 11 d. Similarly, the PV of rapeseed oil, soybean oil, and hardened frying fat increased initially and later declined to baseline levels during 24 h exposure to 150°C (Danowska-Oziewicz and Karpińska-Tymoszczyk, 2005). These results indicate that low PV can be misleading because it may represent minimal peroxidation or that extensive peroxidation has occurred and peroxides have been degraded. Therefore, the practical utility of PV is limited without knowledge of historical values for a lipid. Furthermore, Shahidi and Zhong (2005) suggested that peroxides may be undetectable in lipids exposed to temperatures exceeding 150°C, indicating that PV is of limited utility for lipids subjected to extreme temperatures (e.g. recycled restaurant grease).

Conjugated dienes

Conjugated dienes form after a double bond rearrangement on lipid peroxides, and these primary peroxidation products have intense ultraviolet absorption at 234 nm (Shahidi and Wanasundara, 2008). This assay is relatively quick, simple, and requires no reagent (Shahidi and Wanasundara, 2008). Strong, positive correlations ($r > 0.95$) have been reported for conjugated dienes and PV (Shahidi et al., 1994; Wanasundara et al., 1995). However, caution should be exercised while interpreting results of the conjugated dienes assay because carotenoids also exhibit absorption in the range of 234-236 nm. Furthermore, like peroxides, conjugated dienes may decompose. For example, conjugated dienes increased initially followed by a decline to baseline levels in rapeseed oil, soybean oil, and hardened frying fat heated at 150°C over 24 h (Danowska-Oziewicz and Karpińska-Tymoszczyk, 2005).

p-Anisidine value (AnV)

The AnV assay measures aldehyde content of lipids by using a spectrophotometric procedure. Briefly, *p*-anisidine reacts with aldehydes under acidic conditions to produce a yellowish color (Cd 18-90; AOCS, 2011). However, various 2-alkenals and 2,4-alkedienals react with anisidine under acidic conditions, so this assay is not specific to a particular aldehyde (Shahidi and Wanasundara, 2008). Furthermore, the practical utility of this assay is questionable because aldehydes are created initially and subsequently degraded during peroxidation. For example, DeRouchey et al. (2004) showed the AnV of choice white grease followed a bell-shaped curve over an 11 d exposure to 80°C. Therefore, like PV, low AnV values may be misleading when evaluating the extent of peroxidation, and the usefulness of AnV is limited without knowledge of historical AnV values for a lipid.

2-Thiobarbituric acid reactive substances (TBARS)

The TBARS assay is used commonly to measure MDA, a secondary product of lipid peroxidation (Gray, 1978). In this assay, one mole of MDA reacts with 2 moles of thiobarbituric acid (TBA) under acidic conditions to form an adduct with a pink-red color and an absorption maximum of 530 to 532 nm (Shahidi and Wanasundara, 2008). However, 2-alkenals and 2,4-alkedienals, and other products of peroxidation react with TBA, so this assay is not specific to MDA. Furthermore, inter-laboratory comparisons of TBARS concentrations is challenging because several methodological variants are employed (Pegg, 2001). Like other indicative measures, TBARS follows a bell-shaped curve of production followed by degradation (Liu, 2012; Figure 1-6). As a result, changes

in TBARS concentrations can lead to misinterpretation of low values without knowledge of historical values for the lipid.

TOTOX value

To encompass primary and secondary products of lipid peroxidation, the TOTOX value is calculated as the sum of AnV and two times the PV (Shahidi and Wanasundara, 2008), but TBARS can replace AnV in this equation (Wanasundara and Shahidi, 1995). The TOTOX value compounds the lack of specificity inherent to the constituent assays. However, List et al. (1974) reported good correlation betweent the TOTOX value ($r = -0.80$, $P < 0.05$) and oil flavor.

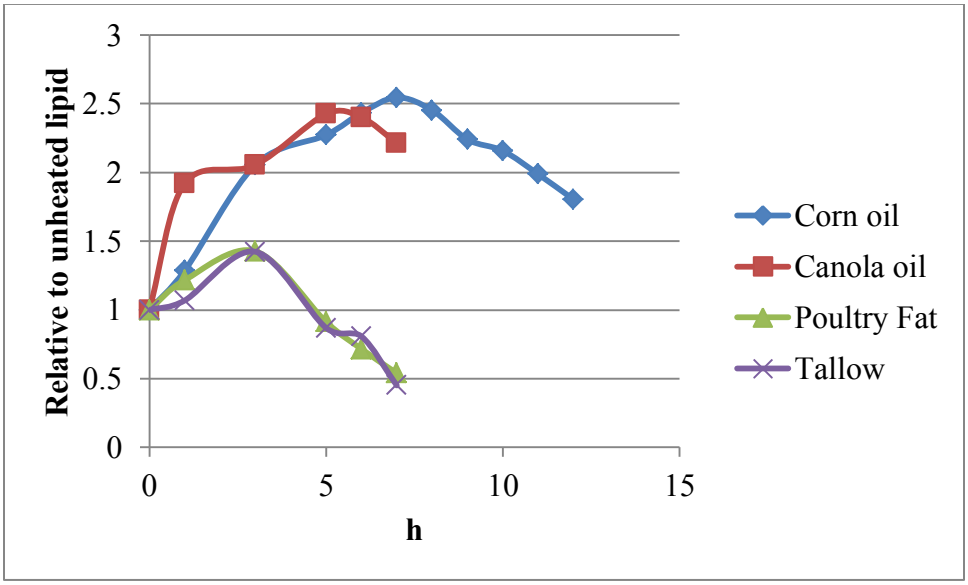

Figure 1-6. Thiobarbituric acid reactive substances increase and later decline in lipids during exposure to 185°C with 12L/min forced air (unpublished data from Liu, 2012).

Total Carbonyls

Secondary products of peroxidation such as aldehydes and ketones can be measured with the carbonyl assay (Shahidi and Zhong, 2005). This assay is a colorimetric procedure which measures colored products formed when the carbonyl compounds react with 2,4-dinitrophenlyhydrazine in alkaline solution. However, total carbonyl content increased initially followed by a later decline in rapeseed oil, soybean oil, and hardened frying fat heated at 150°C over 24 h (Danowska-Oziewicz and Karpińska-Tymoszczyk, 2005). Therefore, evaluation of a carbonyl value at a single time point may be misleading. However, the total carbonyl assay has been criticized for its lack of specificity and tendency to be influenced by compounds other than carbonyls (Shahidi and Zhong, 2005). As an alternative to total carbonyls, concentrations of individual carbonyl compounds can be measured to enhance specificity.

Hexanal and other specific carbonyl compounds

Hexanal is a carbonyl compound formed during the termination phase when linoleic acid or other ω-6 fatty acids are peroxidized (Shahidi and Wanasundara, 2008). Similar to other compounds, hexanal can degrade during peroxidation, making this indicator relatively difficult to interpret. Other specific carbonyl compounds such as propanal, pentanal, HNE, and DDE can also be used to evaluate lipid peroxidation. However, HNE and other carbonyls are produced and later degraded during peroxidation (Seppanen and Csallany, 2002).When selecting an assay, the fatty acid profile of the lipid should be considered because it may affect the relative abundance of specific carbonyls. For example, propanal production exceeds that of hexanal in fish oil during storage, so

the propanal assay is recommended for lipids with high levels of ω-3 fatty acids (Shahidi and Spurvey, 1996). However, hexanal may be more appropriate when evaluating ω-6 fatty acids such as those in corn oil (Shahidi and Wanasundara, 2008).

Triacylglycerol dimers and polymers

As previously described, the PV, TBARS, and AnV assays can provide misleading results because highly peroxidized lipids can have relatively low detectable levels of peroxidation products making their individual usage unreliable to indicate the extent of peroxidation. Alternatively, the formation of dimers and polymers in peroxidized lipids increases with increasing duration of heat (Sánchez-Muniz et al., 1993; Takeoka et al., 1997). These polymeric compounds can be measured with size exclusion chromatography (Sánchez-Muniz et al., 1993) .

Oxirane value

Oxiranes are cyclic compounds produced during lipid peroxidation, and they can be estimated using a colorimetric procedure involving hydrobromic acid (HBr) titration under acidic conditions (Gray, 1978). However, HBr will also react with carbonyls and conjugated dienals, indicating that this reaction is not specific to oxiranes (Shahidi and Wanasundara, 2008).

Non-elutable material

Wiseman (2003) suggested that estimating the total non-elutable material of lipid with quantitative gas-liquid chromatography (Waltking et al., 1975; Edmunds, 1990) collectively measures heat damaged products in a lipid. This procedure incorporates a

correction for glycerol because it appears in the non-elutable material fraction, but is not associated with damaged lipid products. This method may indicate that the lipid has been heated excessively.

Predictive Tests

Predictive tests evaluate the ability of a lipid to withstand peroxidation when exposed to standardized, accelerated conditions to induce peroxidation. Routinely used predictive tests include the active oxygen method (AOM), oil stability index (OSI), and oxygen bomb method (OBM).

Active oxygen method

The AOM, formerly known as the Swift Stability Test (Gearhart et al., 1957), induces peroxidation by passing air through a lipid held at 98 to 100°C. The PV is either assessed periodically until the PV reaches 100 meq O_2/kg, or the PV is evaluated at a set time point such as after 20 h of heating (AOCS, Cd 12-57). However, the time expended to conduct the AOM, particularly for relatively stable lipids, motivated some investigators to modify the procedure. For example, increasing airflow rate by 10% may change the PV determined at 20 h PV by 50% (Shermer and Giesen, 1997). Such modifications may limit validity of inter-laboratory comparisons (Jebe et al., 1993). In fact, some researchers have suggested that this method is outdated (Shahidi and Zhong, 2005), and encourage the use of alternative approaches to estimate lipid stability.

Oil stability index (OSI)

The oil stability index test induces peroxidation by bubbling air through a sample held at a constant, elevated temperature (range = 100 to 140°C). The analyst must select and report the temperature used in the assay. Air passes through the sample and bubbles through a reservoir of deionized water. As a result, volatile acids produced by lipid peroxidation accumulate in water and increase its conductivity (12b-92; AOCS, 2009). The conductivity of the water is monitored, and the OSI value is defined as the time at which conductivity changes exponentially (the induction time). The induction time directly relates to stability, and therefore, OSI values increase with greater stability (Shermer and Giesen, 1997; Shahidi and Zhong, 2005). The OSI may be more efficient than the AOM because multiple samples can be assayed simultaneously with the use of specialized equipment, and good correlation has been reported between AOM and OSI (Läubli and Bruttel, 1986). Furthermore, results from a collaborative study of 14 laboratories revealed high inter-laboratory repeatability for OSI, whereas the repeatability of AOM has been criticized (Jebe et al., 1993).

Oxygen bomb method (OBM)

The oxygen bomb method is approved by the American Society for Testing and Materials to predict stability of both oils and fats in finished products. The change in oxygen pressure of the headspace is measured in a closed system (bomb) containing the sample when submerged in boiling water. As oxygen is incorporated into lipid peroxides, pressure declines. Slower rates of decline indicate greater relative stability. The OBM is unique because it can be conducted on samples without lipid extraction (Gearhart et al.,

1957). This characteristic is advantageous because investigators can account for the action of pro- or antioxidant compounds and reduce the expenditure of time and resources to extract lipids. Gearhart et al. (1957) found that the OBM correlates well (r = 0.89) with, and is more expedient than AOM. However, OBM may be time consuming when evaluating relatively stable samples, and some suggest a catalyst (e.g. Cu) may be needed in such instances (Pohle et al., 1963).

Which assay should be used?

Limited resources prohibit comprehensive evaluation of numerous peroxidation indicator tests, necessitating prioritization of one or a few measures. However, the factors which must be considered when deciding the most appropriate measure are not well established. Most likely, the choice of indicator assay depends on the fatty acid profile of the lipid because the initial fatty acid profile influences the relative production of peroxidation compounds. Additionally, the peroxidative conditions to which lipids are exposed (e.g. storage or processing temperature and duration) should be considered.

Recently, Liu (2012) investigated the effects of lipid composition and peroxidation conditions on the development of peroxidation products in corn oil, canola oil, poultry fat, or tallow when heated for 72 h at 95°C (slow peroxidation; SO) or heated 7 h at 185°C (rapid peroxidation; RO) with a constant forced airflow rate of 12L/min. Samples were obtained after peroxidation and analyzed for PV, AnV, TBARS, hexanal, DDE, HNE, PUFA, and free fatty acids (Table 1-3). Free fatty acids increased and PUFA content in all lipid sources decreased after heating. However the magnitude of change was different for each lipid source. For example, the PUFA content declined in both corn oil (9% decrease) and tallow (35% decrease) when exposed to RO conditions. The

substantial difference in magnitude of change may be related to the initial PUFA content which is relatively greater in corn oil compared with other lipid sources (NRC, 2012). Interestingly, PV increased substantially in lipids exposed to SO conditions, but levels increased to a lesser extent under RO conditions. This finding may indicate that high temperatures (i.e. 185°C) expedite the catabolism of peroxides, as suggested by others (Shahidi and Zhong, 2005). The magnitude of change was also greater for SO relative to RO for concentrations of TBARS, hexanal, and DDE, possibly indicating the occurrence of degradation. However, fluctuations in PV, TBARS, hexanal, and DDE during heating were not monitored. Interestingly, the magnitude of differences under RO conditions compared to SO conditions varied for each lipid source. For example, the hexanal content of SO corn oil increased by 390-fold relative to fresh corn oil, while that of tallow exposed to similar conditions, increased by only 30-fold. This indicates that there is an effect of PUFA content on the products of peroxidation. The magnitude of change relative to fresh lipids was greater for RO compared to SO for AnV and HNE, but only in the vegetable oils. The opposite occurred for tallow or poultry fat. These findings indicate an interactive effect between lipid composition and peroxidation conditions on HNE and AnV. However, little research has been conducted to investigate this putative connection. In summary, these data indicate that measurements of lipid peroxidation respond differently depending on the fatty acid profile of the lipid, as well as the duration and magnitude of exposure to high temperatures.

Table 1-3. Indicative markers of lipid peroxidation measures in original lipids (OL) exposed to slow (SO) or rapid peroxidation (RO) conditions[1]

	Corn oil			Canola oil			Poultry fat			Tallow		
	OL	SO	RO	OL	SO	RO	OL	SO	RO	OL	SO	RO
PUFA	1.00	0.92	0.91	1.00	0.89	0.84	1.00	0.95	0.92	1.00	0.65	0.65
Free fatty acids	1.00	1.71	2.32	1.00	1.58	1.61	1.00	1.01	0.88	1.00	1.56	1.15
Peroxide value	1.00	151.00	2.00	1.00	239.00	12.00	1.00	57.00	2.00	1.00	29.00	3.00
Anisidine value	1.00	61.40	142.90	1.00	37.00	154.80	1.00	29.33	7.33	1.00	30.00	4.75
TBARS[2]	1.00	14.06	7.44	1.00	21.51	13.82	1.00	1.91	0.73	1.00	1.05	0.71
Hexanal	1.00	390.00	83.00	1.00	180.00	59.00	1.00	29.33	7.33	1.00	30.00	4.75
2,4-Decadienal	1.00	51.78	18.68	1.00	155.86	73.00	1.00	14.73	5.63	1.00	5.55	2.66
4-Hydroxynonenol	1.00	194.00	594.00	1.00	105.00	221.00	1.00	2.00	1.00	1.00	13.00	6.00

[1]OL = original unperoxidized lipid; SO = heated at 95°C for 72 h; RO = heated at 185°C for 7 h. Both treatments maintained constant forced air flow of 12L/min. Adapted from Liu (2012). Data are reported relative to OL for each lipid source.

[2]TBARS = thiobarbituric acid reactive substances.

ANTIOXIDANTS

The term “antioxidant” generally describes compounds that impede peroxidation or facilitate the removal of ROS (Wanasundara and Shahidi, 2005). Therefore, antioxidants are added routinely to feed ingredients, complete feeds, and foods to retard lipid peroxidation, but antioxidants are unable to reverse peroxidation (Sherwin, 1978). A wide variety of compounds exhibit antioxidant properties. Carotenoids, flavonoids, phenolic acids, vitamin E, butylated hydroxytoluene (BHT), butlyated hydroxylanisole (BHA), ethoxyquin, propyl gallate, tertiary-butylhydroquinone (TBHQ), vitamin C, lignans, and citric acid function as antioxidants (Wanasundara and Shahidi, 2005). Additionally, nutrients such as Se, riboflavin, niacin, P, amino acids (e.g. Met, Cys, Tau, Glu, Gly, and Trp), Mn, Cu, Fe, and Zn have supporting roles in the metabolic antioxidant system. Antioxidants may be naturally present in ingredients, added as dietary supplements, or synthesized endogenously to function in metabolic oxidative defense.

The dietary addition of antioxidants to foods and animal feeds is regulated by the U.S. Food and Drug Administration. Specifically, BHT, BHA, TBHQ, or propyl gallate can be added at a maximum level of 0.02% of lipid, and ethoxyquin is limited to 0.015% of the diet (AAFCO, 2013) . However, no limits exist for the inclusion of vitamin C, citric acid, tocopherols, and many other natural antioxidant compounds. Lack of regulation and concerns over the safety of synthetic antioxidants has increased interest in using natural antioxidant compounds instead of synthetic compounds (Pokorný, 2007). Various antioxidant compounds are present in wood smoke, cocoa, black pepper, mustard, rosemary, clove, sage, oregano, thyme, mace, allspice, soybeans, tea, peanuts,

rice, oats, onion, sweet potatoes, and other plants (Shahidi et al., 1992). However, a detailed discussion of the numerous antioxidant compounds present in natural sources exceeds the scope of this review, and such information can be found elsewhere (Shahidi et al., 1992).

Individual antioxidant compounds vary in efficacy and mode of action. Generally, antioxidants can be classified by mode of action into primary or secondary antioxidants, but some antioxidants exhibit multiple mechanisms of antioxidant activity and work synergistically (Wanasundara and Shahidi, 2005).

Primary Antioxidants

Primary antioxidants impede peroxidation by inhibiting the propagation or initiation steps of peroxidation by reacting with free radicals (Wanasundara and Shahidi, 2005). Primary antioxidants generally exist as mono- or poly- hydroxy phenolic compounds with various ring substitutions. For example, carotenoids, flavonoids, phenolic acids, ascorbic acid, tocopherols, tocotrienols, lignans, BHT, BHA, ethoxyquin, propyl gallate, TBHQ, and other phenolic compounds act as primary antioxidants (Wanasundara and Shahidi, 2005; Figure 1-7). Primary antioxidants directly quench free radicals, reactive intermediates of peroxidation, or ROS, thus breaking the chain reaction of peroxidation (Figure 1-8). This process yields antioxidant radicals stabilized by the delocalization of the unpaired electron around the phenolic ring (Wanasundara and Shahidi, 2005; Figure 1-9). Primary antioxidant radicals are deactivated by binding with other antioxidant free radicals creating dimers of antioxidant molecules, or they can be regenerated via reduction reactions with other antioxidants (Wanasundara and Shahidi, 2005).

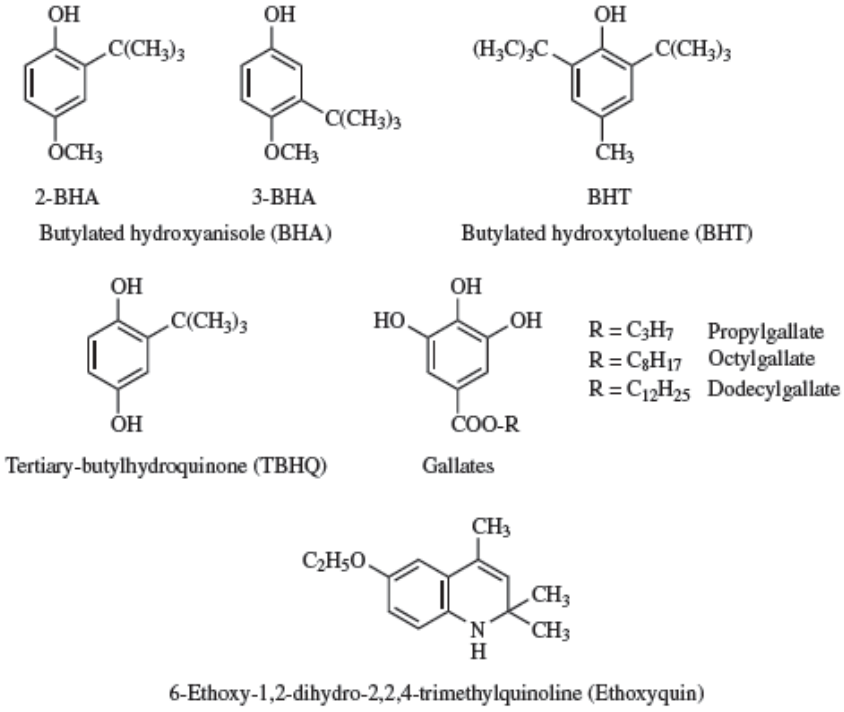

Figure 1-7. Structures of common synthetic antioxidants (adapted from Wanasundara and Shahidi, 2005).

ROO● + AH → ROOH + A●
R● + AH → RH + A●
ROO● + A● → ROOA
RO● + AH → ROH + A●
RO● + A● → ROA
A● + A● → AA

Figure 1-8. Modes of actions for primary antioxidants (AH is an antioxidant molecule;adapted from Wanasundara and Shahidi, 2005).

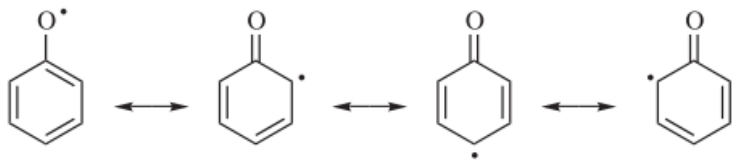

Figure 1-9. Stable resonance structures of phenoxy radical of phenolic antioxidants (adapted from Wanasundara and Shahidi, 2005).

Secondary Antioxidants

Secondary antioxidants retard peroxidation via several mechanisms. They may chelate pro-oxidant metal ions, reduce primary antioxidants, decompose hydroperoxides, deactivate singlet oxygen, or act as oxygen scavengers (Wanasundara and Shahidi, 2005). Secondary antioxidants may require the presence of primary antioxidants to exert antioxidant effects. Some secondary antioxidants prolong the effectiveness of phenolics, and chelators inhibit pro-oxidant effects of metals (Gordon, 1990).

Metal contamination from storage containers or processing equipment can promote peroxidation in lipids (Gordon, 1990). However, chelators form complexes with metals, thereby reducing their pro-oxidant effects. Carboxylic acid compounds such as phosphoric acid derivatives (e.g., phytic acid and polyphosphates), ethylenediamine-tetra-acetic acid (EDTA), and citric acid act as chelators to inhibit the pro-oxidant action of metals (Wanasundara and Shahidi, 2005). Oxidative stability of soybean oil declined with 0.3 ppm added Fe (Frankel et al., 1959) or 3 ppm Cu, Co, Mn, Fe, or Cr (Flider and Orthoefer, 1981), but these effects were attenuated with the addition of 0.01% citric acid. These results indicate that chelators such as citric acid effectively retard peroxidation in the presence of metals.

Other secondary antioxidants work as reducing agents and oxygen scavengers. Vitamin C, carotenoids, some amino acids (e.g taurine), peptides, urates, and phenolic compounds act as reducing agents or oxygen scavengers (Wanasundara and Shahidi, 2005). Specifically, vitamin C functions as a scavenger of reactive oxygen species, and β-carotene quenches singlet oxygen (Gordon, 1990). Addition of 0.46 ppm β-carotene

reduced the PV and conjugated diene concentration of soybean oil stored for 6h at 20°C (Clements et al., 1973).

Synergistic Effects of Antioxidants

Some antioxidants exhibit synergistic activity. Synergism exists when the combined use of 2 antioxidants results in antioxidant activity exceeding that of the sum of the individual antioxidants (Wanasundara and Shahidi, 2005). For example, chelators may be used in combination with antioxidants to effectively inhibit metal-catalyzed peroxidation and improve functionality of the other antioxidant. The TOTOX value of palm oil increased during 1500 h exposure at 50°C with either citric acid or TBHQ, but the TOTOX value stabilized with the use of both compounds (Sherwin, 1978). Other secondary antioxidants exhibit synergy through regeneration of primary antioxidants, thereby extending the functionality of primary antioxidants. Ascorbic acid reduces tocopheroxyl radicals to allow regeneration of functional tocopherol (Cort, 1982).

The diverse array of compounds with antioxidant properties poses a challenge for antioxidant selection. Therefore, Wanasundara and Shahidi (2005) recommended that the following factors be considered when selecting antioxidants: stability to processing conditions (carry through), potency, ease and accuracy of application, synergistic effects, capacity for complete distribution, minimization of discoloration, and ease of handling. Thoughtful consideration of the preceding factors may ensure suitability of an antioxidant for a particular application. Water soluble antioxidants such as vitamin C (ascorbic acid) may have limited value for protecting lipids from peroxidation compared to fat soluble compounds like ascorbyl palmitate (Choe and Min, 2009).

Antioxidants In Vivo

In addition to retarding peroxidation of dietary lipids during storage and processing, numerous antioxidants impede lipid peroxidation *in vivo*. Generally, antioxidants are classified as enzymatic or non-enzymatic depending on function (Table 1-4 and Table 1-5; Sies, 1993). Vitamin E and Se are well known as essential nutrients with major roles in antioxidant defense, but vitamin A, vitamin C (ascorbic acid), riboflavin, niacin, P, amino acids (e.g. Met, Cys, Tau, Glu, Gly, and Trp), Mn, Cu, Fe, and Zn have essential roles in this complex system. Therefore, low intakes or metabolic deficiencies of these micronutrients may impair the metabolic antioxidant system.

Non-enzymatic metabolic antioxidants

Non-enzymatic antioxidants directly impede lipid peroxidation (Table 1-4). Vitamins A and E are dietary antioxidants, but many other antioxidants are synthesized endogenously. Several vitamins play antioxidant roles. Vitamin E (alpha-tocopherol) interferes with the chain reaction of peroxidation by donating hydrogen to reactive oxygen species in the propagation step of peroxidation. The lipophilic nature of vitamin E facilitates its incorporation into cellular membranes where it protects PUFA (Packer et al., 2001). Vitamin E is a generic term which encompasses a family of 8 compounds, the tocopherols and tocotrienols (Figure 1-10). Interestingly, some have suggested that the tocotrienols have greater antioxidant activity than tocopherols in lipid membranes (Packer et al., 2001), but tocopherols have greater relative abundance in porcine plasma (Lauridsen et al., 2002b), porcine tissues (Lauridsen et al., 2002a), and murine tissues (Podda et al., 1996). Antioxidant activity of the tocopherol isomers varies, with $\alpha > \beta > \gamma > \delta$, which is related to the quantity, position, and conformation of methyl groups on the

aromatic ring (Ullrey, 1981). The most common form of vitamin E added to swine diets is synthetic dl-α-tocopheryl acetate, because of enhanced stability relative to the free alcohol form (Chung et al., 1992). However, dl-α-tocopheryl acetate is a racemic mixture of 8 stereoisomers of vitamin E (Mahan, 2001). Researchers have demonstrated that d-α-tocopheryl acetate (Anderson et al., 1995; Mahan et al., 2000; Wilburn et al., 2008; Yang et al., 2009) or d-α-tocopherol (Chung et al., 1992; Anderson et al., 1995) have greater bioavailability than dl-α-tocopheryl acetate for pigs. The variation in bioavailability is related to the α-tocopherol transfer protein which preferentially incorporates d α-tocopherol into very low density lipoproteins in the liver (Meier et al., 2003). Because of this selectivity, α-tocopherol is the most potent metabolic form of vitamin E (Gropper and Smith, 2009), and it has greater abundance *in vivo* relative to other forms (Lauridsen et al., 2002a). Therefore, in this review and subsequent chapters, vitamin E refers to α-tocopherol, unless stated otherwise. The oxidation of vitamin E creates a relatively stable free radical that may be reduced by endogenous antioxidants such as vitamin C, GSH, coenzyme-Q, or other molecules of oxidized vitamin E (Blokhina et al., 2003). More than 500 carotenoids (precursors of vitamin A) have been identified, of which β-carotene is the most prevalent (Sies and Stahl, 1995). Carotenoids are susceptible to peroxidation within the long chain of conjugated double bonds, and thereby quench ROS (Sies and Stahl, 1995). However, the concentration of vitamin A was approximately 10% that of vitamin E in plasma of humans (Sies and Stahl, 1995) and pigs (Boler et al., 2012), indicating there is considerably less vitamin A to be used as a metabolic antioxidant for these species.

Table 1-4. Non-enzymatic antioxidants in biological systems (adapted from Sies, 1993)

System	Comments
α-tocopherol	radical chain-breaking
β-carotene	singlet oxygen quencher
lycopene	singlet oxygen quencher
ubiquinol-10	radical scavenger
ascorbate	diverse antioxidant functions
glutathione (GSH)	diverse antioxidant functions
urate	radical scavenger
bilirubin	plasma antioxidant
flavonoids	plant antioxidants
plasma proteins	metal sequestration (eg. ceruloplasmin)
albumin	plasma antioxidant

Table 1-5. Enzymatic antioxidants in biological systems (adapted from Kohen and Nyska (2002)

Enzyme	Reaction
superoxide dismutase	$O_2^- + O_2^- + 2H^+ \rightarrow 2H_2O_2 + O_2$
catalase	$2H_2O_2 \rightarrow O_2 + 2H_2O$
glutathione peroxidase	$2GSH + ROOH \rightarrow GSSG + RH + 2H_2O$
glutathione reductase	$GSSG + NADPH \rightarrow GSH + NADP$
dehydroascorbate reductase	$DHA + 2GSH \rightarrow 2GSSG + ascorbate$
semidehydroascorbate reductase	$SHA + GSH \rightarrow GSSG + ascorbate$

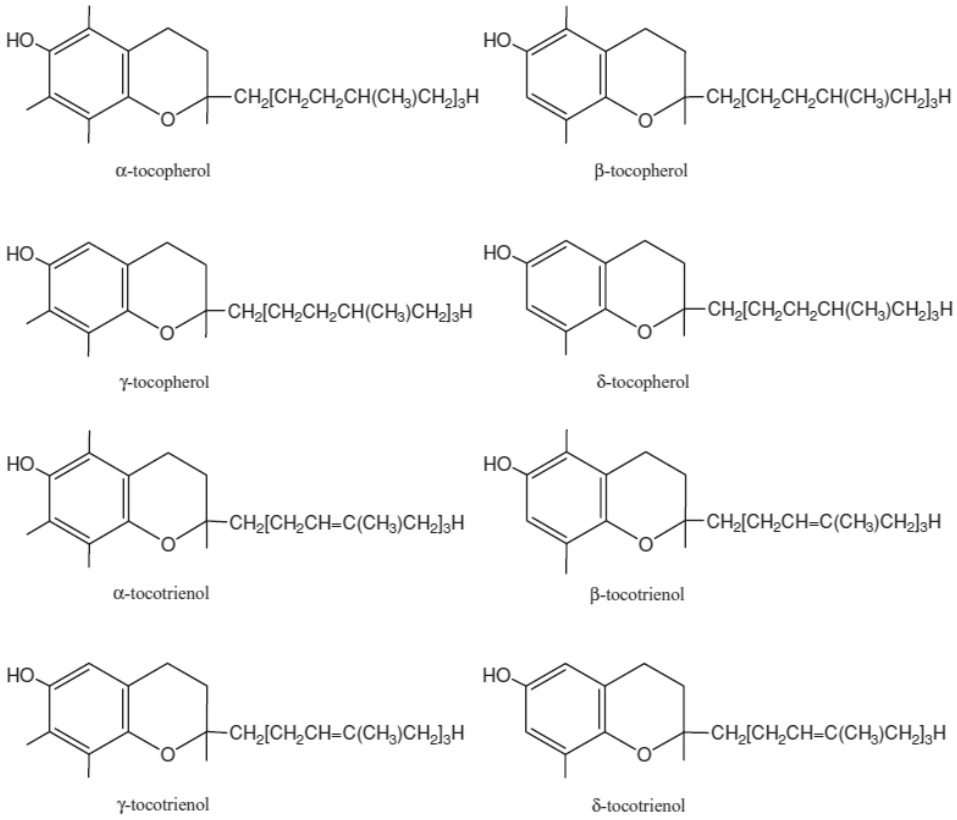

Figure 1-10. Structures of tocopherols and tocotrienols (adapted from Choe and Min, 2009).

Endogenous antioxidants

Many antioxidants are synthesized endogenously. Vitamin C is not a dietary essential for swine because adequate levels are generally synthesized endogenously, except in some instances of stress (NRC, 2012). As ascorbic acid, vitamin C donates up to 2 electrons to reactive species or in the regeneration of other antioxidants (e.g. vitamin E; Figure 1-11). Glutathione is an endogenously synthesized tri-peptide (Glu, Gly, and Cys) and is oxidized in this process. Glutathione provides reducing equivalents during the elimination of peroxides and the regeneration of vitamin C, and also directly scavenges ROS. Reducing equivalents are provided by NADPH to regenerate GSH from its

oxidized form (**GSSG**), and niacin and P are needed for NADPH synthesis. Sulfur-containing amino acids (**SAA**) including Met, Cys, Tau, and homocysteine play direct and indirect roles in the antioxidant system (Figure 1-12). Cysteine plays an indirect role as a structural component of GSH, and it may be rate limiting for endogenous synthesis of GSH (Brosnan and Brosnan, 2006). Conversely, Met, Cys, and Tau directly scavenge ROS (Atmaca, 2004). However, the SAA interconvert. Methionine generates Cys in an irreversible process with homocysteine as an intermediate, and Tau is synthesized from Cys (Brosnan and Brosnan, 2006). Several enzymes such as superoxide dismutase (**SOD**), catalase, glutathione peroxidase (**GPx**) and glutathione reductase (**GSR**) have direct roles in the antioxidant system (Blokhina et al., 2003; Table 1-5). Superoxide dismutase catalyzes the reaction to convert superoxide (O_2^-) to peroxide in the cytosol (Cu/Zn dependent) or mitochondria (Mn dependent). Peroxides are eliminated in a reaction catalyzed by GPx working in cooperation with glutathione (**GSH**). Selenium is a structural component of GPx. Catalase also works to eliminate peroxides, and Fe is a structural component of this enzyme. Other enzymes work to regenerate non-enzymatic antioxidants. For example, vitamin C regenerates vitamin E (Jacob, 1995), and GSR and semidehydroascorbate reductase regenerate the reduced forms of glutathione and ascorbic acid, respectively, with reducing equivalents provided by NADPH. Riboflavin is a structural component of GSR indicating that riboflavin has an indirect role in the antioxidant system.

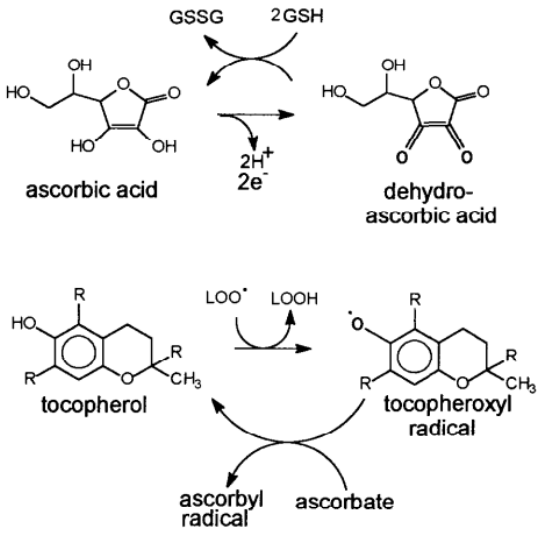

Figure 1-11. Integrated system of vitamin E, vitamin C, and glutathione (GSH; adapted from Jacob, 1995).

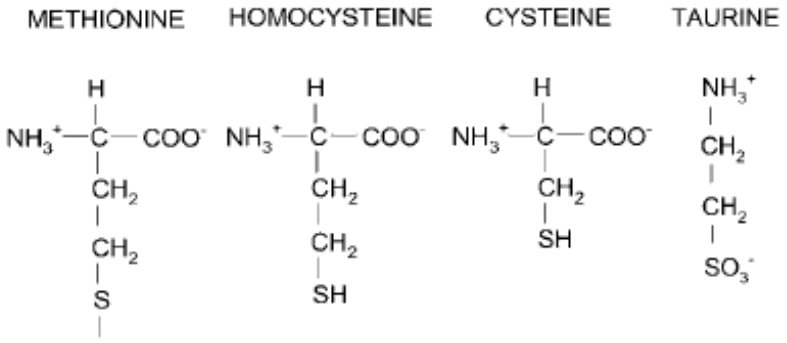

Figure 1-12. Structures of the sulfur containing amino acids (adapted from Brosnan and Brosnan, 2006).

Measuring metabolic status of vitamin E and Se

Vitamin E is an extremely important fat soluble antioxidant. Because vitamin E incorporates into cellular and subcellular lipid membranes, it inhibits peroxidative damage to these structural components. The concentration of d-α tocopherol exceeded

that of 7 other fat soluble antioxidants in human plasma (Sies and Stahl, 1995). Selenium serves as a structural component of GPx which catalyzes the elimination of hydroperoxides in the cytosol. Therefore, vitamin E and Se function cooperatively in the antioxidant system. However, vitamin E and Se cannot be synthesized endogenously, and must be included in diets for swine. Therefore, assessing the vitamin E and Se status of animals is critical to understanding the efficacy of dietary supplementation.

Several techniques may be utilized to assess the vitamin E and Se status of an animal. Generally, the relative concentration of antioxidant compounds in biological samples (e.g. tissue or serum) indicates relative bioavailability. Activity of GPx in liver (Meyer et al., 1981) or serum (Meyer et al., 1981; Kim and Mahan, 2001d) reflects dietary Se concentrations. Similarly, serum or tissue levels of Se increase with increasing dietary Se (Meyer et al., 1981; Mahan and Moxon, 1984). Concentrations of Se in kidney and liver exceed those of other tissues, but liver concentrations relate more consistently to dietary supplementation levels compared with other tissues (Meyer et al., 1981; Mahan and Moxon, 1984). The Se content of hair samples increases with increasing dietary Se in swine (Mahan et al., 1977; Kim and Mahan, 2001b, d; Mahan and Peters, 2004) and steers (Davis et al., 2012). The concentration of Se in sow hair and that of their progeny showed a positive relationship in some studies (Kim and Mahan, 2001d), but not in others (Friendship et al., 1985). However, liver concentrations of Se in neonatal pigs consistently reflect the liver Se concentrations of sows (Mahan et al., 1977; Kim and Mahan, 2001d). Therefore, tissue (generally liver) or serum concentrations of Se are used commonly to indicate Se status of pigs, but the concentration of Se in hair samples may be a simpler, non-invasive alternative.

While there are several forms of vitamin E, the concentration of α-tocopherol is used as a primary indicator of vitamin E status because it is the most abundant form of vitamin E in the body (Podda et al., 1996; Lauridsen et al., 2002a,b). The concentration of α-tocopherol in serum or tissue generally reflects dietary concentrations of vitamin E (Chung et al., 1992; Wilburn et al., 2008). A high correlation ($r > 0.9$) exists among concentrations of α-tocopherol in serum, lung, heart, liver, and longisimus muscle (Chung et al., 1992), suggesting that serum samples can be used to accurately reflect whole body vitamin E status.

While Se and vitamin E have interconnected metabolic functions, the supranutritional supplementation of one does not appear to affect circulating concentrations of the other. Increasing Se supplementation from 0.1 to 2.0 ppm did not affect the α-tocopherol content of pig serum (Meyer et al., 1981). Conversely, increasing supplemental vitamin E from 16 to 96 IU/kg had no effect on the concentration of Se or GPx in serum (Chung et al., 1992). These results indicate that metabolic markers of vitamin E and Se status generally respond independently to the supply of their respective nutrients. Ultimately, pigs without sufficient supplies of vitamin E and Se may develop similar signs of deficiency.

Deficiencies of vitamin E or Se for swine

A wide variety of deficiency signs are responsive to the supplementation of vitamin E, Se, and other antioxidants (NRC, 2012). Vitamin E was discovered initially by Evans and Bishop (1923) as an essential dietary component for reproduction, but with inadequate dietary concentrations of vitamin E or Se, deficiency signs spanning multiple tissues, organs, and metabolic systems may develop (Mahan, 2001). Swine deficient in

vitamin E or Se exhibit wide ranging signs including: splayed rear legs in neonatal pigs, hemolyzed red blood cells, gastric ulcers, post-weaning diarrhea, yellow fat, pericardial fluid retention, edema of intestinal membranes, nutritional muscular dystrophy (pale striated muscles), cecal and colonic hemorrhages, hepatosis dietetica (liver deterioration and hemorrhages), cardiac myopathy (weakened, thin-walled, enlarged heart with pale white striations), and Mulberry Heart Disease (MHD) which results in hemorrhages in the auricle and pericardial fluid retention (Mahan, 2001). Recent field reports have suggested a resurgence of this deficiency disease in pigs (Weaver, 2010a, b).

Mulberry Heart Disease results in the sudden death of fast growing nursery pigs (Rice and Kennedy, 1989; Pettifer, 1998; AASV, 2009). Consequently, its occurrence is of economic importance for pork producers. However, multiple factors seem to contribute to the onset of this disease. Generally, liver concentrations < 2 μg α-tocopherol/g are considered deficient (Rice and Kennedy, 1989), but many have reported MHD in pigs with reserves exceeding that threshold (Pallarés et al., 2002; Desrosiers, 2003; Shen et al., 2011), suggesting that additional factors may contribute to the onset of this deficiency disease. Furthermore, MHD often develops in pigs provided seemingly adequate dietary concentrations of vitamin E and Se, further substantiating the potential involvement of unidentified contributing factors (Rice and Kennedy, 1989; Desrosiers, 2003; Shen et al., 2011). Perhaps established requirements may be insufficient to meet the metabolic demand for vitamin E and Se of animals in particular situations.

Factors Affecting the Dietary Supplementation Strategy for Vitamin E and Se

The required supplemental levels of dietary Se or vitamin E depend on several factors. In his review of the subject, Mahan (2001) succinctly identified 15 contributing factors, which are summarized as follows:

Low Se content in common feed ingredients

Low content of indigenous Se in feedstuffs contributes to the need to supplement Se in commercial swine diets. Grains produced in the Great Lakes, Northwest, or Northeastern regions of the United States may have low (< 0.1 ppm) concentrations of Se (Mahan, 2001). In a survey of swine produced in various regions of the United States, Ku et al. (1972) found that the Se content in loin muscle varied substantially (range = 0.03 to 0.52 ppm), and that tissue concentrations were associated strongly (r = 0.95) with the dietary Se content. To safeguard against potentially low concentrations in feedstuffs, Se is supplemented typically as sodium selenite in swine diets. However, Se may be toxic to animals and humans at excessive dietary levels. Signs of Se toxicity (e.g. reduced growth performance, lethargy, staggered gate, alopecia, and hoof malformations) were evident in nursery pigs fed diets with 5 to 40 ppm Se, and pigs refused to consume diets containing 40 ppm Se (Mahan and Moxon, 1984). Therefore, the United States Food and Drug Administration has established a maximum supplemental level of 0.3 ppm in swine diets (AAFCO, 2013). When organic forms of dietary Se are used, such as Se-enriched yeast, tissue Se retention is increased (Kim and Mahan, 2001b, c, d) and excretion reduced (Kim and Mahan, 2001c) relative to pigs fed similar dietary concentrations of sodium selenite. These results indicate that organic forms of Se have greater bioavailability than inorganic forms for swine.

Low vitamin E content in commonly used feed ingredients

In addition to potentially low indigenous Se, swine diets composed solely of corn and soybean meal may be deficient in vitamin E relative to the pig's requirement. The α-tocopherol content of cereal grains and oilseed protein meals is typically lower than that of forages and supplemental animal fats and vegetable oils (Mahan, 2001). Modern indoor housing and nutritional practices for swine limit access to green forages, alfalfa, and other plants with appreciable concentrations of indigenous vitamin E. Therefore, the indigenous concentrations of vitamin E and Se may vary, and be insufficient to meet the requirements of livestock, necessitating the use of supplements.

In addition to the potential for low levels of indigenous vitamin E in feedstuffs, natural vitamin E (α-tocopherol) is susceptible to degradation. Specifically, degradation of α-tocopherol is enhanced with exposure to pro-oxidant conditions (e.g. heat and transition metals). Feed ingredients and complete diets may be exposed to these pro-oxidants during processing and storage. Liu (2012) reported that vitamin E content of canola or corn oil declined from 29 and 40 IU vitamin E/kg to undetectable levels (< 10 IU vitamin E/kg) after exposure to 95 or 185°C for 72 or 7 h, respectively. However, after exposure to air at 110°C for 3 h in a grain dryer, the vitamin E content of corn declined only 4% (Young et al., 1975). These divergent results indicate that vitamin E is susceptible to degradation, but the extent of degradation is influenced by its external matrix. Therefore, vitamin E is supplemented in practical swine diets because of the potential for degradation and highly variable content of indigenous vitamin E in feed ingredients.

Degradation of vitamin E forms during storage

Vitamin E may degrade during storage, but the extent of degradation depends on its dietary form. Free alcohols (tocopherols) are generally less stable than other chemical forms of vitamin E. Dietary vitamin E activity declined 36% after 60 d of storage when α-tocopherol was supplemented (Waibel et al., 1994). However, dietary vitamin E activity declined less than 15% after 60 (Waibel et al., 1994) or 84 d (Young et al., 1975) of storage when dl-α-tocopheryl acetate was supplemented. Similarly, Chung et al. (1992) reported that dietary retention of vitamin E was greater when dl-α-tocopheryl acetate was used rather than d-α-tocopherol (97 vs. 80% retention, respectively). Stability of dl-α-tocopheryl acetate is enhanced by the methoxy group on the aromatic ring (Mahan, 2001). When a vitamin-trace mineral premix was stored for 6 months, the concentration of α-tocopherol declined 35% per month (Coelho, 2002), but vitamin E losses of 0.2% (Coelho, 2002) to 1.6% (Shurson et al., 2011) per month were observed with dl-α-tocopheryl acetate in vitamin-trace mineral premixes. Therefore, dl-α-tocopheryl acetate is supplemented commonly to provide a stable source of dietary vitamin E for livestock. Supplemental levels of vitamin E generally range from 3 to 5 times the recommended requirement (Hubbard Feeds, personal communication), and the contribution of indigenous vitamin E from other dietary ingredients is often not considered during diet formulation. Furthermore, vitamin E is relatively non-toxic for swine, and levels exceeding the requirement 50 fold were fed to nursery pigs without affecting growth performance (Bonnette et al., 1990).

Dietary form and concentration of pro-oxidant metals

Pro-oxidant metals, such as Cu and Fe, stimulate degradation of vitamin E, particularly in the presence of unsaturated lipids (Dove and Ewan, 1991a). When $CuSO_4$ was added to swine diets to provide 250 ppm Cu, dietary α-tocopherol was undetectable after 14 d of storage, but the concentration of α-tocopheryl acetate was less affected by Cu (Dove and Ewan, 1991b). Consequently, research showed that feeding diets with 250 ppm Cu from $CuSO_4$ reduced serum content of α-tocopherol in nursery pigs compared to those fed 5 ppm Cu (Dove and Ewan, 1990, 1991b). These effects are likely attributable to the degradation of vitamin E and therefore, reduced intake of this nutrient (Dove and Ewan, 1990, 1991b), because free Cu is tightly regulated metabolically presumably to inhibit its promotion of peroxidation *in vivo*. However, when evaluating lower levels of Cu provided by $CuSO_4$ (250 and ≤ 175 ppm), others found no negative effect on vitamin E status of pigs (Myer et al., 1992; Lauridsen et al., 1999b). However, the level of supplemental vitamin E was lower (≤ 11 IU/kg; Dove and Ewan, 1990, 1991a) in studies reporting a negative effect than those that did not (≥ 18 IU/kg; Myer et al., 1992; Lauridsen et al., 1999a). Similarly, Shurson et al. (2011) reported minimal losses (1.1 to 1.3% per month) of vitamin E (supplemented as dl-α-tocopheryl acetate) changed when stored for 120 d in a vitamin-trace mineral premix with Cu present at relatively low levels (8 ppm). Together, these results suggest that high levels of Cu (≥ 250 ppm) may accelerate the catabolism of indigenous dietary vitamin E which can negatively impact the vitamin E status of pigs fed marginal concentrations of vitamin E relative to the requirement.

In addition to Cu, researchers have suggested antagonism between Fe and vitamin E. Dove and Ewan (1991a) reported that the stability of α-tocopheryl acetate declined with 1,000 ppm of supplemental Fe (as $FeSO_4$) in the diet, but such excessive levels of Fe are not common in practical swine diets. Interestingly, vitamin E deficient pigs succumbed to Fe toxicity (> 50% mortality) after intramuscular injection of an organic Fe^{+3} complex (40 to 375 mg/kg), but intramuscular injection of dl-α-tocopheryl acetate (Lannek et al., 1962; Tollerz and Lannek, 1964) or ethoxyquin (Tollerz and Lannek, 1964) eliminated mortality. In addition, Loudenslager et al. (1986) demonstrated that pigs injected with Fe dextran had reduced levels of α-tocopherol and Se in serum compared to pigs who did not receive Fe dextran. However, no pigs developed signs of Fe toxicity. These results suggest antagonism between Fe and vitamin E. However, Hill et al. (1999) found no signs of Fe toxicity after intramuscular injection of iron dextran to pigs from dams fed diets with low (16 IU vitamin E/kg) levels of vitamin E. Together, these results indicate a negative metabolic relationship between vitamin E and Fe that warrants further investigation, but young pigs do not seem to be susceptible to Fe toxicity when sows are fed practical diets containing ≥16 IU vitamin E/kg. While serum concentrations of free Fe are regulated tightly by several metabolic mechanisms, perhaps injectable Fe overloads the capacity of transporters, enabling free Fe to accelerate lipid peroxidation and catabolism of vitamin E. Alternatively, proteins may be susceptible to modification by products of peroxidation, but it is unclear if the functionality of Fe and Cu transporters is impaired by products of peroxidation.

Pig genotype

Genetic background also influences the required level of vitamin E and Se in swine diets. For example, pigs with high growth rates may have greater metabolic activity and demand for these micronutrients. Furthermore, lean pigs generally have lower feed intake than fatter animals, and nutrient density should be increased accordingly (Mahan, 2001). Pigs that succumb to MHD are often fast-growing, seemingly healthy pigs (Rice and Kennedy, 1989; Pettifer, 1998; AASV, 2009) . However, the mechanism by which this deficiency disease specifically targets fast growing nursery pigs is unclear, but it is likely because fastest growing pigs having increased biological demand for vitamin E relative to their slower growing contemporaries. Interestingly, some have linked the phenotypic expression of gain efficiency and residual feed intake with mitochondrial inefficiency and oxidative stress at the mitochondrial level which may increase the biological demand for vitamin E (Bottje and Carstens, 2009). Regardless of the mechanism, the occurrence of MHD has a negative economic impact for pork producers because it results in death of fast-growing pigs.

Pig age affects ability to use certain chemical forms of vitamin E

The age of pigs should also be considered when selecting a dietary vitamin E supplementation strategy. Age not only affects requirements, but it can affect the efficiency of vitamin E utilization. Specifically, dl-α-tocopheryl acetate may be less bioavailable for young pigs compared to the alcohol form. For example, the content of α-tocopherol in tissues and serum was greater when diets were supplemented with d-α-tocopherol compared to dl-α-tocopheryl acetate (Chung et al., 1992). Based on a slope

ratio assay of the concentration of α-tocopherol in several tissues, these authors calculated that α-tocopherol is 2.44 times more bioavailable relative to dl α-tocopheryl acetate, which exceeds the commonly accepted value of 1.49 established by the United States Pharmacopeia (Chung et al., 1992). During digestion, the acetate group of α-tocopheryl acetate is removed by carboxyl ester hydrolase (CEH) prior to absorption of the free alcohol (Mahan, 2001). However, the activity of CEH declines after weaning which may compromise the ability of the pig to utilize the acetate form (Jensen et al., 1997). Simultaneously, dietary vitamin E source changes from d-α-tocopherol in sow milk to dl-α-tocopheryl acetate in typical starter diets (Mahan, 1994; Lauridsen and Jensen, 2007). Consequently, circulating levels of vitamin E decline post-weaning (Chung et al., 1992; Moreira and Mahan, 2002; Lauridsen, 2010), but the loss is attenuated partially by supplementation of d-α-tocopherol (Chung et al., 1992) or supranutritional levels of vitamin E as dl-α-tocopheryl acetate (Moreira and Mahan, 2002; Lauridsen, 2010). Together, these results suggest that pigs have increased metabolic demand for vitamin E after weaning or a diminished capacity to utilize the dl-α-tocopheryl acetate, which is used routinely to provide vitamin E in nursery diets relative to other dietary forms.

Young sows may also require special consideration when selecting supplementary levels of vitamin E. Vitamin E in milk and serum from sows and serum from offspring increased linearly with increasing parity (Mahan et al., 2000). These results suggest that age of sow affects the vitamin E and Se status of sows and progeny, and younger sows seem to have a greater metabolic demand for vitamin E.

Dietary PUFA content

Compared to saturated fat sources, lipids containing high concentrations of PUFA may increase the metabolic demand for vitamin E in swine (Ullrey, 1981) and other species (Horwitt, 1960; Harris and Embree, 1963). This is likely related to increased potential for peroxidation (Hollander, 1981) in diets with high PUFA content relative to other, more saturated, fatty acids. Dietary sources of PUFA like corn oil (Malm et al., 1976) and fish oil (Hidiroglou et al., 1993; Farnworth et al., 1995) reduce the α-tocopherol concentrations in serum of sows and offspring compared to other sources of dietary lipids. Therefore, additional levels of vitamin E may be warranted in diets containing high levels of PUFA (Mahan, 2001).

Conversely, addition of lipids to swine diets may positively affect the vitamin E status of pigs relative to those fed diets without supplemental lipids. As a fat soluble vitamin, vitamin E is absorbed passively in the jejunum after lipid emulsification and micelle formation. Consequently, dietary lipids may facilitate absorption of vitamin E (Gropper and Smith, 2009). Accordingly, the vitamin E status for growing (Lauridsen et al., 1999a,b) and nursery (Myer et al., 1992; Moreira and Mahan, 2002; Liu, 2012) pigs increases when fed diets with supplemental lipid sources relative to pigs fed similar diets without added lipids. However, it is unclear whether increased vitamin E status is caused by improved digestibility or is a result of the vitamin E content in the lipid. Some experimental evidence supports the latter (Lauridsen et al., 1999a,b; Liu, 2012). However, others have shown increased vitamin E status from adding lipids to the diet when diets contain similar analyzed levels of dietary vitamin E (Moreira and Mahan, 2002).

Lipid peroxidation

Lipid peroxidation influences the need for supplemental vitamin E. Feeding diets with peroxidized lipids typically reduces the vitamin E status (serum, liver, or milk levels) of pigs (Boler et al., 2012; Liu et al., 2012a), sows (Nielsen et al., 1973), rats (Liu and Huang, 1995; Eder, 1999; Brandsch and Eder, 2004) and broilers (Lin et al., 1989; Takahashi and Akiba, 1999; Tavárez et al., 2011). However, the specific mechanism is unclear. Some researchers have reported that peroxidized lipids contain less vitamin E than unperoxidized lipids (Liu, 2012) or induce degradation of vitamin E present in digesta (Gorelik et al., 2005), thus confounding actual intake or uptake of vitamin E with metabolic events affecting vitamin E status. Interestingly, some have demonstrated that dietary peroxidized lipids reduce the vitamin E status of animals with similar intake of vitamin E, suggesting that altered uptake or metabolic changes are contributing to this effect (Liu and Huang, 1995). Regardless, evidence suggests the reduction in vitamin E status elicited by feeding peroxidized lipids, is attenuated by additional dietary vitamin E (Nielsen et al., 1973; Lin et al., 1989; Liu and Huang, 1995). Increasing the dietary supply of vitamin E ameliorated the negative effects of dietary peroxidized lipids on growth performance (Oldfield et al., 1963; Rasheed et al., 1963; Lin et al., 1989), indicating the dietary supply of vitamin E may limit growth in diets with peroxidized lipids. Interestingly,Tavárez et al. (2011) found that use of a synthetic antioxidant (Agrado Ultra, Novus, Intl.) increased concentration of vitamin A in liver of broilers fed peroxidized lipids, but vitamin E was not affected. These results suggest that vitamin A status is also altered when feeding peroxidized lipids, and there are likely other biological

and physiological effects that occur as a result of feeding diets containing peroxidized lipids.

BIOLOGICAL IMPLICATIONS OF DIETARY PEROXIDIZED LIPIDS

The extent of lipid peroxidation varies among feed ingredients. Analyzed PV ranged from 0.1 to 180.8 meq O_2/kg (mean = 2.6 meq O_2/kg) among 610 samples of lipid sources such as poultry fat, tallow, lard, canola oil, corn oil, and soybean oil (Dibner, personal communication). These data suggest that some sources of lipids are peroxidized, but the level of PV is generally low. In addition to concentrated sources of lipid, other ingredients with high levels of PUFA are susceptible to peroxidation. The concentration of lipid in DDGS varies, but consists predominantly of PUFA (NRC, 2012). During processing, DDGS is exposed to temperatures as high as 500°C (Rosentrater et al., 2012). In a recent survey, the level of TBARS (1.0 to 5.2 ng MDA eq/mg oil) and PV (4.2 to 84.1 meq O_2/kg oil) varied considerably across 31 sources of DDGS (Song and Shurson, 2013). Therefore, dietary lipid sources can be peroxidized to varying extents and contribute to a situation known as metabolic oxidative stress (Dibner et al., 2011) with negative implications for gain efficiency (McGill et al., 2011a,b) and growth rate (Boler et al., 2012; Liu, 2012) of animals.

Defining Metabolic Oxidative Stress

Reactive oxygen species are produced endogenously by aerobic metabolism and the immune system, but ROS may also be introduced exogenously through diet or generated in the alimentary tract during digestion. Previous discussion in this literature review explained how antioxidants inhibit ROS. Oxidative stress occurs when pro-oxidants overload the antioxidant capacity of an animal (Sies, 1985). Therefore, animals

with inadequate supplies of antioxidants relative to demand *in vivo* may be in a state of metabolic oxidative stress. Furthermore, animals fed peroxidized lipids are in a state of metabolic oxidative stress (Takahashi and Akiba, 1999; Boler et al., 2012; Liu et al., 2012a).

At the cellular level, oxidative stress results in a cascade of events (Figure 1-13), beginning with damage or modification to cellular and subcellular lipid membranes, proteins, nucleic acids, and carbohydrates (Betteridge, 2000; Lykkesfeldt and Svendsen, 2007). Furthermore, some aldehydes present in peroxidized lipids are considered cytotoxic (Esterbauer et al., 1991). Ultimately, such damage may contribute to cellular necrosis, impair cellular function and integrity, contribute to structural damage of tissues, and increase demand for metabolic antioxidants (Lykkesfeldt and Svendsen, 2007).

Dietary inclusion of peroxidized lipids is associated with negative effects for swine and poultry (Robey and Shermer, 1994; Dibner et al., 2011). Specifically, feeding peroxidized lipids causes reduced gain efficiency (McGill et al., 2011a, b; Tavárez et al., 2011) , growth (Boler et al., 2012; Liu, 2012), metabolic oxidative status (Boler et al., 2012; Liu, 2012), energy digestibility (Inoue et al., 1984; Engberg et al., 1996), increased mortality (Takahashi and Akiba, 1999; Anjum et al., 2004), impaired immune function (Dibner et al., 1996b), and reduced meat quality (Asghar et al., 1989; Racanicci et al., 2008; Tavárez et al., 2011). Therefore, dietary peroxidized lipids can substantially influence the efficiency and profitability of livestock and poultry production negatively.

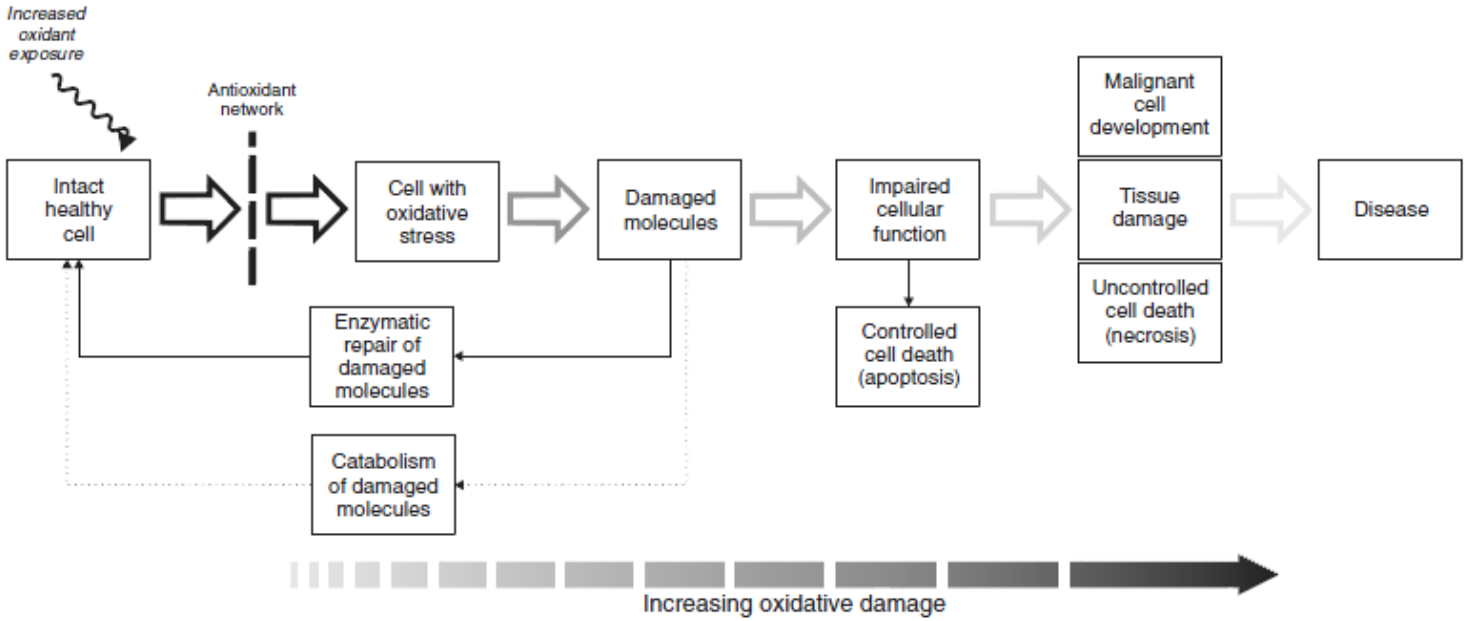

Figure 1-13. Cascade of events in oxidative stress that ultimately contributes to tissue damage and disease (adapted from Lykkesfeldt and Svendsen, 2007).

Measuring Metabolic Oxidative Stress

Several techniques can be used to estimate the extent of metabolic oxidative stress in biological tissues. Biological samples can be used to measure reactive compounds, indicators of biological damage, or antioxidants.

Measuring reactive compounds

Free radicals can be measured with electron spin resonance (**ESR**). However, the short half-life of many free radicals poses a challenge for their enumeration, and ESR requires specialized equipment. This assay may detect relatively stable free radicals generated from antioxidants, indicating it is not specific to ROS (Halliwell et al., 1995). Furthermore, free radicals associated with peroxidation may be present at undetectable levels because of rapid catabolism (Halliwell et al., 1995). Alternative assays to ESR exist which are specific for hydroxy free radicals, but they are not utilized routinely (Halliwell et al., 1995).

Measuring indicators of metabolic peroxidation

The relative quantity of various peroxidation products in a biological sample may indicate that peroxidation that has occurred. Hydrogen peroxide (Lykkesfeldt and Svendsen, 2007), conjugated dienes (Halliwell and Chirico, 1993), and TBARS may be measured, but TBARS and conjugated dienes are criticized because they lack specificity. Specific aldehydes, such as MDA and HNE, can be measured in biological samples along with products of peroxidative damage such as protein carbonyls, 8-hydroxy-deoxyguanosine, and isoprostanes (Halliwell et al., 1995). However, the threshold concentration at which these biological markers should evoke concern is unclear. In their

extensive review, Esterbauer et al. (1991) suggested threshold concentrations for HNE in biological samples and indicated that concentrations exceeding 100 μM are cytotoxic; concentrations from 1 to 20 μM inhibit DNA synthesis, proteogenesis, and cellular growth; and concentrations below 0.1 μM represent basal physiological levels. The concentration of MDA ranges from 0.2 to 0.8 μM in normal human urine (Esterbauer et al., 1991). However, similar threshold levels of MDA acceptability have not been established for livestock or poultry.

Lipids are predominant components of cellular and subcellular membranes. As a result, their peroxidation can affect membrane structure and functionality negatively. Extensive peroxidation of biological membranes increases rigidity, permeability, and cellular rupture (Gutteridge, 1995). Liver damage can be measured indirectly by evaluating transaminase enzymes. Serum concentrations of hepatic transaminase enzymes reflect hepatocytic damage or necrosis (Reichling and Kaplan, 1988), and researchers have reported elevated levels of glutamate-oxalacetate transaminase and glutamate-pyruvate transaminase (Teige et al., 1977) or aspartate transaminase (van Vleet, 1982) in serum of pigs fed inadequate dietary vitamin E, indicating that oxidative stress contributes to hepatocytic damage.

Measuring metabolic antioxidants

In addition to markers of oxidative damage, specific antioxidants can be measured to indicate the metabolic oxidative status of an animal. Vitamin E or vitamin A can be measured in serum or liver where relatively low concentrations may indicate oxidative stress. Research results suggest that a negative correlation exists between vitamin E and TBARS concentrations in biological samples (Hossein Sadrzadeh et al., 1994; Liu and

Huang, 1995; Yanik et al., 1999), indicating that vitamin E is catabolized during metabolic oxidative stress. In addition to vitamin E, the concentration of endogenous antioxidants like GSH and vitamin C, or the activity of enzymes such as GPx, catalase, and SOD can be measured. The relative concentrations of these compounds indicate the ability of the animal to counteract metabolic peroxidative damage. Additionally, a relatively low ratio of GSH/GSSG reflects metabolic oxidative stress because of an increased level of the oxidized form of glutathione (Jones, 2002).

In addition to measuring specific antioxidants, other assays more generally characterize overall metabolic antioxidant status. The total radical-trapping antioxidant parameter, the ferric-oxide reducing antioxidant parameter, and the trolox equivalent antioxidant capacity have been used to measure combined activity of antioxidants in a sample (Antolovich et al., 2002). Generally, these assays induce oxidative conditions in samples, and measure the oxidation of marker molecules added to the assay. However, the application of these assays on biological samples is often criticized because the accelerated pro-oxidant conditions of the assays do not reflect conditions *in vivo* (Sies, 2007). Furthermore, because such assays are not specific to a single antioxidant, they may lack sensitivity to accurately reflect contributions from low-weight molecular antioxidants like α-tocopherol, ascorbic acid, or β-carotene (Sies, 2007).

Numerous assays may indicate the relative level of metabolic oxidative stress, but unfortunately, no single measure effectively characterizes the extent of metabolic oxidative stress because oxidative stress has multifaceted biological and physiological effects. Therefore, researchers must use multiple parameters to assess metabolic oxidative

status, but the relative importance of specific biomarkers for metabolic oxidative stress on the negative effects on health and growth is not well understood.

Effects of Feeding Peroxidized Lipids to Animals

Dietary products of lipid peroxidation contribute to metabolic oxidative stress and reduce efficiency of nutrient utilization as animals attempt to cope with damage at the cellular and systemic levels (Lykkesfeldt and Svendsen, 2007). However, the practical significance of such changes for nutrient metabolism, growth, and health of animals is not clear.

Metabolic oxidative balance

Metabolic oxidative status of animals is affected by dietary peroxidized lipids. Levels of vitamin E in serum or tissue decline with dietary peroxidized lipids for pigs (Boler et al., 2012; Liu et al., 2012a), sows (Nielsen et al., 1973), broilers (Inoue et al., 1984; Engberg et al., 1996; Takahashi and Akiba, 1999; Tavárez et al., 2011), and rats (Liu and Huang, 1995). Interestingly, Upton et al. (2009) reported that GPx activity of broiler liver increased with dietary peroxidized poultry fat. These authors suggest that production of GPx increases as the amount of substrate (i.e. peroxides) in dietary peroxidized lipid increase. Conversely, tissue or serum concentrations of TBARS increase with dietary peroxidized lipids for swine (Boler et al., 2012), rats (Liu and Huang, 1995) and broilers (Lin et al., 1989; Takahashi and Akiba, 1999; Anjum et al., 2004).

Growth performance

Numerous researchers have reported reduced growth rate (Oldfield et al., 1963; Inoue et al., 1984; Lin et al., 1989; Engberg et al., 1996; Takahashi and Akiba, 1999; Anjum et al., 2002, 2004; DeRouchey et al., 2004; Racanicci et al., 2008; Harrell et al., 2010; McGill et al., 2011a; Tavárez et al., 2011; Boler et al., 2012; Liu, 2012) and gain efficiency (Inoue et al., 1984; Takahashi and Akiba, 1999; McGill et al., 2011a,b;Tavárez et al., 2011) in pigs and broilers fed diets containing peroxidized lipids. This response is linked partially to reduced feed intake which is often associated with feeding dietary peroxidized lipids (Oldfield et al., 1963; Engberg et al., 1996; Anjum et al., 2002, 2004; DeRouchey et al., 2004; Tavárez et al., 2011; Boler et al., 2012; Liu, 2012). However, metabolic oxidative stress and cellular damage may also contribute to reduced growth.

In contrast, some researchers found no reduction in gain efficiency when feeding peroxidized lipids to swine and poultry (Oldfield et al., 1963; Engberg et al., 1996; Racanicci et al., 2008; Upton et al., 2009; Harrell et al., 2010; Liu, 2012). Liu (2012) reported that dietary inclusion of rapidly peroxidized (heated 7 h at 185°C) lipids reduced growth rate of nursery pigs, but slowly peroxidized lipids (heated 72 h at 95°C) did not. Therefore, inconsistent findings likely relate partially to the variable fatty acid profiles, dietary inclusion levels, methods employed to induce peroxidation, and inadequate replication among studies.

Some researchers have suggested that antioxidants attenuate the negative effects of peroxidized lipids on growth. Reductions in growth (Tavárez et al., 2011) and gain efficiency (Cabel et al., 1988; McGill et al., 2011b) were recovered partially with the use of antioxidants containing ethoxyquin. Similarly, Oldfield et al. (1963) reported that

adding dl-α tocopheryl acetate or ethoxyquin to swine diets containing peroxidized menhaden fish oil resulted in complete recovery of ADG and ADFI to levels similar to that of control animals. Others have reported similar recovery with d-α tocopherol or BHT and BHA supplementation for broilers fed peroxidized sunflower oil (Lin et al., 1989). Results such as these suggest that inadequate metabolic supplies of antioxidants contribute to reduced growth in animals fed peroxidized lipids, and that partial recovery is possible by increasing the concentration of dietary antioxidants. However, the ameliorative effect of antioxidants is not consistent (Wang et al., 1997; Harrell et al., 2010; McGill et al., 2011a; Boler et al., 2012), indicating that factors other than a metabolic deficiency of antioxidants are involved.

Although the specific physiological mechanism(s) that contribute to reduced growth when feeding peroxidized lipids have not been elucidated, the response may be related to lower efficiency of nutrient digestibility, nutrient utilization, and reduced immune system function.

Animal health and immune responses

Dietary peroxidized lipids have negative effects on the immune system, and consequently reduce animal health. Takahashi and Akiba (1999) reported that feeding peroxidized lipids reduced production of antibodies to *Brucella abortus* in broilers compared those fed unperoxidized lipids. Dibner et al. (1996b) reported that proliferation of intestinal lymphocytes declined when feeding peroxidized lipids to swine. Reduced immune responses may contribute to increased mortality when feeding peroxidized lipids (Takahashi and Akiba, 1999; Anjum et al., 2004), but in many cases, changes in

mortality are not reported in published studies evaluating negative effects of feeding peroxidized lipids.

Changes in gut barrier function have significant implications for animal health. The intestine is the initial barrier against a plethora of dietary components, toxins, bacteria, viruses, and antigens (Walker and Sanderson, 1992; Turner, 2009). Furthermore, PUFA present in intestinal epithelial cell membranes are susceptible to peroxidation. Therefore, the function of the epithelial barrier may be compromised by peroxidative damage. Research results suggest that dietary peroxidized lipids induce metabolic oxidative stress in enterocytes (Reddy and Tappel, 1974; Ringseis et al., 2007). Wang et al. (2000) suggested that lipid peroxides contribute to apoptosis of human intestinal epithelial cells *in vitro*, but a similar response has not been demonstrated in animals. Dibner et al. (1996b) showed histological evidence of reduced half-life of enterocytes when broilers were fed diets containing peroxidized lipids, and suggested that integrity of the gastrointestinal barrier and immune system may be compromised. In contrast, Liu (2012) found no effect of feeding peroxidized lipids on intestinal barrier function or serum concentrations of endotoxins or immunoglobulins. However, the lack of significant differences may relate to lack of statistical replication in that study. In contrast, Weber and Kerr (2011) reported that serum concentrations of IgM and IgA increased substantially (68%) when pigs were fed diets containing DDGS. In that study, the diet containing DDGS had 20% greater concentrations of TBARS relative to those without DDGS. However, DDGS contains several nutrients in addition to lipid which may confound the effect of lipid peroxidation.

Nutrient digestibility and energy metabolism

Some researchers have suggested that the energy value of lipids is reduced by peroxidation (Dibner et al., 2011). Furthermore, the digestibility of certain dietary nutrients was reduced when feeding peroxidized lipids in some studies (Inoue et al., 1984; Liu and Huang, 1995; Engberg et al., 1996; Yuan et al., 2007), but not in others (DeRouchey et al., 2004; Liu, 2012). Specifically, the digestibility of crude fat (Inoue et al., 1984; Liu and Huang, 1995), crude protein (Yuan et al., 2007), and energy (Inoue et al., 1984; Engberg et al., 1996) have declined when feeding peroxidized lipids. However, this effect may depend on the level of peroxidation products present. Feeding diets containing soybean oil with PV of 440 meq O_2/kg to broilers reduced ME, and the digestibility of crude fat and linoleic acid relative to diets with lower (1 to 309 meq O_2/kg) PV (Inoue et al., 1984). In contrast, DeRouchey et al. (2004) and Liu (2012) found no effect of dietary peroxidized lipid on the digestibility of DM, N, or GE. Conflicting research results likely are linked to variable fatty acid profiles and products of peroxidation within the dietary lipids fed. Regardless, digestibility of energy and other nutrients may decline in diets with peroxidized lipids. This reduction may be related to altered digestibility resulting from the presence of products of peroxidation and changes in gastrointestinal physiology.

Peroxidized lipids contain numerous compounds which are absorbed and metabolized to varying extents. Lipid peroxides are absorbed and rapidly catabolized within enterocytes by GPx and GSH (Aw, 2005). However, with insufficient cellular GSH or NADPH, lipid peroxides are exported into the lymphatic system (Aw, 2005). In a review on the fate of peroxidized lipids in the gastrointestinal tract, Márquez-Ruiz et al.

(2008) indicated that mono-peroxides are susceptible to enzymatic hydrolysis by pancreatic lipase, but triacylglycerol dimers and polymers are not. Furthermore, polymers may reduce the digestibility of unperoxidized and monomeric peroxidized fatty acids because of altered micellar solubility (Márquez-Ruiz et al., 2008). Ultimately, these data suggest that reduced digestibility of polymers contribute to reduce the energy value of peroxidized lipids.

Some researchers have suggested that the negative effects on digestibility are related to peroxidative damage to cells of the gastrointestinal tract (Hoerr, 1998). Dietary peroxidized lipids induce metabolic oxidative stress in enterocytes (Reddy and Tappel, 1974; Ringseis et al., 2007), which may have negative implications on their functionality, size, and rate of turnover. The half-life of enterocytes (Dibner et al., 1996b) and villi height (Dibner et al., 1996a) was reduced in broilers fed diets containing peroxidized poultry fat. Enterocyte turnover has an energetic cost (Kelly and McBride, 1990; Nyachoti et al., 1997). Thus, increased enterocyte turnover may reduce energy available for growth or other productive purposes. Diminished villi height reduces the surface area available for nutrient absorption (Mosenthin, 1998) which potentially can reduce nutrient digestibility. Dietary ethoxyquin increased villi height in broilers fed peroxidized lipids (Dibner et al., 1996a), suggesting that dietary antioxidants may have ameliorative effects under oxidative stress. However, it is unclear if ethoxyquin exerts this effect by sparing the peroxidative destruction of lipid soluble antioxidants in the diet during storage and digestion, or if it incorporated into tissue to act as a metabolic antioxidant. Some researchers have suggested that bolus doses of ethoxyquin are metabolized and excreted rapidly (Wilson et al., 1959), but the practical significance of such findings may be

limited for animals fed ethoxyquin for extended periods of time. Ethoxyquin may be added routinely to diets or ingredients and unknowingly not considered when interpreting research results. Detectable levels of ethoxyquin were present in several tissues, milk, and blood of rats fed diets with 50 ppm ethoxyquin for 10 to 11 d, with highest (> 2ppm) levels detected in the liver and kidney (Wilson et al., 1959). Similarly, lambs retain dietary ethoxyquin in tissues, and this effect is dose dependent (deMille et al., 1972). These data suggest that ethoxyquin may be incorporated into tissues for utilization as an antioxidant.

Liver size relative to body weight, which is often described as the hepatosomatic index or relative liver weight, serves as a biological indicator of toxicity (Juberg et al., 2006). Feeding diets containing peroxidized lipids results in increased liver size in swine (Liu, 2012), rodents (Huang et al., 1988; Eder, 1999), and broilers (Anjum et al., 2004). This response may be a result of increased synthesis of enzymes to mitigate toxicity (Huang et al., 1988) or increased proliferation of hepatocytes (Dibner et al., 1996b). However, it is not clear if these alterations in liver size correlate with altered hepatic metabolism and efficiency of nutrient utilization. Hepatic transaminase enzymes in serum may indicate hepatocytic damage or necrosis and liver damage (Reichling and Kaplan, 1988). Engberg et al. (1996) showed that dietary peroxidized lipids did not affect the serum plasma content of aspartate aminotransferase, but liver size was not reported in that study.

The specific mechanism(s) by which growth performance and efficiency of nutrient utilization is reduced when animals are fed diets with peroxidized lipids remains unclear. While negative effects may link to health or altered digestion and absorption,

negative effects may also relate to altered mitochondrial metabolism. Some researchers have linked the phenotypic expression of gain efficiency and residual feed intake with efficiency and oxidative stress at the mitochondrial level (Bottje and Carstens, 2009). Specifically, mitochondrial levels of peroxides, as well as concentrations of protein carbonyls and ubiquitin (a marker of intracellular protein degradation) in muscle were reduced in animals with greater efficiency relative to their less efficient counterparts. Mitochondria produce 90% of ATP in the body, thereby modulating the amount of energy available for metabolic purposes. Normally, up to 4% of the oxygen consumed by a mitochondrion is converted to superoxide and subsequently converted to peroxide by SOD and eliminated via reactions with GPx and GSH (Nelson and Cox, 2008). However, the antioxidant system is reduced during oxidative stress induced by dietary peroxidized lipids (Inoue et al., 1984; Engberg et al., 1996; Takahashi and Akiba, 1999; Tavárez et al., 2011; Boler et al., 2012; Liu, 2012). As previously described, ROS may alter proteins, DNA, and lipids *in vivo* (Yu, 1994). Peroxidative damage may interfere with normal functionality and longevity of mitochondria (Fariss et al., 2005). Perhaps antioxidants which reduce mitochondrial oxidative stress may improve growth and efficiency of nutrient utilization of animals fed diets with peroxidized lipids.

SUMMARY

Lipids are commonly incorporated into swine diets to increase dietary caloric density, improve gain efficiency, improve palatability, control dust, and supply essential fatty acids and vitamins (Azain, 2001). However, lipids with high concentrations of PUFA may become peroxidized during processing and storage when exposed to heat, air, moisture, and other pro-oxidants, but peroxidation is impeded by antioxidants (Belitz et

al., 2009). Furthermore, dietary peroxidized lipids can be further peroxidized and contribute to metabolic oxidative stress and adverse physiological changes in animals. Dietary peroxidized lipids are associated with compromised health and growth performance of swine and poultry (Robey and Shermer, 1994; Dibner et al., 2011). Specifically, reductions in gain efficiency (McGill et al., 2011a; McGill et al., 2011b; Tavárez et al., 2011), growth rate (Boler et al., 2012; Liu, 2012), metabolic oxidative status (Boler et al., 2012; Liu, 2012), energy digestibility (Inoue et al., 1984; Engberg et al., 1996), increased mortality (Takahashi and Akiba, 1999; Anjum et al., 2004), and impaired immune function (Dibner et al., 1996b) have been observed frequently, but not consistently. Furthermore, maximal tolerable limits for dietary inclusion of peroxidized lipids have not been established. Use of dietary antioxidants may attenuate the negative effects of peroxidized lipids partially (Oldfield et al., 1963; Cabel et al., 1988; Lin et al., 1989; McGill et al., 2011b; Tavárez et al., 2011), but relative effectiveness and optimal dietary concentrations of exogenous antioxidants have not been determined.

Therefore, subsequent chapters of this dissertation are focused on developing a better understanding of the impact of lipid peroxidation in DDGS and corn oil on the growth performance, health, and metabolic oxidative status of young pigs. In addition, the extent of lipid peroxidation in DDGS and corn oil was assessed during heating and storage in the presence and absence of antioxidants. This dissertation will provide diet formulators and swine nutritionists with much needed information when sourcing, evaluating, and using feed ingredients containing peroxidized lipids, and offer initial observations on the relative importance of the addition of dietary antioxidants.

CHAPTER 2. PEROXIDIZED LIPIDS REDUCE GROWTH PERFORMANCE AND METABOLIC OXIDATIVE STATUS OF PIGS AND BROILERS

SUMMARY

To evaluate the effects of dietary peroxidized lipids on the growth and metabolic oxidative status of nonruminants, data were summarized from studies that measured growth performance of pigs (n = 16 comparisons) and broilers (n = 26 comparisons) fed diets containing peroxidized lipids. Only studies evaluating supplemental lipid sources in isocaloric diets were included. Dietary thiobarbituric acid reactive substances (**TBARS**) and peroxide value (**PV**) were obtained from each study, along with response variables including ADG, ADFI, G:F, and circulating concentrations of vitamin E and TBARS. Data were evaluated using UNIVARIATE and CORR procedures of SAS. Overall responses for swine and broilers fed diets with peroxidized lipids showed that ADG was 88.8 ± 12.5% (range = 49.8 to 104.6%), ADFI was 92.5 ± 9.0% (range = 67.8 to 109.8%), and G:F was 95.7 ± 7.2% (range = 70.4 to 106.3%) relative to animals fed diets with unperoxidized lipids. The magnitude of reduction from feeding diets with peroxidized lipids relative to diets with unperoxidized lipids was 11.4 and 11.1% for ADG and 8.8 and 6.6% for ADFI for swine and poultry, respectively. For swine, ADG correlated negatively with dietary TBARS content (r = - 0.63, $P = 0.05$), but not PV. Conversely, dietary PV correlated negatively with ADG in broilers (r = - 0.78, $P < 0.01$), but dietary TBARS concentrations were not reported in any of the 26 broiler studies included in this review. The difference in magnitude of change for ADG (11.2%) compared to ADFI (7.5%) suggests that factors in addition to caloric intake contribute to reduced ADG when feeding peroxidized lipids. For swine and broilers fed peroxidized

lipids, serum content of vitamin E was 53.7 ± 26.3% (range = 15.2 to 105.8%, n = 18) and TBARS was 119.7 ± 23.3% (range = 97.0 to 174.8%, n = 12) relative to animals fed unperoxidized lipids, indicating that inclusion of peroxidized lipids in diets contributes to changes in metabolic oxidative status. Historically, PV has been used to assess lipid peroxidation, but TBARS may be a better measure for predicting the effects of lipid peroxidation on growth in swine. Future research is necessary to develop an accurate model for predicting reductions in growth performance and metabolic oxidative status when feeding diets containing peroxidized lipids.

KEYWORDS: broiler, fat, lipid peroxidation, oil, peroxide value, swine

INTRODUCTION

Energy is the most expensive dietary component for swine and poultry. Corn and dried distillers grains with solubles (**DDGS**) commonly provide a substantial portion of dietary energy, but supplemental lipids are often added to swine and poultry diets to increase energy density. About 22% of fats and oils used for industrial purposes are incorporated into animal diets in the United States (USDA, 2010). However, the quality of dietary lipids is variable. Peroxide value (**PV**) has been used as an indicator of lipid quality, but a recent industry survey showed that PV ranged from 0.1 to 180.8 meq O_2/kg (mean = 2.6 meq O_2/kg) among 610 samples of dietary lipids (Dibner, 2013; personal communication). These data indicate that highly peroxidized lipids are present in the marketplace.

Dietary lipids are susceptible to peroxidation. Lipid peroxidation is associated with the degree of unsaturation of lipids positively, and is accelerated by exposure to heat, air, moisture, and pro-oxidant metals (Belitz et al., 2009). Therefore, lipids in feed

ingredients may become peroxidized depending on storage or processing conditions (Dibner et al., 2011; Song and Shurson, 2013). Peroxidation converts fatty acids into numerous products (Spiteller et al., 2001; Seppanen and Csallany, 2002; Belitz et al., 2009), and degrades antioxidants such as vitamin E (Seppanen and Csallany, 2002; Liu, 2012). In addition to PV, several analytical assays exist to evaluate dietary lipid peroxidation, but the accuracy of using these peroxidation tests to predict animal growth performance and health of animals is unclear.

Feeding peroxidized lipids reduces gain efficiency (McGill et al., 2011a; McGill et al., 2011b; Tavárez et al., 2011), growth rate (Boler et al., 2012; Liu, 2012), metabolic oxidative status (Boler et al., 2012; Liu, 2012), and energy digestibility (Inoue et al., 1984; Engberg et al., 1996), while increasing mortality (Takahashi and Akiba, 1999; Anjum et al., 2004), and impairing immune function (Dibner et al., 1996b) of swine and broilers. However, these responses have not been observed consistently, and the magnitude of response varies among experiments.

Therefore, the objectives of this study were to compile and summarize data from published studies which evaluated the effects of peroxidized lipids on growth performance of pigs and broilers, and determine the association between measures of lipid peroxidation and growth performance of animals fed peroxidized lipids.

MATERIALS AND METHODS

Publications with growth performance data from pigs and broilers fed peroxidized lipids were obtained by searching online indexes (e.g. Google Scholar), scholarly journal archives, and conference proceedings using keywords including oxidized lipids, growth, broilers, or swine. Additional publications were identified by retrieving citations within

many of the published papers. The main criteria for inclusion of experiments in this review were: (1) animals were fed isocaloric diets with peroxidized and unperoxidized lipids, and (2) growth performance responses (i.e. G:F) were evaluated regardless of whether the responses were positive or negative.

Thirteen journal articles, 1 abstract, 1 proceedings paper, and 1 dissertation (n = 16 studies) were obtained (Oldfield et al., 1963; Inoue et al., 1984; Cabel et al., 1988; Lin et al., 1989; Engberg et al., 1996; Takahashi and Akiba, 1999; Anjum et al., 2002, 2004; DeRouchey et al., 2004; Racanicci et al., 2008; Harrell et al., 2010; McGill et al., 2011a,b; Tavárez et al., 2011; Boler et al., 2012; Liu, 2012). Some studies evaluated the effects of multiple diets containing peroxidized lipids relative to the effect of similar diets containing unheated lipids. This resulted in 16 and 26 comparisons for pigs and broilers, respectively. Lipid sources included corn oil, vegetable oil, canola oil, sunflower oil, soybean oil, beef tallow, poultry fat, fish oil, and choice white grease. Temperatures to induce peroxidation (reported by 83% of comparisons) ranged from 22 to 185°C for 7 to 98 h (mean = 103.9°C for pig experiments and 66.8°C for broiler experiments). Of the 42 comparisons utilized, 100% reported PV, 23% reported thiobarbituric acid reactive substances (**TBARS**), and 28% reported *p*-anisidine value (**AnV**). Because AnV is a unitless measure, it was excluded from the current investigation. The PV of lipids ranged from 1 to 440 meq O_2/kg when reported (mean = 175 for pig experiments and 58 meq O_2/kg for broiler experiments). The TBARS content ranged from 7 to 155 mg malondialdehyde (**MDA**) eq/kg fat when reported (mean = 58 mg MDA eq/kg fat for pigs), and this measure was not reported in studies involving broilers. Control

(unperoxidized) lipids used in these experiments were not subjected to conditions (e.g. heat or forced air) which induced peroxidation.

From each comparison, the following data were extracted or calculated: reported temperature to induce peroxidation, dietary TBARS and PV concentrations, supplemental dietary vitamin E and Se concentrations, duration of the experiment, initial age (broilers) or BW (swine), ADG, ADFI, G:F, and circulating concentrations of α-tocopherol and TBARS. Data were tabulated in a spreadsheet (Excel 2010, Microsoft Corporation; Redmond, WA) with a separate row for each comparison. Within each comparison, dependent variables were expressed as a percentage relative to animals fed the same dietary lipid source which was unperoxidized.

Statistical Analysis

The UNIVARIATE and CORR procedures of SAS (v9.3; SAS Inst. Inc., Cary, NC) were used to summarize the data separately and together for swine and broilers. Means, standard deviations, correlation coefficients, and *P* values of correlations are reported. Significance was declared at $P < 0.05$, whereas values between $0.05 \leq P \leq 0.15$ were considered statistical trends.

RESULTS AND DISCUSSION

Feeding peroxidized lipids reduces gain efficiency of animals in some studies (Takahashi and Akiba, 1999; Anjum et al., 2004; McGill et al., 2011a; McGill et al., 2011b; Tavárez et al., 2011), but not in others (Oldfield et al., 1963; Racanicci et al., 2008; Upton et al., 2009). Inconsistent findings may relate to the variable fatty acid profiles, dietary inclusion levels, methods employed to induce peroxidation, or inadequate replication among studies. After compiling data from 42 evaluations

comparing responses from feeding peroxidized and unperoxidized lipids to swine and broilers, statistically significant responses varied among experiments for growth performance (Table 2-1). One experiment was omitted from Table 2-1 (DeRouchey et al., 2004) because data were not subjected to mean separation procedures. Mean value for ADG was 88.8 ± 12.5% (range = 49.8 to 104.6%), ADFI was 92.5 ± 9.0% (range = 67.8 to 109.8%), and G:F was 95.7 ± 7.1% (range = 70.4 to 106.3%) for animals fed dietary peroxidized lipids relative to those fed unperoxidized lipids (Table 2-2). The magnitude of reduction from feeding diets with peroxidized lipids relative to diets with unperoxidized lipids for ADG (11.4 vs. 11.1%), ADFI (8.8 vs. 6.6%), and G:F (3.4 vs. 4.6%) were similar for swine and poultry, respectively. These results suggest that growth performance of both species is reduced to a similar extent when feeding peroxidized lipids. Reduced growth rate is linked partially to the reduced ADFI which some have associated with feeding dietary peroxidized lipids (Oldfield et al., 1963; Engberg et al., 1996; Anjum et al., 2002, 2004; DeRouchey et al., 2004; Tavárez et al., 2011; Boler et al., 2012). Reduced ADFI may relate to undesirable odor or flavor of aldehydic compounds generated during peroxidation (Esterbauer et al., 1991; Halliwell and Chirico, 1993). Regardless, the greater magnitude of change for ADG (11.2%) relative to ADFI (7.5%) suggests that factors in addition to ADFI contribute to reduce ADG when feeding peroxidized lipids. Metabolic oxidative stress and physiological changes resulting from feeding peroxidized lipids may also contribute to reduced growth performance. Although a specific mechanism affecting growth when feeding peroxidized lipids has not been elucidated, reduced efficiency of nutrient utilization with dietary peroxidized lipids may be related to reduced energy and nutrient digestibility (Inoue et al., 1984; Engberg et al.,

1996) as well as impaired immune function (Dibner et al., 1996b). Ultimately, understanding the factors that contribute to varied growth performance responses from feeding peroxidized lipids is critical for nutritionists to avoid unexpected reductions in animal performance.

Dietary peroxidation measures that accurately predict reduction in growth performance have not been established. Lipid hydroperoxides are produced initially during lipid peroxidation, but they subsequently react to form numerous products including aldehydes, ketones, acids, esters, hydrocarbons, epoxides, polymers, lactones, furans, and aromatic compounds (Belitz et al., 2009). Numerous assays are available to assess peroxidation in dietary lipids. Peroxide value and TBARS are commonly reported, but other markers such as AnV, conjugated dienes, hexanal, 4-hydroxynonenol, 2,4-decadienal, and hexanal can be measured. Currently, the relationship of these markers with growth performance and health of animals is unclear. Dietary TBARS content correlated negatively with ADG (r = - 0.63, $P = 0.05$; Figure 2-1) for swine, but PV did not (Table 2-3). Conversely, dietary PV correlated negatively with ADG in broilers (r = -0.78, $P < 0.01$; Table 2-4 and Figure 2-2), but dietary TBARS concentrations were not reported in any of the 26 broiler studies. Initially, these data suggest that dietary TBARS concentration partially predicts the effects of lipid peroxidation on growth rate of swine, and PV may predict changes in growth rate of broilers. However, these relationships seem to be influenced strongly by a few (1 to 2) extreme data points, so these results should be considered with caution. In fact, when the most extreme data point was removed (dietary TBARS = 16.2 mg MDA eq/kg), the correlation between ADG and dietary TBARS was not significant (r = -0.38, $P = 0.31$). Interestingly, there was no

correlation between temperature (reported by 83% of comparisons) used to produce peroxidized lipids and measures of growth performance (Tables 2-3 and 2-4) across these experiments. However, lower temperatures (mean = 66.8 ± 24.8˚C) were used to create peroxidation of experimental lipids used in the broiler experiments compared to those used in the swine experiments (mean = 103.9 ± 55.3˚C). Peroxides are degraded at high temperatures (Shahidi and Zhong, 2005). Consequently, researchers report that PV increases initially and later declines in lipids exposed to high temperatures (≥ 80°C; (DeRouchey et al., 2004; Danowska-Oziewicz and Karpińska-Tymoszczyk, 2005). Conversely, others have reported that PV increases continuously in lipids during peroxidation at lower temperatures (≤ 65°C; Wanasundara and Shahidi, 1994; Naz et al., 2005; Winkler-Moser and Breyer, 2011; Chen et al., 2014). Therefore, rather than suggesting a species dependent response to PV, our results indicate that PV has limited utility for predicting growth performance responses when excessively heated lipids (i.e. those used for swine) are fed. Regardless, future research is necessary to develop multifactorial models to accurately predict growth performance when feeding diets containing peroxidized lipids.

Currently, nutritionists lack practical guidelines for maximal tolerable limits for adding peroxidized lipids to swine and poultry diets. However, some researchers have suggested acceptable thresholds levels of specific peroxidation products. Azain (2001) suggested that lipids with PV below 5 meq/kg are "not currently rancid," implying that such lipids are of acceptable quality. Conversely, Gray and Robinson (1941) suggested that lipids with PV greater than 20 meq/kg are "definitely rancid," and their dietary inclusion should be limited to 10 to 15% of the diet. However, experimental data to

support these claims was not provided (Gray and Robinson, 1941). Similarly, DeRouchey et al. (2004) suggested feeding diets with PV levels greater than 2.4 meq O_2/kg can result in reduced growth performance of pigs. However, the worst growth performance response in their experiment was achieved when pigs were fed diets with PV below 1 meq O_2/kg (DeRouchey et al., 2004). Liu (2012) also showed substantial reductions in growth performance when diets contained low (<1 meq O_2/kg) levels of PV (Liu, 2012). In the current summary, dietary PV ranged from 0.06 to 44.0 meq O_2/kg (mean = 8.6 ± 9.6 meq O_2/kg). Gain efficiency declined significantly in 39% of the comparisons, and declined numerically in 74% of the comparisons. Perhaps had additional statistical replication been employed, a greater proportion of differences would have been significant.

These data suggest that PV predicts ADG when peroxidized lipids exposed to low temperatures are fed to broilers. However, PV was the only measure used to estimate the extent of lipid peroxidation reported in some studies (Inoue et al., 1984; Cabel et al., 1988; Lin et al., 1989; Takahashi and Akiba, 1999; Anjum et al., 2002, 2004; Upton et al., 2009; McGill et al., 2011a,b; Tavárez et al., 2011) which prohibits assessment of the utility of using other measures of lipid peroxidation. To facilitate the development of predictive models, researchers should report more comprehensive data on the composition and extent of peroxidation (including the use of multiple peroxidation measures) of experimental lipids in future experiments.

The metabolic oxidative status of animals generally declines as indicators of peroxidation (e.g. TBARS or protein carbonyls) increase. Consequently, levels of vitamin E in serum or tissue decline with increasing concentrations of dietary peroxidized lipids

for pigs (Boler et al., 2012; Liu et al., 2012a), sows (Nielsen et al., 1973), rats (Liu and Huang, 1995; Eder, 1999; Brandsch and Eder, 2004) and broilers (Takahashi and Akiba, 1999; Tavárez et al., 2011). This effect may partially relate to degradation of vitamin E in peroxidized lipids resulting in reduced intake (Liu, 2012), altered uptake, or increased metabolic utilization (Liu and Huang, 1995). Conversely, tissue or serum concentrations of TBARS increase with dietary peroxidized lipids for swine (Boler et al., 2012), rats (Liu and Huang, 1995), and broilers (Lin et al., 1989; Takahashi and Akiba, 1999; Anjum et al., 2004). In the current study, the effect of peroxidized lipids on measures of metabolic oxidative status varied (Table 2-5). However, the serum content of vitamin E was 53.7 ± 26.3% (range = 15.2 to 105.8%, n = 18) and TBARS was 119.7 ± 23.3% (range = 97.0 to 174.8%, n = 12) for broilers and swine fed diets with peroxidized lipids relative to animals fed unperoxidized lipids (Table 2-2). Upton et al. (2009) showed that the activity of the antioxidant enzyme glutathione peroxidase (**GPx**) increased in the liver of broilers fed peroxidized poultry fat. These authors suggested that the production of GPx increased in response to peroxides in dietary peroxidized lipid. Ultimately, the increased concentration of cirulating TBARS accompanied by reduced circulating concentrations of vitamin E suggests that metabolic oxidative stress occurred when feeding dietary peroxidized lipids in these studies.

Dietary constituents that can be used to predict changes in metabolic oxidative status have not been identified, and the relationships of these dietary markers with changes in metabolic markers and growth performance are not clear. The circulating concentrations of TBARS and vitamin E correlated negatively (r = -0.66, P = 0.02), which is similar to correlations reported by others (Hossein Sadrzadeh et al., 1994; Liu

and Huang, 1995; Yanik et al., 1999). In pigs, serum concentration of TBARS was associated positively with dietary PV (r = 0.64, P = 0.06) and TBARS (r = 0.78, P = 0.02), but associations between serum vitamin E content and dietary markers were not observed. Furthermore, a similar relationship was not observed for broilers (P > 0.05), but relatively few (< 10%) broiler experiments reported serum concentrations of TBARS. Although these biomarkers indicate that metabolic oxidative stress occurred, their use as predictors of subsequent growth performance appears to be limited. For example, circulating content of vitamin E tended to associate positively with ADG (r = 0.41, P = 0.07) and ADFI (r = 0.46, P = 0.05) for swine and broilers, but these effects are a function of increased ADFI and daily intake of vitamin E, respectively. It appears that comprehensive targeted metabolomics analysis is warranted to identify more reliable metabolites that can be used to more accurately predict animal growth performance responses when feeding peroxidized lipids.

Some researchers have suggested antioxidants may alleviate some of the negative effects of peroxidized lipids on growth performance. Reductions in growth rate (Tavárez et al., 2011) and gain efficiency (Cabel et al., 1988; McGill et al., 2011b) were recovered partially when ethoxyquin was added as an antioxidant to diets. Similarly, Oldfield et al. (1963) reported that adding dl-α tocopheryl acetate or ethoxyquin to swine diets containing peroxidized menhaden fish oil resulted in complete recovery of ADG and ADFI to levels similar to control animals. Lin et al. (1989) reported similar recovery when d-α tocopherol or other antioxidants (butylated hydroxytoluene and butylated hydroanisole) were added to broiler diets containing peroxidized sunflower oil. These results suggest that inadequate dietary or metabolic amounts of antioxidants contribute to

reduced growth performance of animals fed peroxidized lipids, and that partial recovery is possible by increasing dietary antioxidants. However, the ameliorative effect of antioxidants is not consistent (Wang et al., 1997; Harrell et al., 2010; McGill et al., 2011a; Boler et al., 2012). In the current study, the concentration of supplemental vitamin E correlated positively (r = 0.61, $P = 0.03$) with G:F for swine. Superficially, this finding seems to support the idea that the reduction in growth performance when feeding peroxidized lipids can be mitigated partially by dietary antioxidants. However, this effect is likely an artifact of the study rather than a cause and effect relationship because only 2 studies (DeRouchey et al., 2004; Liu, 2012) reported the supplemental dietary concentrations of vitamin E (39 or 44 IU/kg) for pigs. A similar relationship was not found for broilers. Therefore, future research studies should provide more information about vitamin E, antioxidants, and other nutrients that have antioxidant properties to better characterize the antioxidant status of the diet and its potential relationship to growth performance responses when feeding peroxidized lipids.

In summary, results from experiments reported in published studies show that feeding peroxidized lipids reduces growth rate and gain efficiency of swine and broilers. While the specific mode(s) of action remain unknown, these findings may relate to the antioxidant capacity of the diet and the metabolic oxidative stress status of pigs and broilers. There is a dearth of knowledge regarding the relative value and accuracy of using a wide variety of indicator and predictive assays to define the degree of lipid peroxidation in animal feed ingredients and diets. Furthermore, thresholds for the dietary inclusion of peroxidized lipids are poorly defined. Therefore, multifactorial and

metabolomics-based approaches must be utilized to identify the most accurate measures of lipid peroxidation and more appropriate biological indicators for prediction of animal growth performance reductions when feeding peroxidized lipids to swine and broilers.

Table 2-1. Statistical significance of the effects of peroxidized lipid on growth performance of swine and broilers

		Response to dietary peroxidized lipids, no. of experiments[1]			
Item	n	Increased[2]	Decreased[2]	Not changed[3]	Not reported or analyzed statistically[4]
ADG	38	0	20	12	6
ADFI	38	0	8	25	5
G:F	38	1	15	22	0

[1]Data from experiments by Oldfield et al., 1963; Inoue et al., 1984; Cabel et al., 1988; Lin et al., 1989; Engberg et al., 1996; Takahashi and Akiba, 1999; Anjum et al., 2002, 2004; Racanicci et al., 2008; Harrell et al., 2010; McGill et al., 2011a,b; Tavárez et al., 2011; Boler et al., 2012; and Liu, 2012. Data from DeRouchey et al. (2004) were excluded because mean separation tests were not performed.

[2]$P < 0.05$, relative to isocaloric diet with unperoxidized lipid. Some comparisons increased numerically, but the response was not statistically significant.

[3]$P > 0.05$, relative to isocaloric diet with unperoxidized lipid.

[4]For some experiments, ADG was calculated from other available data, and was therefore not analyzed statistically.

Table 2-2. Summary of 42 evaluations of dietary peroxidized lipids for broilers (n = 26) and pigs (n = 16)

Citation	Species	Lipid source[1]	PV, meq/kg diet[2]	ADG[3]	ADFI[3]	G:F[3]	Serum vitamin E[3]	Serum TBARS[3,4]
Inoue et al., 1984	broilers	soy oil	44.0	49.8	70.1	70.4	23.1	.
Inoue et al., 1984	broilers	soy oil	30.9	60.7	76.4	78.7	50.0	.
L'Estrange et al., 1966	broilers	beef	10.9	68.9	67.8	101.2	.	.
Takahashi and Akiba, 1999	broilers	soy oil	10.0	80.2	90.6	88.5	19.6	174.8
Inoue et al., 1984	broilers	soy oil	22.0	85.8	92.8	92.0	47.8	.
Inoue et al., 1984	broilers	soy oil	15.5	87.4	92.8	94.4	62.3	.
Wang et al., 1997	broilers	poultry fat	26.8	89.3	92.3	91.7	.	.
Engberg et al., 1996	broilers	vegetable	17.2	92.2	92.8	99.4	61.4	.
Takahashi and Akiba, 1999	broilers	soy oil	10.0	92.5	99.1	93.3	15.2	156.5
Anjum et al., 2002	broilers	soy oil	1.5	93.7	98.8	94.6	.	.
Tavárez et al., 2011	broilers	soy oil	6.8	93.9	98.4	95.5	41.5	97.0
Inoue et al., 1984	broilers	soy oil	11.7	94.2	103.2	90.4	105.8	.
Lin et al., 1989	broilers	sunflower	22.0	95.4	98.2	97.1	.	.
Anjum et al., 2004	broilers	soy oil	1.0	95.8	98.6	97.1	.	.
McGill et al., 2011a	broilers	A-V blend	3.5	96.8	99.3	97.8	.	.
Anjum et al., 2002	broilers	soy oil	1.0	97.3	98.9	98.2	.	.
Inoue et al., 1984	broilers	soy oil	5.9	97.5	96.0	101.9	60.9	.
McGill et al., 2011a	broilers	A-V blend	7.1	97.8	99.1	98.4	.	.
McGill et al., 2011b	broilers	A-V blend	7.1	99.3	99.3	98.4	.	.
McGill et al., 2011b	broilers	A-V blend	3.5	99.3	100.0	98.9	.	.
Racanicci et al., 2008	broilers	poultry fat	1.6	100.0	97.8	101.9	.	.
Upton et al., 2009	broilers	poultry fat	3.0	.[5]	.	101.5	.	.
Upton et al., 2009	broilers	poultry fat	6.0	.	.	104.6	.	.
Cabel et al., 1988	broilers	poultry fat	7.2	.	.	96.5	.	.
Cabel et al., 1988	broilers	poultry fat	2.1	.	.	99.4	.	.
Cabel et al., 1988	broilers	poultry fat	4.1	.	.	99.4	.	.
Mean, broilers				**88.9**	**93.4**	**95.4**	**48.8**	**142.8**

Table 2-2 *(continued)*. Summary of 42 evaluations of dietary peroxidized lipids for broilers (n = 26) and pigs (n = 16)

Oldfield et al., 1963	pigs	fish oil	6.1	64.6	77.1	83.5	.	.
Liu, 2012	pigs	canola oil	23.9	74.6	89.5	84.1	48.9	122.8
Liu, 2012	pigs	canola oil	1.2	75.7	78.8	96.8	60.0	120.4
Liu, 2012	pigs	corn oil	0.2	76.9	84.4	91.3	18.9	114.8
Liu, 2012	pigs	poultry fat	0.2	77.3	87.1	88.6	70.0	103.4
Liu, 2012	pigs	tallow	0.3	86.3	89.4	95.7	89.7	102.0
DeRouchey et al., 2004	pigs	ch. white grease	0.1	91.0	90.8	100.3	.	.
Liu, 2012	pigs	corn oil	15.1	91.6	88.8	102.9	29.0	121.0
Boler et al., 2012	pigs	corn oil	7.5	93.4	95.0	95.3	46.3	113.1
Oldfield et al., 1963	pigs	fish oil	1.6	93.7	90.0	104.0	.	.
Harrell et al., 2010	pigs	corn oil	7.5	94.7	94.8	99.8	.	.
DeRouchey et al., 2004	pigs	ch. white grease	6.3	95.4	92.1	103.6	.	.
DeRouchey et al., 2004	pigs	ch. white grease	0.1	96.8	91.1	106.3	.	.
DeRouchey et al., 2004	pigs	ch. white grease	2.4	100.2	98.1	102.1	.	.
Liu, 2012	pigs	poultry fat	5.7	101.4	102.0	97.1	73.6	107.0
Liu, 2012	pigs	tallow	2.9	104.6	109.8	94.3	96.6	103.1
Mean, swine				**88.6**	**91.2**	**96.6**	**59.2**	**112.0**
Mean, broilers and swine				**88.8**	**92.5**	**95.9**	**53.7**	**119.7**

[1]Lipid source = source of supplemental fat, A-V blend = blend of animal fat-vegetable oil, and ch. white grease = choice white grease.

[2]PV = peroxide value of diet containing peroxidized lipid was calculated as: [fat inclusion level,% x PV of fat source] or reported as stated by researchers.

[3]All response variables were calculated by dividing the mean of the animals fed diets with peroxidized lipids by those fed unperoxidized lipids and multiplying by 100. Therefore, the values presented represent the percentage of the variable relative to controls.

[4]TBARS = thiobarbituric acid reactive substances.

[5]A period indicates that this variable was not reported or estimable from the research reports.

Table 2-3. Pearson correlation matrix of measures of dietary lipid peroxidation, metabolic oxidative status, and growth performance in swine[1]

	Diet TBARS[2]	Diet PV[3]	Lipid processing temperature	Plasma or serum Vitamin E	Plasma or serum TBARS	ADG	ADFI	G:F	Initial BW[4]	Supplemented dietary Vitamin E	Supplemented dietary Se
Diet TBARS	1.00 10										
Diet PV	0.27 0.44 10	1.00 16									
Lipid processing temperature	***0.59*** ***0.07*** ***10***	-0.27 0.32 15	1.00 15								
Plasma or serum Vitamin E	-0.28 0.50 8	-0.35 0.35 9	0.01 0.98 9	1.00 9							
Plasma or serum TBARS	**0.78** **0.02** **8**	***0.65*** ***0.06*** ***9***	-0.21 0.59 9	**-0.75** **0.02** **9**	1.00 9						
ADG	***-0.63*** ***0.05*** ***10***	-0.16 0.55 16	-0.28 0.32 15	0.43 0.24 9	-0.45 0.22 9	1.00 16					
ADFI	***-0.55*** ***0.10*** ***10***	0.02 0.95 16	-0.21 0.45 15	0.54 0.14 9	-0.52 0.15 9	**0.87** **< 0.01** **16**	1.00 16				
G:F	-0.50 0.14 10	-0.31 0.25 16	-0.21 0.45 15	-0.02 0.96 9	0.00 0.99 9	**0.74** **< 0.01** **16**	0.31 0.24 16	1.00 16			
Initial BW	**0.72** **0.02** **10**	0.06 0.83 16	-0.33 0.23 15	-0.19 0.63 9	0.05 0.89 9	-0.05 0.86 16	-0.06 0.83 16	-0.12 0.65 16	1.00 16		
Supplemented dietary vitamin E	. . 8	-0.21 0.50 13	**-0.58** **0.04** **13**	-0.19 0.63 9	0.05 0.89 9	0.45 0.12 13	0.14 0.65 13	**0.61** **0.03** **13**	0.37 0.22 13	1.00 13	
Supplemented dietary Se	. . 8	-0.10 0.74 13	0.14 0.66 13	0.19 0.63 9	-0.05 0.89 9	-0.11 0.72 13	-0.11 0.72 13	0.07 0.81 13	**-1.00** **< 0.01** **13**	-0.37 0.22 13	1.00 13

[1]The Pearson correlation coefficient (r value), *P* value, and number of observations are listed in the top, middle, and bottom of each cell. Outcome variables (circulating vitamin E and thiobarbituric acid reactive substances [TBARS], ADG, ADFI, and G:F) were expressed as a percentage relative to animals fed unperoxidized lipids.

[2]TBARS = thiobarbituric acid reactive substances.
[3]PV = peroxide value.
[4]Initial BW was included to account for initial age of pigs.

Table 2-4. Pearson correlation matrix of measures of dietary lipid peroxidation, metabolic oxidative status, and growth performance in broilers[1]

	Diet PV[2]	Lipid processing temperature	Plasma or serum Vitamin E	Plasma or serum TBARS[3]	ADG	ADFI	G:F	Initial age	Supplemented dietary Vitamin E	Supplemented dietary Se
Diet PV	1.00 26									
Temperature	0.20 0.41 20	1.00 20								
Plasma or serum Vitamin E	-0.22 0.55 10	-0.33 0.67 4	1.00 10							
Plasma or serum TBARS	0.97 0.14 3	-0.97 0.14 3	-0.93 0.24 3	1.00 3						
ADG	**-0.78** **< 0.01** **21**	-0.20 0.48 15	0.39 0.26 10	-0.75 0.46 3	1.00 21					
ADFI	**-0.65** **< 0.01** **21**	0.29 0.30 15	0.41 0.24 10	-0.62 0.57 3	**0.93** **< 0.01** **21**	1.00 21				
G:F	**0.80** **< 0.01** **26**	0.22 0.36 20	0.32 0.37 10	-0.87 0.33 3	**0.81** **< 0.01** **21**	**0.55** **0.01** **21**	1.00 26			
Initial age	-0.04 0.83 26	0.00 0.99 20	-0.30 0.40 10	0.97 0.14 3	0.11 0.63 21	0.07 0.75 21	0.08 0.71 26	1.00 26		
Supplemented dietary Vitamin E	0.02 0.95 18	-0.14 0.59 18	***0.94*** ***0.06*** ***4***	-0.97 0.14 3	***0.48*** ***0.09*** ***13***	0.35 0.25 13	0.22 0.37 18	-0.19 0.45 18	1.00 18	
Supplemented dietary Se	0.41 0.11 16	-0.11 0.68 16	1.00 . 2	. . 1	0.46 0.15 11	0.37 0.27 11	0.05 0.86 16	**0.84** **< 0.01** **16**	0.36 0.18 16	1.00 16

[1]The Pearson correlation coefficient (r value), *P* value, and number of observations are listed in the top, middle, and bottom of each cell. Outcome variables (circulating vitamin E and thiobarbituric acid reactive substances [TBARS], ADG, ADFI, and G:F) were expressed as a percentage relative to animals fed unperoxidized lipids.

[2]PV = peroxide value.

[3]TBARS = thiobarbituric acid reactive substances.

Table 2-5. Statistical significance of effects of feeding dietary peroxidized lipids on metabolic oxidative balance in swine and broilers

		Response to dietary peroxidized lipids, no. of experiments[1]			
Item	n	Increased[2]	Decreased[2]	Not changed[3]	Not reported
Vitamin E[4]	38	0	14	5	19
TBARS[5]	38	3	0	14	21
GPx[6]	38	2	0	1	35

[1]Data from experiments by Oldfield et al., 1963; Inoue et al., 1984; Cabel et al., 1988; Lin et al., 1989; Engberg et al., 1996; Takahashi and Akiba, 1999; Anjum et al., 2002, 2004; Racanicci et al., 2008; Harrell et al., 2010; McGill et al., 2011a,b; Tavárez et al., 2011; Boler et al., 2012; and Liu, 2012. Data from DeRouchey et al. (2004) were excluded because mean separation tests were not performed.

[2]$P < 0.05$; relative to isocaloric diet with unperoxidized lipid.

[3]$P > 0.05$; relative to isocaloric diet with unperoxidized lipid.

[4]Tissue, serum, or plasma level of α-tocopherol.

[5]Tissue, serum, or plasma level of thiobarbituric acid reactive substances.

[6]Glutathione peroxidase activity of liver.

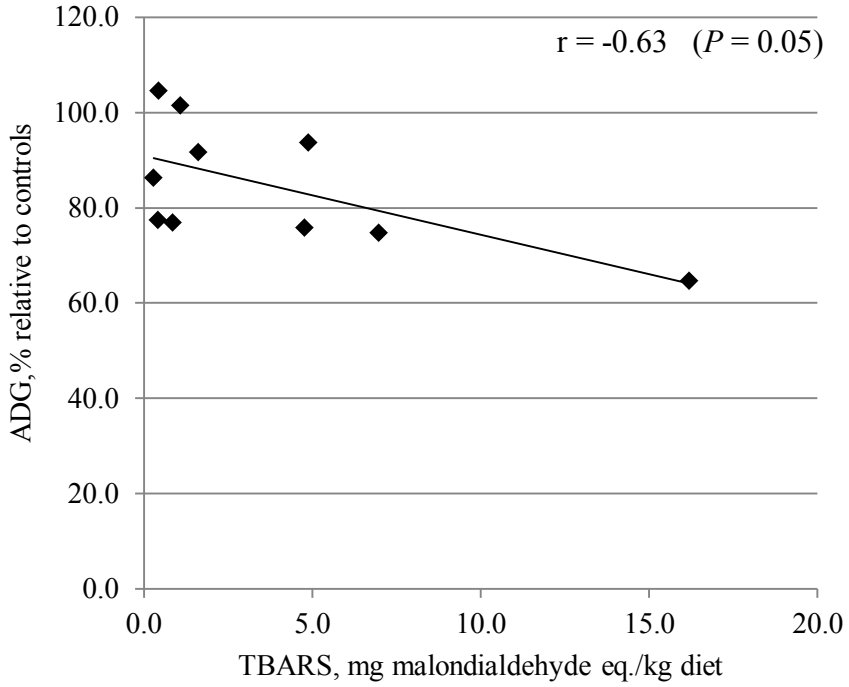

Figure 2-1. **Pearson correlation between dietary thiobarbituric acid reactive substances (TBARS, meq malondialdehyde eq/kg diet) and ADG of swine**

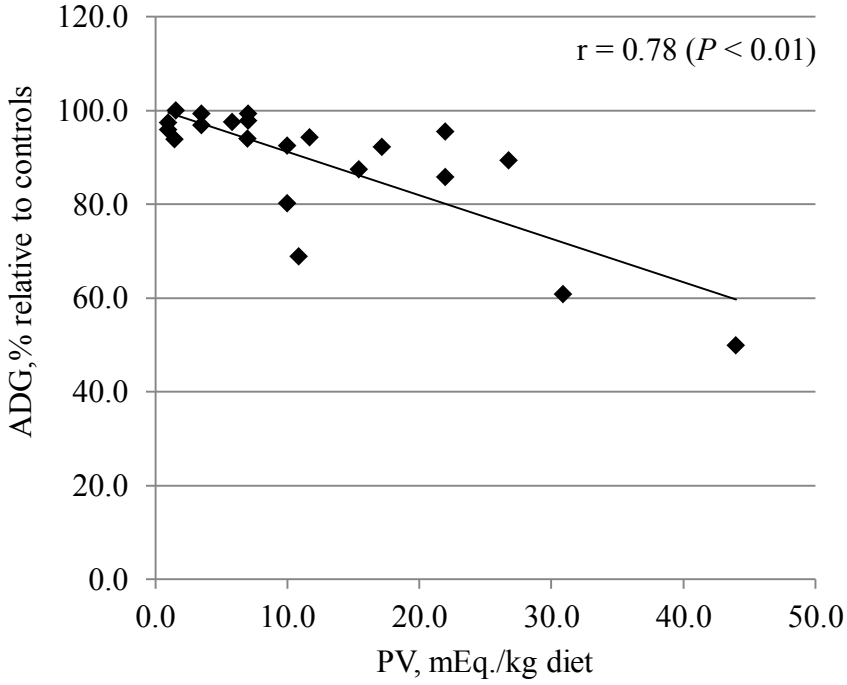

Figure 2-2. Pearson correlation between dietary peroxide value (PV; meq/kg diet) and ADG for broilers

CHAPTER 3. COMPARISON OF LIPID PEROXIDATION MEASURES FOR CORN OIL SUBJECTED TO VARYING THERMAL CONDITIONS

SUMMARY

Lipid peroxidation results in the production and degradation of numerous compounds, but assays commonly used to evaluate the extent of peroxidation measure a small proportion of these compounds. Therefore, a study was conducted to compare several indicators of peroxidation when heating refined corn oil at 185°C for 12 h (RO) or 95°C for 72 h (SO) sampled hourly or at 8 h intervals, respectively. Air flow was maintained at 12 L/min. Samples were assayed for PUFA, FFA, peroxide value (PV), anisidine value (AnV), thiobarbituric acid reactive substances (TBARS), hexanal, 4-hydroxynonenol (HNE), and 2,4-decadienal (DDE). The correlation procedure of SAS was used to evaluate associations among assays and within assays over time for each heat treatment. Unperoxidized refined corn oil (0 h) contained 53.9% PUFA, 1.1% FFA, 2.1 meq O_2/kg PV, 0.24 AnV, 17.8 mg malondialdehyde eq/kg TBARS, 1.7 ug hexanal/g, 1.1 ug HNE/mL, and 12.6 uM DDE. Regardless of treatment, PUFA was correlated negatively ($r < -0.9$, $P < 0.05$), whereas TBARS, hexanal, and HNE were correlated positively ($r > 0.6$, $P < 0.05$) with time. However, FFA was associated positively ($r = 0.86$, $P < 0.01$) with time for RO, but not for SO, and AnV was correlated positively ($r = 0.96$, $P < 0.01$) with time for SO, but not for RO. Peroxide value was correlated negatively ($r = -0.81$, $P < 0.01$) with time for RO, but correlated positively ($r = 0.94$, $P < 0.01$) with time for SO. Regardless of thermal treatment, hexanal ($r < -0.9$) and HNE ($r < -0.6$) were associated negatively ($P < 0.05$) with PUFA. Anisidine value was correlated negatively with PUFA ($r = -0.95$, $P < 0.01$) for SO, but not RO. In RO, FFA content was correlated positively

($P < 0.02$) with TBARS (r = 0.87), hexanal (r = 0.79), and HNE (r = 0.64), but not for SO. These results indicate that thermal processing and storage conditions should be considered when selecting indicators of peroxidation, but this information is seldom available. Peroxide value, AnV, and FFA measurements are variable indicators of peroxidation in oil exposed to different heating duration and temperatures. However, HNE and hexanal increased with heating duration and were reflected in PUFA degradation for both RO and SO, indicating that hexanal and HNE are reliable indicators of peroxidative damage in corn oil regardless of time and temperature conditions.

KEYWORDS: corn oil, lipid peroxidation, lipid stability, lipid quality, peroxidation assays, rancidity

INTRODUCTION

Polyunsaturated fatty acids are highly susceptible to peroxidation. Peroxidation is accelerated upon exposure to heat, air, moisture, and pro-oxidant metals which may be introduced during storage and processing (Belitz et al., 2009). Rendering exposes lipids to 115 to 145°C for 40 to 90 minutes (Meeker and Hamilton, 2006), and vegetable oils recycled for animal feed use from restaurants may be exposed to temperatures exceeding 180°C for variable durations (Belitz et al., 2009). Therefore, animal fats, vegetable oils, and dried distillers grains with solubles may be peroxidized to varying extents depending on the temperature and duration of thermal exposure (Dibner et al., 2011; Song and Shurson, 2013).

During peroxidation, fatty acids are degraded into lipid hydroperoxides which subsequently react to form numerous products including aldehydes, ketones, acids, esters, hydrocarbons, epoxides, polymers, lactones, furans, and aromatic compounds (Belitz et

al., 2009). Metabolically, endogenous and exogenous products of peroxidation may further promote peroxidative damage to physiological lipids within cellular and subcellular membranes, and can also modify enzymes, protein, and DNA (Yu, 1994). Certain aldehydes produced during peroxidation such as malondialdehyde (**MDA**) and 4-hydroxynonenal (**HNE**) are cytotoxic and mutagenic (Esterbauer et al., 1991). Ultimately, characterizing the extent of peroxidation in dietary lipids is important because peroxidation can have significant negative physiological effects on food animal production. Specifically, dietary inclusion of peroxidized lipids reduces growth performance of pigs and broilers (Hanson et al., 2014).

The dynamic nature of the peroxidation process over time creates challenges for accurate assessment of deteriorating lipid quality and fluctuating concentrations of primary, secondary, and tertiary peroxidation compounds. Therefore, some researchers have recommended the use of multiple measures to describe the peroxidation status of a lipid (Shahidi and Zhong, 2005; NRC, 2012). Several assays are available to measure peroxidation or predict the stability of lipids exposed to peroxidative conditions. Peroxide value (**PV**) and thiobarbituric acid reactive substances (**TBARS**), and *p*-anisidine value (**AnV**) are commonly used to assess primary and secondary products of peroxidation. The suitability of these measures likely depends on temperature and duration of thermal exposure, as well as, fatty acid profile (Liu, 2012).

There is no universally accepted, standard measure of lipid peroxidation which accurately assesses peroxidation of various lipids subjected to varying thermal conditions. Few studies (Naz et al., 2005; Liu, 2012) have been conducted to compare results of various analytical assays of corn oil heated at different temperatures and

duration. Therefore, the objective of this experiment was to evaluate the characteristics of corn oil exposed to 2 temperature conditions during 2 time periods. Corn oil was selected because of its high susceptibility to peroxidation resulting from its high concentration of PUFA relative to other dietary lipids (NRC, 2012).

MATERIALS AND METHODS

Refined, deodorized, and bleached corn oil (without added antioxidants) was obtained from Stratas Foods (Memphis, TN; Table 3-1). Approximately 26.5 L were heated in an aluminum pot over a flame at a target temperature of at 185°C for 12 h (**RO**) and 75.7 L were heated at a target temperature of 95°C for 72 h (**SO**) using immersion heaters. Constant ambient air was bubbled through the corn oil (flow rate = 12 L/min). Samples (≤ 1 L) were obtained and temperature was measured hourly or at 8 h intervals for RO and SO, respectively. Samples were stored at -20°C until further analysis. Actual average temperatures during heating were 92.8 ± 5.7°C and 196 ± 12.3°C for SO and RO, respectively.

Laboratory Analysis

Oil samples were analyzed for PV (method Cd 8-53; AOCS, 1992), *p*-anisidine value (method Cd 18-90; AOCS, 2011), hexanal (Elisia and Kitts, 2011), moisture (method Ca 2c-25; AOCS, 2009), impurities (method Ca 3a-46; AOCS, 2011), unsaponifiables (method Ca 6a-40; AOCS, 2011), free fatty acid (method Ca 5a-40; AOCS, 2012), and fatty acid profile (methods Ce 2-66; AOCS, 1997 and 996.06; AOAC, 2001) at the UMO-AESCL (Columbia). The TBARS were also measured using a modified version of the AOCS procedure (Cd 19-90; AOCS, 2001) with malonaldehyde used as a standard as described by Pegg (2001).

In addition, concentrations of HNE, and 2,4-decadienal (**DDE**) were determined by liquid chromatography–mass spectrometry (LC-MS). Briefly, 4 μL of samples and standards were mixed with 20 μL of 10 mM triphenylphosphine, 20 μL of 10 mM of 2, 2'-dipyridyl disulfide, 20 μL of 10 mM HQ 2-hydrazinequinoline, 2 μL of 100 mM d6-acetone, 2 μL of 100 mM chlorobutyric acid, and 140 μL of acetonitrile. Mixtures were incubated at 60°C for 30 min, cooled on ice, and centrifuged (18,000 × *g* at 4°C) for 15 min. An aliquot of 100 μL of the top layer was mixed with an equal volume of cold, deionized water, centrifuged (18,000 × *g* at 4°C). Five microliters of supernatant was injected into an ultra-performance liquid chromatography column and separated as described by Lu et al. (2013). Mass chromatograms and mass spectral data were acquired and processed by MassLynx software (Waters, Milford, MA) in centroid format. Concentrations of HNE and DDE in oil were determined by calculating the ratio between peak areas of compounds (HNE and DDE) and internal standards (d6-Acetone and chlorobutyric acid), using QuanLynx^{TM} software (Waters, Milford, MA).

Statistical Analysis

Data were summarized and analyzed independently for SO and RO using CORR and UNIVARIATE procedures, respectively, in SAS (v9.3, SAS Inst. Inc., Cary NC). Significance was declared at $P < 0.05$, whereas values between $0.05 \leq P \leq 0.15$ were considered statistical trends.

RESULTS AND DISCUSSION

Polyunsaturated fatty acids are highly susceptible to peroxidation (Belitz et al., 2009). During peroxidation, lipids are ultimately degraded into a variety of primary, secondary, and tertiary products. At least 19 volatile compounds are formed by the

peroxidation of linoleic acid (Belitz et al., 2009). Consequently, concentrations of linoleic (C18:2) declined 5.2% and 14.7%, and concentrations of linolenic acid (C18:3) declined 15.7% and 41.6% in SO and RO, respectively, after heating (Table 3-1). These decreases in C18:2 and C18:3 resulted in a 5% and a 15% reduction in total PUFA concentration in SO and RO, respectively (Table 3-2). The PUFA concentration was associated negatively with duration of heating for both SO and RO ($r < -0.9$, $P < 0.01$; Table 3-3 and able 3-4). Others have reported that PUFA decline in oil after peroxidation (DeRouchey et al., 2004; Liu, 2012) . Our data are in agreement with the fact that PUFA are degraded preferentially compared with other fatty acids during peroxidation because SFA concentration changed minimally (2.5% and 4.7%) for SO or RO, respectively, and the concentration of MUFA increased 9.3% and 17.3% after heating for SO and RO, respectively.

Free fatty acid content has been commonly used as an indicator of lipid quality. Concentration of FFA increased by 63% and 250% for SO and RO, respectively. However, FFA correlated positively with time for RO ($r = 0.86$, $P < 0.01$), but not SO ($r = 0.45$, $P = 0.17$). Similarly, FFA correlated negatively with PUFA for RO ($r = -0.85$, $P < 0.01$), but not SO ($r = -0.46$, $P = 0.18$). This seems to indicate that FFA may indicate peroxidation in lipids exposed to high temperatures (i.e. 185°C), but may have limited suitability as an indicator of peroxidation at lower processing temperatures.

Lipid hydroperoxides are produced initially during lipid peroxidation, but they subsequently react to form numerous products including aldehydes, ketones, acids, esters, hydrocarbons, epoxides, polymers, lactones, furans, and aromatic compounds (Belitz et al., 2009). However, the rate of degradation of peroxides seems to be dependent on the

thermal conditions used. The PV initially increased in SO and later appeared to plateau by 40 h of heating, ultimately increasing by 6.4 fold from baseline after 72 h of heating. The PV of SO was associated strongly with time for SO (r = 0.94, $P < 0.01$; Figure 3-1). Conversely, PV declined after only 1 h of heating under RO conditions (correlation with time, r = -0.81, $P < 0.01$; Figure 3-2), and ultimately declined by 74% after 12 h. Consequently, PV was associated positively (r = 0.73, $P < 0.01$) with PUFA for SO, but negatively with PUFA for RO (r = -0.78, $P < 0.01$). Others have reported a bell shaped curve of PV production and degradation in lipids exposed to a low (80°C; DeRouchey et al., 2004) temperature for 11 d or a high (150°C; Danowska-Oziewicz and Karpińska-Tymoszczyk, 2005) temperature for 24 h. In addition, some researchers have suggested that peroxides are undetectable when exposed to temperatures greater than 150°C (Shahidi and Zhong, 2005). Perhaps had the duration of heating in the current study been extended for SO, the PV would have declined. However, our current results suggest PV is a poor indicator of peroxidation in peroxidized corn oil.

Concentrations of TBARS and AnV are often used to evaluate aldehydes in peroxidized lipids, but neither method is specific to a particular aldehyde (Shahidi and Wanasundara, 2008). The AnV increased substantially (22-fold) in SO after 72 h of heating, and AnV correlated with duration of heating for SO (r = 0.96, $P < 0.01$). Conversely, AnV declined 21% in RO after 12 h of heating, and was associated negatively with duration of heating (r = -0.46, $P < 0.11$). Consequently, AnV was not associated with PUFA for RO ($P > 0.25$), and correlated negatively with PUFA for SO (r = -0.95, $P < 0.01$). Interestingly, others have reported a bell shaped curve of AnV that peaks at 7 d when heating choice white grease for 11 d at 80°C (DeRouchey et al., 2004).

However, a similar curve of production and degradation was not found in the current experiment, and this may relate to the reduced duration of heating. Our data suggest that the utility of using AnV is limited as a peroxidation measure for corn oil heated at 185°C for 12h. Ultimately the practical utility of AnV is limited because formulators and nutritionists seldom know the historical conditions of lipid handling. Concentration of TBARS increased in both SO after 72 h and RO (r = 0.72, $P = 0.02$) after 12 h (r = 0.82, $P < 0.01$) of heating. However, TBARS was more closely associated with the declining concentration PUFA for SO (r = -0.78, $P < 0.01$) than RO (r = -0.52, $P = 0.12$). These findings suggest that TBARS may be an indicator of peroxidation in SO and RO, but the TBARS concentration was less strongly associated with PUFA in RO. Some researchers have reported that the concentration of TBARS follows a bell-shaped curve in lipids exposed to 150°C for 24 h (Danowska-Oziewicz and Karpińska-Tymoszczyk, 2005) or 185°C for 10 h (Seppanen and Csallany, 2002). While this finding was not observed in the results from the current study, the TBARS concentration appeared to plateau after approximately 5 to 6 h in RO.

In addition to non-specific markers of peroxidation, specific compounds such as DDE, hexanal, and HNE were evaluated. The concentration of DDE increased substantially in either SO after 72 h (> 467 fold) or RO (> 149 fold) after 12 h of heating. However, DDE was associated positively with time for SO (r = 0.90, $P < 0.01$), but not RO (r = 0.08, $P = 0.79$). These results indicate that DDE may not be a reliable indicator of peroxidation in corn oil exposed to 185°C for 12h because DDE followed a bell-shaped curve of production and degradation during heating (Figure 3-3). Other researchers have reported that DDE degrades to hexanal, 2-butenal, 5-oxodecanal, and 2-

buten-1,4-dial upon exposure to air (Spiteller et al., 2001). Hexanal increased in SO after or RO after heating, and was strongly correlated with duration of heating for both SO and RO ($r \geq 0.94$, $P < 0.01$; Table 3-1 and 3-2). Consequently, hexanal had a strong negative correlation with PUFA for SO and RO ($r \leq -0.91$, $P < 0.01$). Hexanal is a carbonyl compound formed during the termination phase when linoleic acid or other ω-6 fatty acids are peroxidized (Shahidi and Wanasundara, 2008). Similarly, the concentration of HNE increased substantially (> 19-fold) in either SO or RO after heating (Figure 3-4; $r = 0.66$, $P < 0.01$). 4-hydroxynonenal was negatively correlated with PUFA for SO ($r = -0.94$, $P < 0.01$) and RO ($r = -0.61$, $P = 0.03$). However, Seppanen and Csallany (2002) reported that the concentration of HNE increases initially and later declines in soybean oil exposed to 185°C.

Of the indicators evaluated in this experiment, hexanal and HNE most consistently indicated the peroxidation status of corn oil exposed to temperatures of 95°C or 185°C. Both measures correlated negatively with PUFA ($r \leq -0.61$) and positively with time in both RO and SO ($r \geq 0.66$), but these correlations were greater for SO. Under the conditions of this experiment, PV, AnV, and FFA measurements were not reliable indicators of peroxidation in corn oil exposed to different heat treatments, but additional experiments are needed to verify these findings. These data suggest that temperature and duration of thermal processing should be considered when selecting the optimal metric for assessing peroxidation. Unfortunately, this information is seldom available for lipids used to formulate animal diets. Establishing and implementing universally accurate measures of peroxidative damage of lipids will facilitate investigation of physiological

connections between products of peroxidation and the growth, efficiency, and health of animals.

Table 3-1. Characteristics of corn oil

	Corn oil[1]		
Parameter	UN	SO	RO
Peroxide value, meq O_2/kg	2.11	13.57	0.55
TBARS, mg MDA eq/kg	17.83	32.03	43.45
p-Anisidine value	0.24	5.4	0.19
Hexanal, µg/g	1.70	6.60	2.73
2,4-decadienal, µMol	1.05	27.73	20.40
4-hydroxynonenol, µg/mL	12.60	588.50	1,877.70
Free fatty acids,%	1.12	1.84	2.81
Moisture,%	< 0.10	< 0.10	< 0.10
Impurities,%	< 0.15	< 0.15	< 0.15
Unsaponifiables,%	0.78	0.83	0.71
Fatty acids,% of lipid			
C14:1	0.00	0.00	0.00
C15:0	0.00	0.15	0.00
C16:0	14.36	13.02	13.84
C16:1	0.14	0.11	0.13
C18:0	1.75	2.07	2.32
C18:1	28.93	31.6	33.98
C18:2	53.06	50.32	45.25
C18:3	0.89	0.75	0.52
Total PUFA,% of lipid	53.95	51.07	46.08
Total SFA,% of lipid	16.51	16.10	17.28
Total MUFA,% of lipid	29.33	32.06	34.41

[1]UN = corn oil was not subjected to heat treatment, SO = corn oil heated at 95°C for 72 h with air flow rate of 12L/min, RO = corn oil heated at 185°C for 12 h with air flow rate of 12L/min.

Table 3-2. Characteristics of heated corn oil relative to unheated corn oil[1]

Parameter	SO[2]	RO[3]
PUFA	0.95[a]	0.85[a]
Free fatty acids	1.63	2.50[a,b]
Peroxide value	6.43[a,b]	0.26[a,b]
Anisidine value	22.50[a,b]	0.79
Thiobarbituric acid reactive substances	1.80[a]	2.44[a,b]
Hexanal	3.88[a,b]	1.61[a,b]
4-Hydroxynonenal	20.70[a,b]	19.43[a,b]
2,4-Decadienal	467.34[a,b]	149.02

[1]Data expressed as ratio to unheated corn oil.

[2]SO = heated at 95°C for 72 h (air flow rate = 12 L/min).

[3]RO = heated at 185°C for 12 h (air flow rate = 12 L/min).

[a]Correlated with sampling time ($P < 0.05$).

[b]Correlated with PUFA ($P < 0.05$).

Table 3-3. Pearson correlation coefficients for characteristics of slowly peroxidized corn oil[1]

	Free fatty acids	Peroxide value	*p*-Anisidine value	TBARS	Hexanal	PUFA	HNE	DDE	Time
Free fatty acids	1.00 10								
Peroxide value	0.33 0.35 10	1.00 10							
***p*-Anisidine value**	*0.58* *0.07* *10*	**0.84** **< 0.01** **10**	1.00 10						
TBARS	0.47 0.18 10	**0.81** **< 0.01** **10**	**0.65** **0.04** **10**	1.00 10					
Hexanal	0.47 0.17 10	**0.95** **< 0.01** **10**	**0.94** **< 0.01** **10**	**0.73** **0.02** **10**	1.00 10				
PUFA	-0.46 0.18 10	**-0.78** **< 0.01** **10**	**-0.95** **< 0.01** **10**	*-0.52* *0.12* *10*	**-0.91** **< 0.01** **10**	1.00 10			
HNE	*0.53* *0.12* *10*	**0.89** **< 0.01** **10**	**0.98** **< 0.01** **10**	**0.74** **0.01** **10**	**0.96** **< 0.01** **10**	**-0.94** **< 0.01** **10**	1.00 10		
DDE	*0.59* *0.07* *10*	**0.74** **0.02** **10**	**0.97** **< 0.01** **10**	*0.59* *0.07* *10*	**0.86** **< 0.01** **10**	**-0.92** **< 0.01** **10**	**0.95** **< 0.01** **10**	1.00 10	
Time	0.47 0.17 10	**0.94** **< 0.01** **10**	**0.96** **< 0.01** **10**	**0.72** **0.02** **10**	**0.99** **< 0.01** **10**	**-0.90** **< 0.01** **10**	**0.97** **< 0.01** **10**	**0.90** **< 0.01** **10**	1.00 10

[1]Corn oil was heated at 95°C for 72 h (air flow rate = 12 L/min). Abbreviations: TBARS = thiobarbituric acid reactive substances, HNE = 4-hydroxynonenol, and DDE = 2,4-decadienal. Data were expressed as ratio relative to initial (unheated) corn oil. The correlation coefficient (r value), *P* value, and number of observations are listed in the top, middle, and bottom of each cell.

Table 3-4. Pearson correlation coefficients for characteristics of rapidly peroxidized corn oil[1]

	Free fatty Acids	Peroxide value	*p*-Anisidine value	TBARS	Hexanal	PUFA	HNE	DDE	Time
Free fatty acids	1.00 13								
Peroxide value	**-0.75** **< 0.01** **13**	1.00 13							
***p*-Anisidine value**	*-0.48* *0.13* *13*	**0.85** **< 0.01** **13**	1.00 13						
TBARS	**0.87** **< 0.01** **13**	**-0.80** **< 0.01** **13**	**-0.60** **0.03** **13**	1.00 13					
Hexanal	**0.79** **< 0.01** **13**	**-0.69** **< 0.01** **13**	-0.26 0.40 13	**0.72** **0.01** **13**	1.00 13				
PUFA	**-0.85** **< 0.01** **13**	**0.73** **< 0.01** **13**	0.34 0.25 13	**-0.78** **< 0.01** **13**	**-0.96** **< 0.01** **13**	1.00 13			
HNE	**0.64** **0.02** **13**	**-0.64** **0.02** **13**	**-0.55** **0.05** **< 0.01**	**0.78** **< 0.01** **13**	*0.49* *0.09* *13*	**-0.61** **0.03** **13**	1.00 13		
DDE	0.26 0.40 13	-0.29 0.33 13	*-0.49* *0.09* *13*	*0.45* *0.13* *13*	-0.13 0.75 13	0.00 0.99 13	**0.95** **< 0.01** **13**	1.00 13	
Time	**0.86** **< 0.01** **13**	**-0.81** **< 0.01** **13**	-0.46 0.11 13	**0.82** **< 0.01** **13**	**0.94** **< 0.01** **13**	**-0.99** **< 0.01** **13**	**0.66** **0.01** **13**	0.08 0.79 13	1.00 13

[1]Corn oil was heated at 185°C for 12 h (air flow rate = 12 L/min). Abbreviations: TBARS = thiobarbituric acid reactive substances, HNE = 4-hydroxynonenol, and DDE = 2,4-decadienal. Data were expressed as ratio relative to initial (unheated) corn oil, and time was expressed as hours. The correlation coefficient (r value), *P* value, and number of observations are listed in the top, middle, and bottom of each cell.

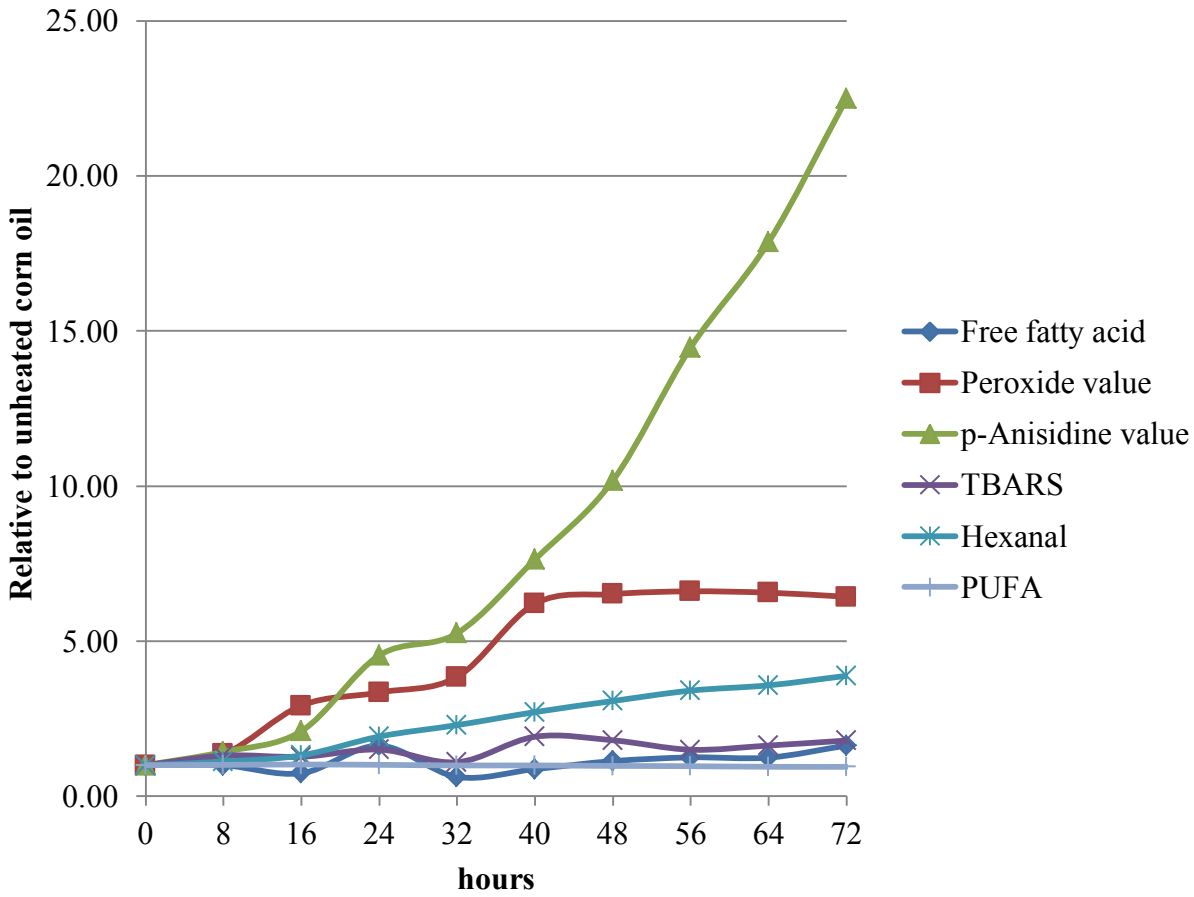

Figure 3-1. Changes in characteristics of corn oil heated at 95°C for 72 h (air flow rate = 12 L/min)

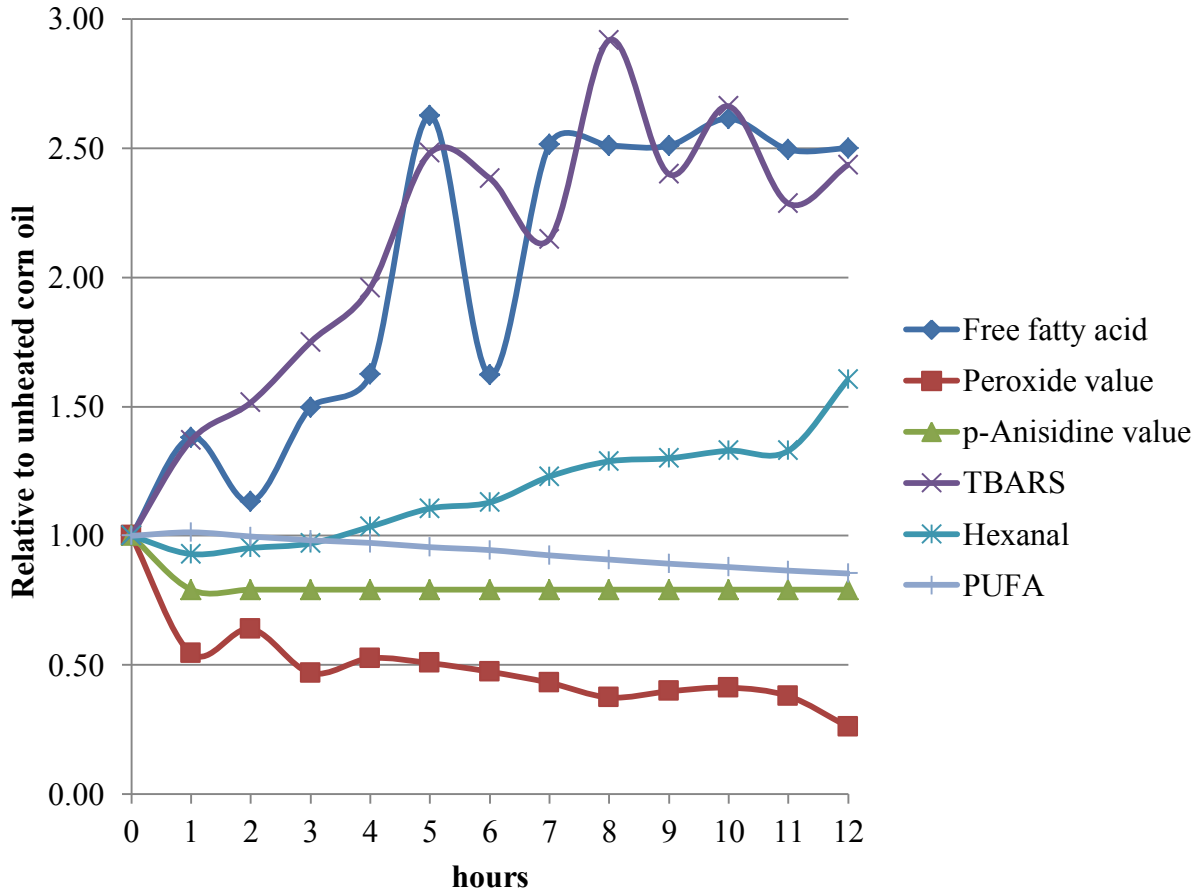

Figure 3-2. Characteristics of corn oil heated 185°C for 12 h (air flow rate = 12 L/min)

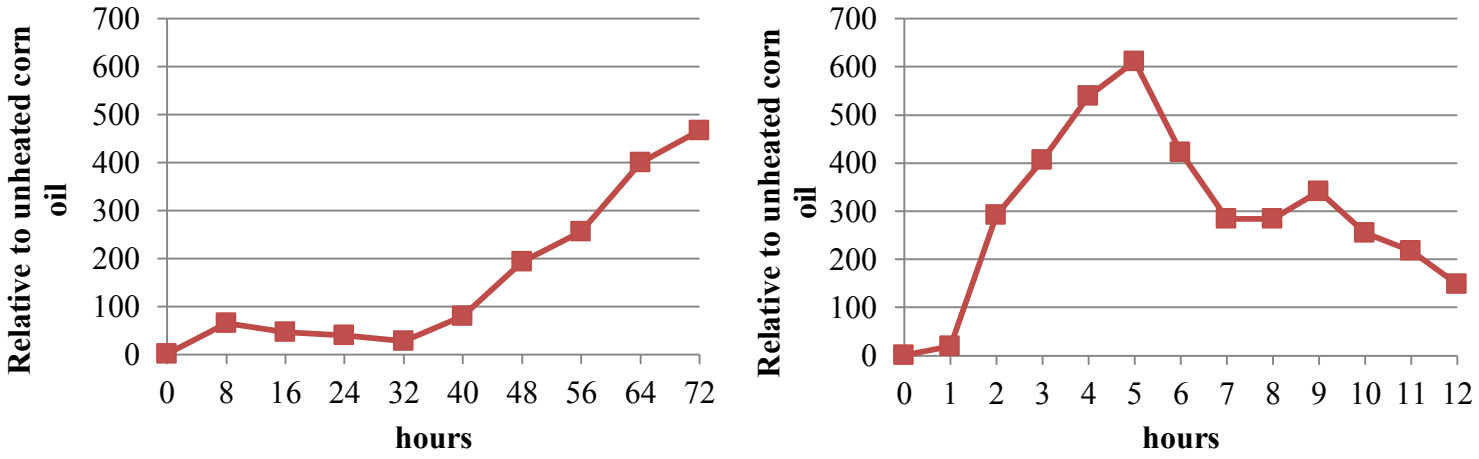

Figure 3-3. Relative concentration of 2,4-decadienal in corn oil exposed to 95°C for 72 h (left) or 185°C for 12 h (right) with air flow rate of 12L/min

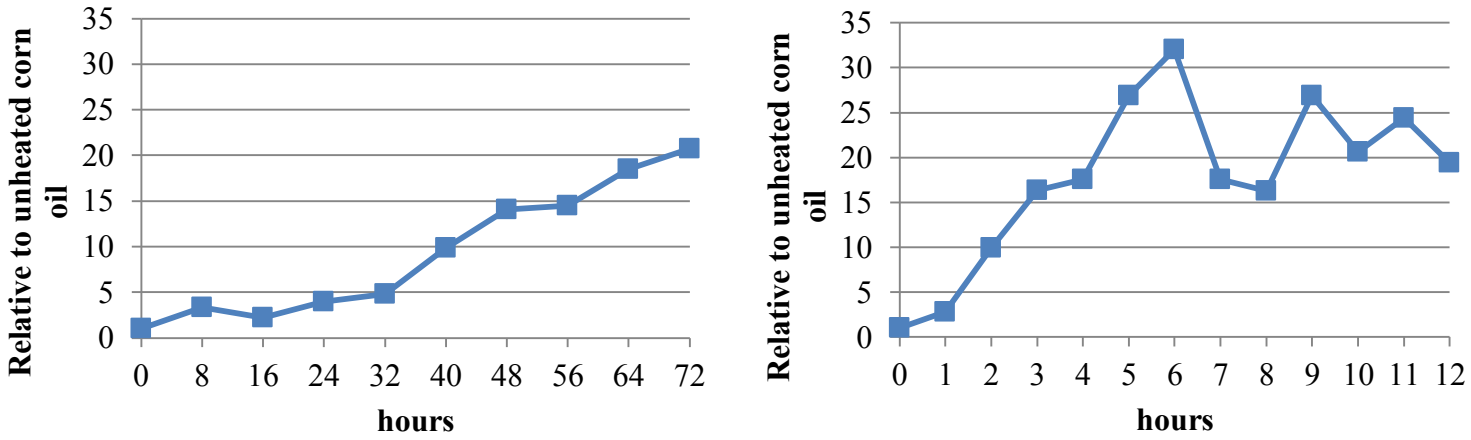

Figure 3-4. Relative concentration of 4-hydroxynonenal in corn oil exposed to 95°C for 72 h (left) or 185°C for 12 h (right) with air flow rate of 12 L/min

CHAPTER 4. IMPACT OF ANTIOXIDANTS ON LIPID PEROXIDATION OF DRIED DISTILLERS GRAINS WITH SOLUBLES AND DISTILLER'S CORN OIL STORED IN EXTREME TEMPERATURE AND HUMIDITY CONDITIONS

SUMMARY

Lipid peroxidation is accelerated by exposure to heat, light, oxygen, and metal, and it is inhibited by antioxidants. Susceptibility to peroxidation increases with increasing degree of unsaturation. An experiment was conducted to assess markers of peroxidation in distillers corn oil (DCO) and dried distillers grains with solubles (DDGS) during storage. Reduced oil-DDGS (RO-DDGS; 5.0 ± 0.15% crude fat), high oil DDGS (HO-DDGS; 13.0 ± 0.19% crude fat), and 2 sources of DCO (DCO-1, 1.2, 0.08, and 0.48% moisture, impurities, and unsaponifiables [MIU]; and DCO-2, 1.2, 0.01, and 0.1% MIU) were obtained. Each ingredient was divided into 18 representative subsamples (~908 g for DDGS or 2 kg of corn oil). Six subsamples were mixed with either no supplemental antioxidants (**CON**), Rendox-CQ® (**REN**; active ingredient: tertiary butyl hydroquinone [**TBHQ**]; Kemin, Industries, Des Moines, IA), or Santoquin-Q4T® (**SAN**; active ingredient: ethoxyquin and TBHQ; Novus Intl., St. Louis, MO) for a total of 72 batches. Batches were formulated to contain 0 (CON), 1,000 mg REN/kg crude fat, or 1,500 mg SAN/kg crude fat. Three samples of each batch were obtained. One sample was immediately frozen (-20°C) for later analysis. Two samples were placed in an environmental chamber set to maintain 38°C and 90% relative humidity (actual = 38.6 ± 0.1°C and 94.0 ± 0.3%), and removed after 14 or 28 d. Peroxide value (PV), *p*-anisidine value (AnV), and thiobarbituric acid reactive substances (TBARS) of DCO and DDGS

increased ($P < 0.05$) from d 14 to 28 of storage. The PV of DCO-1 and HO-DDGS (12.3 and 12.6 ± 0.3 meq O_2/kg oil, respectively) exceeded ($P < 0.05$) that of DCO-2 or RO-DDGS (9.6 and 9.3 ± 0.3 meq O_2/kg oil, respectively) overall (averaged across d 14 and 28), and on d 14 and 28. Similarly, the TBARS concentration of DCO-2 was less ($P < 0.05$) than DCO-1, and less ($P < 0.05$) for RO-DDGS than HO-DDGS overall and on d 14. The AnV was lower ($P < 0.05$) in DCO-1 compared to the other ingredients across sampling days. Concentrations of PV overall, and on d 14 or 28 declined ($P < 0.05$) relative to CON by adding either REN or SAN to ingredients. However, the PV was lower ($P < 0.05$) overall and on d 14 and 28 for ingredients treated with REN compared to SAN. On d 14 and 28, the concentration of TBARS and AnV declined ($P < 0.05$) relative to CON by adding either REN or SAN, but there was no difference between the 2 antioxidants (overall mean = 11.0, 6.1, and 5.9 ± 0.2 mg malondialdehyde eq/kg oil and 6.5, 1.9 and 1.8 ± 0.2, respectively for TBARS and AnV). These data suggest that lipids in DCO and DDGS peroxidize during 28 d of storage at elevated temperature and relative humidity. Based on these findings, peroxidation of DCO is influenced negatively by MIU content. Antioxidants effectively impeded peroxidation, but neither antioxidant evaluated completely stabilized the ingredients in this experiment.

KEYWORDS: corn oil, dried distillers grains with solubles, lipid, lipid peroxidation, peroxidation assays, storage,

INTRODUCTION

Energy is the most expensive component of swine diets. Corn or dried distillers grains with solubles (**DDGS**) commonly provide a substantial portion of dietary energy in swine diets, but supplemental sources of concentrated lipids are more energy dense and

increasingly available. More than 85% of American ethanol plants currently produce corn oil from thin stillage, generating more than 105,000 tonnes of distiller's corn oil (**DCO**) in 2013 for use in the production of biodiesel and livestock diets (Renewable Fuels Association, 2014).

Corn oil is high in PUFA relative to other dietary lipids (NRC, 2012), and PUFA are highly susceptible to peroxidation. Lipid peroxidation is accelerated by exposure to heat, air, moisture, and pro-oxidant metals which may be introduced during processing and storage, but peroxidation is impeded by antioxidants (Belitz et al., 2009). Therefore, animal fats, vegetable oils, and lipid rich ingredients may be peroxidized to varying extent depending on storage or processing conditions (Dibner et al., 2011; Song and Shurson, 2013).

During peroxidation, fatty acids degrade into lipid hydroperoxides which subsequently react to form numerous products including aldehydes, ketones, acids, esters, hydrocarbons, epoxides, polymers, lactones, furans, and aromatic compounds (Belitz et al., 2009). Therefore, products of peroxidation are generally measured to assess extent of peroxidation. The rate of peroxidation of corn germ oil and DCO was greater than oil extracted from DDGS during 14 d of storage at 40°C, likely because of the protective action of antioxidants indigenous to corn which concentrated in DDGS (Winkler-Moser and Breyer, 2011).

We are aware of no published evaluations of the effects of synthetic antioxidants on lipid peroxidation in DCO and DDGS during storage under extreme temperatures and humidity. The objective of this experiment was to investigate lipid peroxidation in DDGS

or DCO stored with or without antioxidants during exposure to elevated temperature and relative humidity.

MATERIALS AND METHODS

Reduced oil-DDGS (RO-DDGS; 5.0 ± 0.15% crude fat) and DCO (Voila™ [**DCO-1**], POET Nutrition, Sioux Falls, SD) were obtained from the same ethanol plant (Lake Crystal, MN). A second source of DDGS (13.0 ± 0.19% crude fat) was obtained from CHS Inc.(Inver Grove Heights, MN) which was produced by Highwater Ethanol (Lamberton, MN). Another DCO source was obtained from a second ethanol plant (**DCO-2;** Sioux Falls, SD). The DCO-1 contained 1.2, 0.08, and 0.48% moisture, impurities, and unsaponifiables (**MIU**), respectively, and DCO-2 contained 1.2, 0.01, and 0.1% MIU, respectively.

The 4 ingredients were each divided into 18 representative subsamples (~908 g for DDGS or 2 kg of corn oil). Six subsamples were mixed with either no supplemental antioxidants (**CON**), Rendox-CQ® (**REN**; active ingredient: tertiary butyl hydroquinone [**TBHQ**]; Kemin, Industries Des Moines, IA), or Santoquin-Q4T® (**SAN**; active ingredient: ethoxyquin and TBHQ; Novus Intl., St. Louis, MO) using a stationary food mixer (Kitchen Aid®; Benton Harbor, MI) on the lowest setting for at least 3 minutes (total = 72 batches). Batches were formulated to contain 0 (CON), 1,000 mg REN/kg crude fat, or 1,500 mg SAN/kg crude fat according to the maximum level recommended by the manufacturer (Table 4-1).

Each batch was divided into 3 samples of approximately 300 g of DDGS or 600 mL of corn oil. Within each batch, 2 portions were placed in two plastic containers, and the remainder was placed in screw top bottles and frozen (-20°C) until later analysis (d 0).

Therefore, similar volumes of material were placed in each container, assuming a bulk density of 0.4896 g/mL for DDGS (Letsche et al., 2009). Plastic containers (n = 144) were covered with synthetic cheesecloth material secured by rubber band and placed in an environmental chamber (model z-32; Cincinnati Sub Zero, Cincinnati, OH). Samples were blocked across the chamber to account for potential environmental gradients, and samples representing the same batch were placed adjacently. The chamber was set to maintain 38˚C and 90% relative humidity. Temperature and humidity were monitored at 5 min intervals using a ThermaData Logger (Thermoworks, Lindon, UT). Light inside the environmental chamber was turned off except during sample collection, and the window on the unit was covered to prevent exposure to external light.

Sampling

One randomly selected container from each batch was removed at d 14, and the remaining sample in each batch was removed at d 28. Samples were placed in screw top bottles, covered with N gas to minimize peroxidation, and frozen (-20°C) until analysis.

Laboratory Analyses

Samples of DDGS were ground, and lipids were extracted as described by Folch et al. (1957). Oil samples and DDGS oil extracts from d 0 were analyzed for Rendox-CQ® and Santoquin-Q4T® using gas chromatography mass spectroscopy as described by Guo et al. (2006). All samples were analyzed for fatty acid profile (methods Ce 2-66; AOCS, 1997 and 996.06; AOAC, 2001), thiobarbituric acid reactive substances (**TBARS**), peroxide value (**PV,** method Cd 8-53; AOCS, 1992), and *p*-anisidine value (**AnV,** method Cd 18-90; AOCS, 2011). The TBARS assay was a modified version of the AOCS procedure (Cd 19-90; AOCS, 2001) using malonaldehyde as a standard as

described by Pegg (2001). Initial samples of corn oil were evaluated for moisture (method Ca 2c-25; AOCS, 2009), impurities (method Ca 3a-46; AOCS, 2011), unsaponifiables (method Ca 6a-40; AOCS, 2011), free fatty acid (method Ca 5a-40; AOCS, 2012). All assays were conducted at the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia) unless noted otherwise.

Mold was observed in all containers with DDGS after 14 d or 28 d of storage. When these samples were removed, the apparently moldy fraction was separated from the fraction that did not appear moldy, each portion was weighed, and the apparent mold growth was calculated using the following equation: apparent mold, % = $\left(\left[\frac{moldy\ wt}{moldy+non\ moldy\ wt.}\right] x\ 100\right)$. Moldy and non-moldy fractions were homogenized for later analyses. Samples were submitted for total mold count according using the plate dilution technique described by Tournas et al. (2001) at Minnesota Valley Testing Laboratories (New Ulm).

Statistical Analysis

Data were analyzed using the Mixed procedure of SAS (v9.3; SAS Institute, Cary, NC) with repeated measures to evaluate the fixed effects of ingredient (HO-DDGS, RO-DDGS, DCO-1, DCO-2), antioxidant inclusion (CON, REN, SAN), time (d 14 or 28), and interactions in a 4 × 3 × 2 complete factorial arrangement. Block was included as a random effect. Initial (d 0) values were used as covariates for each variable, and apparent mold concentration was used as a covariate for analysis of all variables except total mold count. To facilitate mean separation, the PLM procedure of SAS (v9.3; SAS Institute, Cary, NC) was used with the Tukey adjustment. Significance was declared at $P < 0.05$, whereas values between $0.05 \leq P \leq 0.10$ were considered statistical trends.

RESULTS AND DISCUSSION

The PUFA concentration of corn oil enhances its susceptibility to peroxidation relative to more saturated sources of lipid. Numerous accelerants of peroxidation such as heat, air, moisture, and pro-oxidant metals may be introduced during processing and storage, and peroxidation is impeded by antioxidants (Belitz et al., 2009). Others report that lipids in DDGS (Song and Shurson, 2013) and concentrated sources of dietary lipids such as corn oil (Dibner et al., 2011) are peroxidized to varying extents. Song and Shurson (2013) reported that PV and TBARS of DDGS varies from 4.2 to 84.1 meq O_2/kg oil and 1.0 to 5.2 mg malondialdehyde eq/kg of oil, respectively. Relatively low initial values of PV (1.4 and 1.2 ± 0.01 meq O_2/kg) and TBARS (2.0 and 2.0 ± 0.01 mg MDA eq/kg of oil) for HO-DDGS and RO-DDGS in the present study indicate that the DDGS was not excessively peroxidized before use in this experiment (Table 4-2).

The actual temperature and humidity varied slightly during the 28 d storage period (actual = 38.6 ± 0.1°C and 94.0 ± 0.3%), and little is known about peroxidation in DCO or DDGS during storage under these conditions. In this experiment, the AnV, PV, and TBARS of DCO and DDGS increased from d 14 to 28 ($P < 0.05$; Table 4-2) of storage. Others have reported increased concentrations of PV (Naz et al., 2005; Winkler-Moser and Breyer, 2011), AnV (Naz et al., 2005) in corn oil stored at ≤ 40°C. Similarly, PV and TBARS increased in canola oil heated at 65°C for 17 d (Wanasundara and Shahidi, 1994), and PV, TBARS, and AnV increased in sunflower oil heated at 60°C for 21 d (Chen et al., 2014). However, peroxidation is a dynamic process resulting in the production and degradation of numerous compounds. Lipid hydroperoxides are produced initially during peroxidation, but react subsequently to form other secondary and tertiary

products that are subject to further degradation (Belitz et al., 2009). The rate of degradation of peroxides seems dependent on thermal conditions. Others have reported PV follows a bell shaped curve of production and degradation in lipids exposed to moderate (80°C; DeRouchey et al., 2004) or high (150°C; Danowska-Oziewicz and Karpińska-Tymoszczyk, 2005) temperatures. Similarly, TBARS concentration followed a bell-shaped curve in corn oil, canola oil, tallow, or poultry fat during heating at 185°C for 12 h (Liu, 2012). Consequently, PV and TBARS may be misleadingly low in lipids exposed to moderate or high temperatures. Accordingly, some researchers have suggested employing multiple markers of lipid peroxidation to assess extent of peroxidation comprehensively in dietary lipids (Shahidi and Zhong, 2005; NRC, 2012). Together, this information and the current findings suggests PV, TBARS, and AnV are acceptable markers of peroxidation in corn oil exposed to storage conditions with low temperature (≤ 65°C), but these assays have limitations in lipids exposed to high temperatures (≥ 80°C). Unfortunately, knowledge of historical conditions of lipid handling is often unknown to animal nutritionists.

The PV of DCO-1 and HO-DDGS exceeded ($P < 0.05$) that of DCO-2 or RO-DDGS. Similarly, the TBARS concentration of DCO-2 was less ($P < 0.05$) than DCO-1, and less ($P < 0.05$) for RO-DDGS than HO-DDGS. Perhaps the corn oil which remains in RO-DDGS is more resistant to peroxidation compared to the lipid fraction extracted to yield DCO. Others have found that extracted oils are utilized with increased efficiency than intact oil by pigs, likely because the oil in grains is encapsulated in fiber matrices making it less accessible to lipases (Adams and Jensen, 1984; Kil et al., 2010; Kim et al., 2013). However, whether the oil which remains in RO-DDGS is encapsulated and

protected from peroxidation is unknown. Despite the fact that DCO-1 and RO-DDGS were manufactured at the same ethanol plant, the PV was 25% lower ($P < 0.05$) in RO-DDGS than DCO-1 after 28 d of storage. This finding supports the theory that the oil in DCO is more easily peroxidized than intact oil in remaining in RO-DDGS. However, the AnV was actually 25% lower ($P < 0.05$) in DCO-1 compared to the other ingredients, and the reason for this result is unclear. The relative reduction in PV and TBARS for DCO-2 relative to DCO-1 suggests that the concentration of MIU affects the peroxidation status of DCO during storage. The MIU concentration of DCO-2 was 26% lower than DCO-1, with the main differences being the concentration of insoluble impurities (87.5% reduction) and unsaponifiables (80% reduction). Contaminants and metals such as Fe, Cu, and Mn enhance peroxidation of unsaturated fats (Flider and Orthoefer, 1981). Furthermore, differences in peroxidation between ingredients may reflect variable PUFA content. Concentrations of linoleic acid and total PUFA as a percentage of lipid were lower ($P < 0.05$) in DCO-2 and RO-DDGS compared to DCO-1 or HO-DDGS, but these differences were relatively small in magnitude (<2.5%). However, these findings suggest that a reduced availability of substrate exists for peroxidation in DCO-2 and RO-DDGS. Linoleic acid is the predominant PUFA present in corn oil (NRC, 2012), and PUFA are highly susceptible to peroxidation relative to more saturated sources (Belitz et al., 2009). Conversely, the concentrations of linolenic acid, SFA, and MUFA varied ($P < 0.05$) among ingredients, but the practical significance of these findings is unclear (Table 4-2).

Antioxidants such as TBHQ and ethoxyquin retard peroxidation (Wanasundara and Shahidi, 1994; Wanasundara and Shahidi, 2005; Chen et al., 2014). In the current experiment, REN and SAN inhibited peroxidation of lipids in DCO and DDGS.

Concentrations of PV declined ($P < 0.05$) overall and on d 14 or 28 by adding either REN or SAN to ingredients. However, the PV was approximately 10% lower ($P < 0.05$) overall and on d 14 or 28 for ingredients treated with REN compared to SAN. While the difference was relatively small in magnitude, others have reported that TBHQ is more effective than ethoxyquin for stabilizing fish oil (Sanhueza et al., 2000) and soybean oil (Valenzuela et al., 2002). On d 14 and 28, the concentration of TBARS and AnV declined ($P < 0.05$) relative to CON by adding either REN or SAN, but there was no difference between the 2 antioxidants. The ingredient x antioxidant interaction affected the concentration of linoleic acid, PUFA, and SFA ($P < 0.05$). However, these differences have limited practical significance because they are relatively small in magnitude, and are generally related to ingredient. When DCO-1 was treated with SAN, PUFA concentrations increased ($P < 0.05$) relative to CON.

The crude fat content of RO-DDGS (5.0%) was substantially less than HO-DDGS (13.0%), as intended by experimental design. Surprisingly, however, the crude fat concentration of both DDGS sources declined ($P < 0.05$) in HO-DDGS (22% decline) and RO-DDGS (5.2% decline), from d 14 to 28 (Table 4-4). This result may be linked to visible mold that developed on all DDGS after 14 and 28 d of storage. Others have shown that crude fat content of corn declined after experimental infection with mold and 8 wks of storage, and suggested that mold degrades lipids (Reed et al., 2007). In the current experiment, the apparent concentration of mold $\left(\left[\frac{moldy\ wt}{moldy+non\ moldy\ wt.}\right] x\ 100\right)$ increased ($P < 0.05$) from d 14 to 28, and the apparent concentration of mold was greater ($P < 0.05$) on d 14 and 28 in RO-DDGS relative to HO-DDGS. Elevated moisture content and temperature conditions promote growth of mold (Milton and Pawsey, 1988). The

moisture content of DDGS increased ($P < 0.05$) over time, but the moisture content of HO-DDGS exceeded ($P < 0.05$) that of RO-DDGS on d 14 and 28. Superficially, these findings do not support the theory that apparent mold growth associates positively with moisture content, but the magnitude of difference in moisture content was small (22.2 vs. 20.4% for HO and RO-DDGS averaged across d 14 and 28, respectively). It is likely that the combination of high relative humidity and exposure to heat contributed to the development of mold in DDGS during storage. The total mold count was less ($P < 0.05$) on d 14 and 28 in RO-DDGS relative to HO-DDGS, and this may indicate that mold fraction of RO-DDGS had reduced ability to generate new colonies compared to mold in HO-DDGS.

These data indicate that lipid peroxidation occurs when DCO and DDGS are exposed to excessive heat and humidity which may have practical implications for storage, handling and transportation of these ingredients. Lipids in HO-DDGS were more susceptible to peroxidation than RO-DDGS after 28 d of storage. The MIU value of DCO negatively affects the peroxidation of lipids. In this experiment, antioxidants effectively reduced lipid peroxidation in DDGS and DCO, but did not completely prevent lipid peroxidation.

Table 4-1. Initial concentration of antioxidant in high oil DDGS (HO-DDGS), reduced oil DDGS (RO-DDGS), and distillers corn oil (DCO) prior to storage at 38°C and 90% relative humidity[1,2]

	Ingredient					
Item	HO-DDGS	RO-DDGS	DCO-1	DCO-2	PSEM[3]	P-value
Rendox-CQ®, mg/kg crude fat	976.9[a]	945.6[b]	1003.7[c]	961.7[a,b]	4.2	< 0.01
Santoquin, mg/kg crude fat	1,463.2[a]	1,454.8[a]	1,486.2[b]	1,476.0[ab]	5.5	< 0.01

[1]Rendox-CQ® (active ingredient is tertiary butyl hydroquinone [TBHQ]; Kemin Industries, Des Moines, IA) was added at 1000 ppm of lipid.

[2]Santoquin-Q4T® (active ingredients are ethoxyquin and TBHQ; Novus Intl., St. Louis, MO) was added at 1500 ppm of lipid.

[3]PSEM = pooled standard error of mean.

[a,b]Means with different superscripts differ ($P < 0.05$).

Table 4-2. Main effects of ingredient, antioxidant, and day on characteristics of high oil DDGS (HO-DDGS), reduced oil DDGS (RO-DDGS), and distillers corn oil (DCO) stored at 38°C and 90% relative humidity[1]

	Ingredient[2]					Antioxidant[3]				P-Values[4]			
Item	HO-DDGS	RO-DDGS	DCO-1	DCO-2	PSEM[5]	CON	REN	SAN	PSEM	I	A	I × day	A × day
Peroxide value, meq/kg lipid[6]													
d 14	4.63^{a}	3.33^{b}	4.42^{a}	3.42^{b}	0.11	5.80^{a}	2.87^{b}	3.18^{c}	0.09				
d 28	20.55^{a}	15.26^{b}	20.24^{a}	15.79^{b}	0.43	26.29^{a}	13.12^{b}	14.47^{c}	0.32				
Mean[7]	12.59^{a}	9.30^{b}	12.33^{a}	9.60^{b}	0.26	16.04^{a}	8.00^{b}	8.82^{c}	0.17	< 0.01	< 0.01	< 0.01	< 0.01
TBARS, meq MDA/kg lipid[6,8]													
d 14	3.46^{a}	2.82^{bc}	3.27^{ab}	2.77^{c}	0.14	4.45^{a}	2.49^{b}	2.30^{b}	0.13				
d 28	13.19	11.80	12.97	11.32	0.49	17.66^{a}	9.76^{b}	9.55^{b}	0.32				
Mean	8.32^{a}	7.31^{bc}	8.12^{ab}	7.05^{c}	0.27	11.05^{a}	6.12^{b}	5.92^{b}	0.16	< 0.01	< 0.01	0.06	< 0.01
p-Anisidine value[6]													
d 14	1.98^{a}	1.79^{ab}	1.46^{b}	1.71^{ab}	0.10	3.78^{a}	0.61^{b}	0.82^{b}	0.12				
d 28	5.14^{ab}	6.23^{a}	4.08^{b}	5.02^{ab}	0.37	9.3^{a}	3.24^{b}	2.81^{b}	0.24				
Mean	3.56^{a}	4.01^{a}	2.77^{b}	3.37^{a}	0.18	6.54^{a}	1.92^{b}	1.82^{b}	0.15	< 0.01	< 0.01	< 0.01	< 0.01
Linoleic Acid,% of lipid[6]													
d 14	53.05^{a}	52.66^{b}	53.28^{a}	52.26^{b}	0.07	52.75	52.86	52.83	0.06				
d 28	53.53^{a}	52.9^{bc}	53.2^{ab}	52.33^{c}	0.11	52.98	52.94	53.04	0.06				
Mean	53.29^{a}	52.78^{b}	53.24^{a}	52.29^{b}	0.08	52.86	52.9	52.94	0.03	< 0.01	0.37	< 0.01	0.21
Linolenic Acid,% of lipid[6]													
d 14	1.65^{a}	1.71^{b}	1.33^{c}	1.37^{d}	0.03	1.51	1.52	1.52	0.01				
d 28	1.6^{a}	1.72^{b}	1.33^{c}	1.37^{d}	0.03	1.50	1.49	1.52	0.01				
Mean	1.62^{a}	1.71^{b}	1.33^{c}	1.37^{d}	0.03	1.50	1.51	1.52	< 0.01	< 0.01	0.16	< 0.01	0.04
PUFA,% of lipid[6]													
d 14	54.72^{a}	54.39^{b}	54.58^{ab}	53.61^{c}	0.08	54.26	54.37	54.34	0.06				
d 28	55.15^{a}	54.64^{bc}	54.50^{bc}	53.67^{d}	0.12	54.48	54.44	54.56	0.06				
Mean	54.93^{a}	54.52^{b}	54.54^{b}	53.64^{c}	0.09	54.37	54.41	54.45	0.04	< 0.01	0.29	< 0.01	0.17

MUFA,% of lipid[6]													
d 14	27.65	26.41	28.47	29.27	0.12	27.96	27.93	27.95	0.03				
d 28	27.83	26.61	28.55	29.27	0.14	28.07	28.08	28.04	0.05				
Mean	27.74[a]	26.51[b]	28.51[c]	29.27[d]	0.13	28.02	28.01	28.00	0.03	< 0.01	0.86	0.33	0.69
SFA,% of lipid[6]													
d 14	17.44[a]	18.85[b]	16.29[c]	16.38[c]	0.10	17.27	17.22	17.23	0.03				
d 28	16.75[a]	18.44[b]	16.34[a]	16.42[a]	0.12	17.01	16.98	16.97	0.03				
Mean	17.10[a]	18.64[b]	16.31[c]	16.40[c]	0.11	17.14	17.10	17.10	0.02	< 0.01	0.31	< 0.01	0.89

[1]Data were covariate adjusted for baseline (d 0) values and apparent mold concentration [(Apparently moldy fraction / (apparently moldy fraction + apparently fresh fraction)) x 100].

[2]DCO-1 = 1.2, 0.08, and 0.48% moisture, insoluble impurities, and unsaponifiables, respectively. DCO-2 = 1.2, 0.01, and 0.1% moisture, insoluble impurities, and unsaponifiables, respectively.

[3]CON = no added antioxidant, REN = Rendox-CQ® (active ingredient is tertiary butyl hydroquinone [TBHQ]; Kemin Industries, Des Moines, IA) at 1,000 ppm of lipid, and SAN = Santoquin-Q4T® (active ingredients are ethoxyquin and TBHQ; Novus Intl., St. Louis, MO) at 1,500 ppm of lipid.

[4]I = effect of ingredient, A = effect of antioxidant.

[5]PSEM = pooled standard error of mean.

[6]Effect of time ($P < 0.05$).

[7]Main effect mean of ingredient or antioxidant, regardless of day.

[8]TBARS = thiobarbituric acid reactive substances, MDA = malondialdehyde.

[a,b,c,d]Within a row, means without a common superscript differ ($P < 0.05$).

Table 4-3. Interactive effects of ingredient, antioxidant, and sampling day on characteristics of high oil DDGS (HO-DDGS), reduced oil DDGS (RO-DDGS), and distillers corn oil (DCO) stored at 38°C and 90% relative humidity[1,2,3]

Ingredient:	HO-DDGS			RO-DDGS			DCO-1			DCO-2			
Antioxidant:	CON	REN	SAN	CON	REN	SAN	CON	REN	SAN	CON	REN	SAN	PSEM[4]
Item													
Peroxide value, meq/kg lipid[5,6]													
d 14	7.13^{a}	3.12^{bc}	3.63^{b}	4.49^{d}	2.68^{c}	2.83^{c}	6.86^{a}	2.99^{bc}	3.40^{bc}	4.73^{d}	2.68^{c}	2.84^{bc}	0.37
d 28	31.37^{a}	13.93^{bc}	16.35^{b}	20.50^{d}	11.73^{bc}	13.55^{bc}	31.02^{a}	14.57^{bc}	15.14^{bc}	22.26^{d}	12.26^{c}	12.83^{c}	0.37
Mean[7]	19.25^{a}	8.52^{cde}	9.99^{c}	12.49^{b}	7.21^{e}	8.19^{de}	18.94^{a}	8.78^{cde}	9.27^{cd}	13.5^{b}	7.47^{e}	7.84^{de}	0.21
TBARS, meq MDA/kg lipid[5,6,8]													
d 14	5.05^{a}	2.90^{cd}	2.41^{d}	3.75^{bc}	2.37^{d}	2.34^{d}	4.83^{a}	2.61^{cd}	2.39^{d}	4.16^{ab}	2.08^{d}	2.06^{d}	0.20
d 28	21.06^{a}	9.53^{b}	8.99^{b}	14.26^{d}	11.00^{bc}	10.13^{bc}	21.24^{a}	8.41^{b}	9.27^{b}	14.08^{cd}	10.08^{b}	9.81^{b}	0.69
Mean	13.05^{a}	6.22^{b}	5.70^{b}	9.00^{c}	6.69^{b}	6.24^{b}	13.04^{a}	5.51^{b}	5.83^{b}	9.12^{c}	6.08^{b}	5.93^{b}	0.37
p-Anisidine value[5,6]													
d 14	3.94^{a}	0.96^{b}	1.04^{b}	3.76^{a}	0.65^{b}	0.97^{b}	3.79^{a}	0.18^{b}	0.40^{b}	3.62^{a}	0.64^{b}	0.87^{b}	0.17
d 28	9.10^{a}	3.43^{bc}	2.88^{bc}	9.48^{a}	4.96^{b}	4.26^{b}	9.69^{a}	1.22^{c}	1.33^{c}	8.95^{a}	3.35^{bc}	2.77^{bc}	0.48
Mean	6.52^{a}	2.19^{b}	1.96^{bcd}	6.62^{a}	2.80^{b}	2.61^{b}	6.74^{a}	0.70^{d}	0.87^{cd}	6.29^{a}	2.00^{bc}	1.82^{bcd}	0.29
Linoleic Acid,% of lipid[5]													
d 14	53.02	53.14	52.99	52.74	52.63	52.61	52.99	53.4	53.44	52.24	52.27	52.27	0.11
d 28	53.59	53.56	53.45	53.03	52.85	52.83	53.07	53.13	53.39	52.23	52.23	52.51	0.14
Mean[4]	53.31^{a}	53.35^{a}	53.22^{ab}	52.88^{abc}	52.74^{bc}	52.72^{bc}	53.03^{ab}	53.26^{ab}	53.41^{a}	52.24^{c}	52.25^{c}	52.39^{c}	0.10
Linolenic Acid,% of lipid													
d 14	1.65	1.65	1.63	1.7	1.71	1.71	1.32	1.34	1.34	1.36	1.37	1.38	0.03
d 28	1.6	1.58	1.62	1.72	1.7	1.73	1.31	1.33	1.34	1.36	1.36	1.38	0.03
Mean	1.63	1.62	1.63	1.71	1.7	1.72	1.31	1.34	1.34	1.36	1.37	1.38	0.03
PUFA,% of lipid[5]													
d 14	54.7	54.81	54.64	54.47	54.36	54.35	54.28	54.72	54.75	53.58	53.62	53.63	0.12
d 28	55.21	55.16	55.08	54.77	54.57	54.58	54.36	54.43	54.71	53.57	53.57	53.87	0.15
Mean	54.96^{a}	54.99^{a}	54.86^{ab}	54.62^{abc}	54.47^{bc}	54.46^{bcd}	54.32^{cd}	54.57^{abc}	54.73^{ab}	53.58^{e}	53.59^{e}	53.75^{de}	0.11

MUFA,% of lipid													
d 14	27.57	27.65	27.72	26.44	26.39	26.41	28.52	28.46	28.43	29.32	29.24	29.25	0.13
d 28	27.77	27.88	27.84	26.62	26.56	26.66	28.65	28.57	28.44	29.26	29.31	29.24	0.16
Mean	27.67	27.76	27.78	26.53	26.47	26.54	28.58	28.52	28.43	29.29	29.27	29.25	0.13
SFA,% of lipid[5]													
d 14	17.47	17.4	17.47	18.79	18.87	18.88	16.42	16.22	16.22	16.39	16.4	16.35	0.13
d 28	16.76	16.71	16.78	18.4	18.4	18.53	16.38	16.36	16.29	16.5	16.47	16.29	0.14
Mean	17.12[a]	17.05[a]	17.12[a]	18.59[b]	18.63[b]	18.70[b]	16.4[c]	16.29[c]	16.26[c]	16.45[c]	16.44[c]	16.32[c]	0.11

[1]DCO-1 = 1.2, 0.08, and 0.48% moisture, insoluble impurities, and unsaponifiables, respectively. DCO-2 = 1.2, 0.01, and 0.1% moisture, insoluble impurities, and unsaponifiables, respectively.

[2]CON = no added antioxidant, REN = Rendox-CQ® (active ingredient is tertiary butyl hydroquinone [TBHQ]; Kemin Industries, Des Moines, IA) at 1,000 ppm of lipid, and SAN = Santoquin-Q4T® (active ingredients are ethoxyquin and TBHQ; Novus Intl., St. Louis, MO) at 1,500 ppm of lipid.

[3]Data were covariate adjusted for baseline (d 0) values and apparent mold concentration [(Apparently moldy fraction / (apparently moldy fraction + apparently fresh fraction)) x 100].

[4]PSEM = pooled standard error of mean.

[5]Ingredient × antioxidant ($P < 0.05$).

[6]Ingredient × antioxidant x day ($P < 0.05$).

[7]Main effect mean of ingredient x antioxidant, regardless of day.

[8]TBARS = thiobarbituric acid reactive substances, MDA = malondialdehyde.

[a,b,c,d,e] Within a row, means without a common superscript differ ($P < 0.05$).

Table 4-4. Main effects of ingredient, antioxidant, and day on characteristics of high oil DDGS (HO-DDGS), reduced oil DDGS (RO-DDGS) stored at 38°C and 90% relative humidity

Item	Ingredient			Antioxidant[1]				P-Values[2]			
	HO-DDGS	RO-DDGS	PSEM[3]	CON	REN	SAN	PSEM	I	A	I × day	A × day
Crude fat,%[4]											
d 14	11.45[a]	2.86[b]	0.53	7.08	7.14	7.24	0.53				
d 28	8.98[a]	2.74[b]	0.53	5.98	5.86	5.75	0.55				
Mean[5]	10.21[a]	2.8[b]	0.51	6.53	6.5	6.5	0.09	< 0.01	0.96	< 0.01	0.05
Apparent mold,%											
d 14	13.46[a]	24.2[b]	2.1	19.4	17.5	19.58	2.20				
d 28	46.08[a]	70.12[b]	3.2	56.33	57.81	60.17	3.60				
Mean	29.77[a]	47.16[b]	2.34	37.86	37.66	39.88	2.50	< 0.01	0.60	< 0.01	0.68
Total mold count, CFU[6] × 10^6/g											
d 14	2.47	2.08	0.55	2.73	1.65	2.44	0.64				
d 28	11.36[a]	1.72[b]	0.62	6.93	7.45	5.25	0.71				
Mean	6.91[a]	1.90[b]	0.42	4.83[x]	4.55[x,y]	3.85[y]	0.47	< 0.01	0.11	< 0.01	0.12
Moisture,%[4]											
d 14	21.33[a]	19.66[b]	0.31	20.44	20.35	20.7	0.33				
d 28	23.05[a]	21.25[b]	0.32	22.02	22.04	22.38	0.35				
Mean	22.19[a]	20.45[b]	0.23	21.23	21.19	21.54	0.26	< 0.01	0.05	< 0.01	0.66

[1]CON = no added antioxidant, REN = Rendox-CQ® (active ingredient: tertiary butyl hydroquinone [TBHQ]; Kemin Industries, Des Moines, IA) at 1,000 ppm of lipid, and SAN = Santoquin-Q4T® (active ingredient: ethoxyquin and TBHQ; Novus Intl., St. Louis, MO) at 1,500 ppm of lipid.

[2]I = effect of ingredient, A = effect of antioxidant.

[3]PSEM = pooled standard error of mean..

[4]Data were covariate adjusted for baseline (d 0) values and apparent mold concentration [(Apparently moldy fraction / (apparently moldy fraction + apparently fresh fraction)) × 100].

[5]Main effect mean of ingredient or antioxidant, regardless of day.

[6]CFU = colony forming unit.

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

Table 4-5. Interactive effects of ingredient, antioxidant, and sampling day on characteristics of high oil DDGS (HO-DDGS) and reduced oil DDGS (RO-DDGS) stored at 38°C and 90% relative humidity[1]

Ingredient:	HO-DDGS			RO-DDGS			
Antioxidant:	CON	REN	SAN	CON	REN	SAN	PSEM[2]
Item							
Crude fat,%[3,4,5,6]							
d 14	11.04[a]	11.52[a,b]	11.79[b]	3.12[c]	2.77[c]	2.70[c]	0.53
d 28	9.03[a]	8.85[a]	9.06[a]	2.93[b]	2.86[b]	2.43[b]	0.53
Mean[7]	10.03[a]	10.18[a]	10.43[a]	3.02[b]	2.81[b]	2.57[b]	0.52
Apparent mold,%[3]							
d 14	13.63	12.03	14.71	25.17	22.98	24.46	2.5
d 28	43.61	46.89	47.76	69.05	68.73	72.58	4.79
Mean	28.62	29.46	31.23	47.11	45.85	48.52	3.06
Total mold count, CFU[3,8,9] × 10^6/g							
d 14	3.03	2.03	2.33	2.43	1.26	2.55	0.83
d 28	12.28[a]	12.67[a]	9.13[a]	1.57[b]	2.23[b]	1.37[b]	0.96
Mean	7.66[a]	7.35[a,b]	5.73[b]	2.00[c]	1.75[c]	1.96[c]	0.57
Moisture,%[3,6]							
d 14	21.64	21.23	21.12	19.25	19.46	20.28	0.43
d 28	23.22	23.06	22.87	20.83	21.02	21.89	0.47
Mean	22.43	22.15	21.99	20.04	20.24	21.08	0.35

[1]CON = no added antioxidant, REN = Rendox-CQ® (active ingredient: tertiary butyl hydroquinone [TBHQ]; Kemin Industries, Des Moines, IA) at 1,000 ppm of lipid, and SAN = Santoquin-Q4T® (active ingredient: ethoxyquin and TBHQ; Novus Intl., St. Louis, MO) at 1,500 ppm of lipid.

[2]PSEM = pooled standard error of mean.

[3]Effect of time ($P < 0.05$).

[4]Ingredient × antioxidant ($P < 0.05$).

[5]Ingredient × antioxidant x day ($P < 0.05$).

[6] Data were covariate adjusted for baseline (d 0) values and apparent mold concentration [(Apparently moldy fraction / (apparently moldy fraction + apparently fresh fraction)) × 100].

[7]Main effect mean of ingredient x antioxidant, regardless of day.

[8]Ingredient × antioxidant ($P < 0.1$).

[9]CFU = colony forming unit.

[a,b,c]Within a row, means without a common superscript differ ($P < 0.05$).

CHAPTER 5. EFFECT OF FEEDING PEROXIDIZED DRIED DISTILLERS GRAINS WITH SOLUBLES TO SOWS AND PROGENY ON GROWTH PERFORMANCE AND INCIDENCE OF MULBERRY HEART DISEASE

SUMMARY

The objective of this experiment was to evaluate the effects of including peroxidized corn dried distillers grains with solubles (DDGS) in diets for sows and nursery pigs on growth performance, vitamin E (VE) and Se status, and the incidence of Mulberry Heart Disease (MHD) of nursery pigs. Sows (n = 12) were fed corn-soybean meal diets (C-SBM) or C-SBM diets with DDGS (40 and 20% in gestation and lactation, respectively) for 3 parities. In the third parity, 108 weaned pigs (BW = 6.6 ± 0.36 kg) were blocked by BW within litter, assigned to pens (2 littermates/pen), and pens were assigned 1 of 3 nursery dietary treatments: 1) corn-soybean meal (CON), 2) 30% peroxidized DDGS (Ox-D), and 3) 30% Ox-D with 5× NRC (1998) level of VE (Ox-D+5VE) for 7 wks, in a 2 × 3 factorial arrangement of sow and nursery diets (n = 9 pens/treatment). The peroxidized DDGS source added to nursery diets contained concentrations of thiobarbituric acid reactive substances (TBARS) and peroxide value that were 25 and 27 times greater than a reference corn sample. Sow colostrum, milk, and serum, as well as pig serum and liver samples were analyzed for α-tocopherol and Se concentrations. Pig serum was also analyzed for glutathione peroxidase activity (GPx), TBARS, and sulfur-containing amino acids (SAA). Pig hearts were evaluated for gross and histopathological lesions, and there was no evidence of MHD among all dietary treatments. Pigs from sows fed DDGS tended to have reduced (P = 0.07) VE in serum during lactation and reduced VE at weaning (P < 0.01; 5.6 vs. 6.7 ± 0.1 μg/mL) compared with pigs from sows fed CON.

Pigs fed Ox-D ($P = 0.04$) or Ox-D+5VE ($P = 0.08$) had greater ADFI than pigs fed C, but ADG was not affected ($P > 0.05$) by nursery diet. Feeding Ox-D or Ox-D+5VE increased ($P < 0.05$) serum α-tocopherol compared with CON (2.5, 2.8, and 3.4 ± 0.09 μg /mL, respectively), but TBARS and GPx were not affected by nursery diet. Serum concentration of SAA was 40 to 50% greater ($P < 0.01$) for pigs fed Ox-D or Ox-D+5VE compared with those fed C, which was likely related to greater ($P < 0.01$) SAA intake for pigs fed Ox-D. The antioxidant properties of SAA may have spared VE and Se and masked any effect of Ox-D on metabolic oxidation status. Therefore, increasing the dietary VE concentration was unnecessary in nursery diets containing Ox-D. Including DDGS in sow diets reduced the VE status of pigs during lactation, but not in the nursery when MHD can be a concern.

KEYWORDS: dried distillers grains with solubles, growth performance, Mulberry Heart Disease, pigs, sows, vitamin E

INTRODUCTION

Lipid peroxidation is a complex process which produces and degrades a variety of toxic compounds associated with cellular damage (Bartosz and Kolakowska, 2011). Peroxidation is accelerated with increasing amounts of unsaturated fatty acids in lipids and exposure to heat, light, moisture, and oxygen, but antioxidants impede peroxidation (Belitz et al., 2009). Linoleic acid and other PUFA in corn dried distillers grains with solubles (**DDGS**) can be readily peroxidized depending on temperature and duration of drying (Song and Shurson, 2013). In fact, Song and Shurson (2013) reported that DDGS may contain thiobarbituric acid reactive substances (**TBARS**) value and peroxide values

(**PV**) exceeding 25 times those in corn, but levels vary, depending on the source of DDGS.

Dietary inclusion of peroxidized lipids reduces growth (DeRouchey et al., 2004; Boler et al., 2012; Liu et al., 2012b) and vitamin E content of serum and tissues (Yuan et al., 2007; Boler et al., 2012; Liu et al., 2012a), potentially impairing the metabolic antioxidant system. A deficiency of vitamin E or Se is associated with Mulberry Heart Disease (**MHD**), which typically results in the sudden death of fast growing nursery pigs (AASV, 2009). Weaver (2010a,b) reported an increased incidence of MHD in the U.S. pork industry and suggested that feeding diets containing DDGS was a contributing factor. However, Song et al. (2013) reported improved vitamin E status in pigs fed diets with 30% DDGS, but pigs were not evaluated for MHD. Furthermore, the impact of including DDGS in sow diets on antioxidant status of their offspring has not been determined.

We hypothesized that adding high levels of DDGS to diets for sows during gestation and lactation over three parities, and feeding a highly peroxidized source of DDGS to their progeny after weaning would reduce growth and antioxidant status of nursery pigs leading to an increased incidence of MHD, and that supranutritional supplementation of vitamin E would mitigate these effects.

MATERIALS AND METHODS

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

Animal management

This experiment was conducted at the University of Minnesota Southern Research and Outreach Center Swine Research Facility in Waseca. Two farrowing groups of 6 (n = 12) third-parity sows (Landrace × Yorkshire; Topigs, Winnipeg, Manitoba) mated to Duroc boars (Compart Boar Store, Nicollet, MN) and their progeny (n = 108) were used. On d 109 of gestation, sows were moved into environmentally controlled farrowing rooms and placed in individual farrowing stalls (2.13 m long × 0.97 m high × 0.66 m wide) with fully slatted floors. Each farrowing stall was equipped with a feeder, heat pad (Osborne Industries Inc., Osborne, KS), heat lamp, and nipple waterers for sows and piglets. Piglets were identified with ear notches at birth. When necessary, pigs were cross-fostered within sow dietary treatment before 24 h of age (target litter size = 10 to 11 piglets per sow). Pigs were weaned on d 19.3 ± 1.3 of age and housed in pens (1.2 m x 1.2 m) where they were provided *ad libitum* access to water and experimental diets for 7 wks.

Dietary treatments

Sows were fed one of two gestation and lactation diets: corn-soybean meal diets (**C-SBM**) or diets containing DDGS (40 and 20% in gestation and lactation, respectively; **DDGS**) for three successive parities (Table 5-1) as part of a larger experiment (Li, 2012). In the third parity, 108 weaned pigs (n = 60 and 48 for groups 1 and 2, respectively), half from each sow dietary treatment were selected. Pigs were blocked by BW and litter, and placed in pens of 2 littermates. Each pen was assigned to 1 of 3 nursery diets (**ND;**

Table 5-2): 1) Control (**CON**), 2) 30% oxidized DDGS (**Ox-D**), and 3) **Ox-D+5VE** (as Ox-D with 5× NRC [1998] recommended level of vitamin E as dl-α-tocopheryl acetate). This design resulted in a 2 × 3 factorial arrangement of sow diets and nursery diets with 9 replications for each treatment.

Diet composition

Diets were formulated to meet or exceed NRC (1998) requirements for sows and nursery pigs, and were provided in meal form. Nursery diets were fed over 3-phases with intervals of 1, 2, and 4 wks for phases 1, 2, and 3, respectively. Nursery diets were formulated to contain similar concentrations of standardized ileal digestible AA and available P.

The DDGS source used to formulate sow diets contained a level of peroxidation that was representative of the level typically observed among DDGS sources (1.6 ng Malondialdehyde [**MDA**] eq./mg oil; Song and Shurson, 2013). The DDGS source used to formulate nursery diets was selected based on evaluation of 31 corn DDGS sources produced by U.S. ethanol plants (Song and Shurson, 2013), and contained the greatest thiobarbituric acid reactive substances (**TBARS**) value (5.2 ng MDA eq./mg oil) and peroxide value (**PV;** 84.1 meq O_2/kg oil) relative to 30 other DDGS sources (mean values = 1.8 ng MDA eq./mg oil and 11.5 meq O_2/kg oil, respectively). Coincidentally, this source also contained the highest total S concentration (0.95%) compared with 7 other DDGS sources evaluated (mean = 0.50%).

Sow and litter performance

Throughout gestation, sows were fed approximately 2.04 kg/d of their assigned experimental diets, which was adjusted to achieve a body condition score of 3 at farrowing (scale: 1 = thin, 5 = obese; Coffey et al., 1999). Sows were fed 2.25 kg/d of their assigned lactation diet from d 109 of gestation to farrowing. After parturition, feeding level increased steadily to allow *ad libitum* consumption from d 5 to 19 of lactation. Orts were collected at weaning, weighed, and recorded. Sows were weighed and backfat depth was determined ultrasonically (Lean-Meater, Renco Corp., Minneapolis, MN) at the last rib 6.5 cm off the dorsal mid-line on both the right and left side on d 109 of gestation, within 24 h after farrowing, and at weaning. Sow feed disappearance, pig mortality, and sow and pig weights at farrowing and weaning were recorded. After weaning, pigs were weighed individually at each dietary phase change, and pen feed disappearance was recorded. These data were used to calculate ADFI, ADG, and G:F of each pen.

Milk, blood, and tissue collection

Colostrum (20 to 30 mL) was collected (without exogenous oxytocin) by manual expression of functional glands during farrowing. On d 7 and d 19 of lactation, milk was collected within 10 minutes after injection of 20 USP of oxytocin (Bimeda-MTC–Animal Health Inc., Cambridge, Ontario, Canada). Samples were frozen (-20°C) until further analysis.

Focal pigs (at least 2 per litter, total = 27 pigs) were selected from each litter at birth based on the criteria of being the first apparently healthy pigs born regardless of sex. On d 0, blood samples were collected from pigs before initial suckling, and from sows (<

24 h post-farrowing) via jugular venipuncture using serum separator vacutainer tubes coated with silicone and micronized silica particles (Becton Dickson, Franklin Lakes, NJ). Blood was again collected on from sows and pigs on d 7 and 19. In the nursery, blood samples were obtained from the same focal pigs, but additional focal pigs were selected randomly to achieve 1 focal pig per pen (n = 54 focal pigs). On d 19 (weaning), 47, and 68 of age, blood samples were collected as previously described. After collection, blood was placed on ice, stored at 4°C, and centrifuged at 1,400 x *g* for 10 min at room temperature. Serum was transferred into microcentrifuge tubes and frozen at -20°C.

Focal pigs were euthanized with a sodium pentobarbital overdose on d 68 to obtain heart and liver samples. Livers were placed immediately on ice and frozen at -20°C. A veterinarian blinded to treatments evaluated each intact heart and assigned a score for gross heart lesions (0 = normal heart, 1 = abnormal heart, without characteristics of MHD, 2 = abnormal heart with mild characteristics of MHD, and 3 = abnormal heart with severe characteristics of MHD). Heart tissue was then excised from right and left ventricles and septum, and placed in 10% neutral buffered formalin. Heart sections were trimmed, embedded in paraffin, mounted onto slides, and stained with haematoxylin and eosin according to procedures described by Carson and Hladik (2005). A veterinary pathologist, blinded to treatments, evaluated heart sections histologically for lesions characteristic of MHD.

Laboratory Analysis

Diet nutrient composition

Feed samples were retained, frozen at -20°C, and analyzed for DM (method 930.15; AOAC, 2005), crude fat (method 920.39; AOAC, 2005), NDF (method 2002.04; AOAC, 2005, and Methods 5.1 and 5.2; NFTA, 1993), ADF, (method 973.18; AOAC, 2005 modified according to Tecator Application Note 3429), ash (method 942.05; AOAC , 2008), N (method 990.03; AOAC, 2005), Ca (method 985.01; AOAC, 2005), P (method 985.01; AOAC, 2005), S (method D4239; ASTM, 2011), Se, and α-tocopherol at Minnesota Valley Testing Laboratories (New Ulm). After digestion in nitric acid, Se was analyzed according to procedures described by Wahlen et al. (2005) using an Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer (Agilent Technologies Inc, Santa Clara, CA). The α-tocopherol content of feeds was measured using a modified AOAC method (971.30; AOAC, 2006) with high-performance liquid chromatography (**HPLC**) and a fluorescence detector. Amino acids (method 982.30; AOAC , 2006) were analyzed at the University of Missouri Agricultural Experiment Station Chemistry Laboratory (Columbia).

Milk, liver, and serum Se and α-tocopherol concentrations

The Se and α-tocopherol concentrations in sow serum and milk and in pig serum and liver samples were analyzed at the Michigan State University Diagnostic Center for Population and Animal Health (East Lansing). One gram of liver tissue was digested overnight in 2 mL nitric acid, and Se concentrations were determined according to the procedure of Wahlen et al. (2005) using an Agilent 7500ce Inductively Coupled Plasma

Mass Spectrometer (Agilent Technologies Inc, Santa Clara, CA). For α-tocopherol analysis, liver samples were weighed and homogenized in distilled, deionized water (1:4 w/v). Serum samples and liver homogenates were mixed with equal volumes of hexane and a solution of butylated hydroxytoluene in ethanol (10% w/v). Mixtures were centrifuged at 1,900 × *g* for 10 min, and a known aliquot of the hexane layer was removed and dried under vacuum. Samples were dissolved in a chromatographic mobile phase (7:2:1, acetonitrile, methylene chloride, methanol) and analyzed by HPLC (Separation Module 2690) using a Waters Symmetry C18, 3.5 mm, 4.6 x 75 mm analytical column with detection by UV absorbency at 292 nm (Waters, Milford, MA). Trans-β-APO-8"-carotenal was used as an internal standard.

Serum TBARS

Pig serum was analyzed for TBARS concentration according to methods adapted from the Animal Models of Diabetic Complications Consortium (Feldman, 2004). Briefly, 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 12,000 × *g* for 15 min at 4ºC. Two hundred microliters 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 dry block heater maintained at 100°C. Each vial was then cooled in an ice bath, and an aliquot was read at 532 nm using a spectrometer (SpectraMax 250, Molecular Devices, Sunnyvale, CA).

Hepatic glutathione (GSH)

Total GSH concentrations in liver were analyzed using a commercial kit (catalog number: CS0260, Sigma-Aldrich, St. Louis, MO). Fifty milligrams of liver was homogenized in 500 μL of 5% 5-sulfosalcyclic acid followed by centrifugation at 10,000 × *g* for 10 min at 4ºC, and duplicate 10 μL aliquots of supernatant and standards were analyzed according to kit instructions. Briefly, GSH was measured indirectly by a coupled reaction with glutathione reductase after adding 5',5'-dithio-bis(2-nitrobenzoic acid) and NADPH. Formation of 2-Nitro-5-thiobenzoic acid was monitored colorimetrically using a spectrometer (SpectraMax 250, Molecular Devices, Sunnyvale, CA) at 412 nm for 5 min.

Serum glutathione peroxidase activity

Glutathione peroxidase (**GPx**) activity in pig serum was determined using a commercial kit (catalog number: 703102, Cayman Chemical, Ann Arbor, MI). Briefly, GPx activity was measured indirectly by a coupled reaction with GSH reductase after addition of cumene hydroperoxide to duplicate serum samples and standards. Oxidation of NADPH was measured colorimetrically using a spectrometer (SpectraMax 250, Molecular Devices, Sunnyvale, CA) at 340 nm for 5 min. Glutathione peroxidase activity was expressed as μmol/min/mL of serum and compared to a bovine erythrocyte GPx standard curve.

Serum sulfur-containing amino acids

Concentrations of Met, Cystine, and Tau were determined in pig serum on d 68 of age by liquid chromatography–mass spectrometry (LC-MS). Briefly, 5 μL of samples

and standards were mixed with 5 μL of 100 μM *p*-chlorophenylalanine (internal standard), 50 μL of 10 mM sodium carbonate, and 100 μL of dansyl chloride (3 mg/mL in acetone). The mixture was incubated at 25°C for 15 min and centrifuged (18,000 × *g*) for 10 min. Five microliters of supernatant were injected and separated in an Acquity BEH C18 column (Waters, Milford, MA) by a gradient of mobile phase ranging from water to 95% aqueous acetonitrile containing 0.1% formic acid for 10 min. The eluent was introduced into a SYNAPT quadrupole time-of-flight mass spectrometer (Waters, Milford, MA) for mass detection. Mass chromatograms and spectral data were acquired and processed by MassLynx™ software (Waters, Milford, MA) in centroid format. The concentrations of Met, Cystine, and Tau in serum were determined by calculating the ratio between their peak areas and the peak area of *p*-chlorophenylalanine using QuanLynx™ software (Waters, Milford, MA).

Statistical Analyses

The MIXED procedure of SAS (v9.3; SAS Inst. Inc., Cary NC) was used to evaluate the effect of sow dietary treatment on the measures obtained during the lactation period using farrowing group as a random effect. Sow (or litter) was the experimental unit for measures from the lactation phase. A separate ANOVA was used to analyze data collected in the nursery period using the MIXED procedure of SAS (SAS Inst. Inc., Cary NC) in a 2 x 3 factorial arrangement of sow and nursery pig diet in a split-plot design. Sow was the whole plot and nursery pen was the subplot. Pen was used as the experimental unit for data from the nursery phase. Group and nursery BW block were included as random factors in the model. The repeated measures option was used to evaluate the effect of time and its interactions. Normality of model residuals was

evaluated using the UNIVARIATE procedure of SAS. The association of gross heart lesion score with dietary treatment was evaluated using Chi-square analysis. Results are reported as least squares means. Comparisons were performed using the PDIFF option of SAS with the Tukey-Kramer adjustment. Treatment effects were significant at $P < 0.05$, whereas values between $0.05 \leq P \leq 0.10$ were considered statistical trends.

RESULTS AND DISCUSSION

Six pigs were removed from the experiment for reasons unrelated to dietary treatment. One pig died of intestinal torsion and five pigs were euthanized because of substantial body weight loss resulting from post-weaning feed refusal. Two of these pigs were from DDGS/Ox-D, 2 pigs were from C-SBM/CON, and 2 pigs were from DDGS/CON sow and nursery dietary treatments, respectively. No pigs developed MHD during the course of this experiment, and gross heart lesion score was not associated with dietary treatment.

Growth Performance

A summary of these performance results is available elsewhere (Li, 2012). The primary focus of the current work was on the antioxidant status and growth of nursery pigs.

Including DDGS in sow diets for 3 reproductive cycles did not affect ADG, ADFI, and gain:feed of nursery pigs (Table 5-3). Few have investigated the effect of sow diet on the growth performance of progeny, but our results suggest long-term dietary inclusion of DDGS for sows does not affect the growth performance of their offspring in the nursery phase.

The concentration of lipids in DDGS can be as much as three-fold greater than corn, and linoleic acid is the predominant fatty acid (NRC, 2012). Linoleic acid and other PUFA are highly susceptible to lipid peroxidation, which is accelerated by heat and moisture (Bartosz and Kolakowska, 2011). During drying, DDGS is typically exposed to temperatures as high as 500°C (Rosentrater et al., 2012), but temperatures may vary from 371-593°C (Song and Shurson, 2013). Such extreme and variable temperatures contribute to substantial ranges in the concentrations of lipid peroxidation products in DDGS. Analysis of PV and TBARS in 31 DDGS samples revealed ranges of 4.2 to 84.1 meq O_2/kg oil and 1.0 to 5.2 ng MDA eq/kg of oil, respectively (Song and Shurson, 2013). The DDGS source in the nursery diets of the current experiment had the greatest concentrations of PV and TBARS among 31 samples evaluated, but the DDGS in sow diets had intermediate concentrations. Lipid peroxidation products contribute to damage of organs and cells (Griffiths, 2005; Bartosz and Kolakowska, 2011), and may lead to a metabolic deficiency of antioxidants known as oxidative stress (Lykkesfeldt and Svendsen, 2007).

When dietary peroxidized lipids are fed to swine (DeRouchey et al., 2004; Boler et al., 2012; Liu et al., 2012b), poultry (Dibner et al., 1996b), and rats (Liu and Huang, 1995; Eder, 1999) ADG often declines. Conversely, we observed no negative effects of Ox-D in nursery diets on ADG. DeRouchey et al. (2004) suggested a threshold of 2.4 meq O_2/kg diet, above which growth performance is compromised. In the current experiment, nursery diets contained 1.7 meq O_2/kg of diet (30% inclusion of DDGS [6.9% crude fat] with 84.1 meq O_2/kg of oil). Therefore, it is possible that our nursery diets had insufficient levels of peroxidation products to reduce pig growth. Furthermore,

other researchers evaluated concentrated lipid sources (e. g. corn oil, fish oil, canola oil) containing peroxidation products (DeRouchey et al., 2004; Boler et al., 2012; Liu et al., 2012b), but lipids represent < 12% of the total chemical composition in DDGS. Our findings are in agreement with others indicating that dietary DDGS does not affect ADG (Whitney and Shurson, 2004; Spencer et al., 2007; Barbosa et al., 2008), but our results are not directly comparable to the results from these studies because they fed transition diets without DDGS for 1 to 3 wks after weaning. Our experimental diets were first offered on the day after weaning. Pigs fed Ox-D ($P = 0.04$) or Ox-D+5VE ($P = 0.08$) had greater ADFI compared with pigs fed CON (Table 5-3). Therefore, gain:feed was improved ($P = 0.08$) by feeding CON compared to Ox-D, but not ($P > 0.05$) Ox-D+5VE. The effect of feeding diets containing DDGS on ADFI in the current study was likely related to overestimation of the ME content of the DDGS for nursery pigs because ADG was not affected. Many factors are involved in the production of DDGS and vary among ethanol plants, contributing to variable energy content of DDGS (Shurson and Alhamdi, 2008). Dietary energy density is related inversely to feed intake (Ellis and Augspurger, 2001). Furthermore, fibrous feedstuffs like DDGS contribute to expansion of the gastrointestinal tract and increased feed intake (Pond et al., 1988). In the current study, nursery diets containing Ox-D had 70% higher concentrations of NDF (mean weighted by duration of phase) than C. Therefore, in the current study, increased ADFI for pigs fed DDGS may be related to caloric density and fiber content.

Metabolic Oxidative Balance

Compared to saturated fat sources, lipids containing high concentrations of PUFA increase the metabolic demand for vitamin E in swine (Ullrey, 1981) and other species

(Horwitt, 1960; Harris and Embree, 1963) because of the increased potential for peroxidation or reduced absorption of vitamin E (Hollander, 1981). Research results suggest that dietary sources of PUFA like corn oil (Malm et al., 1976) and fish oil (Hidiroglou et al., 1993; Farnworth et al., 1995) reduce the α-tocopherol concentrations in serum of sows and fetuses compared to other dietary lipids. Concentrations of α-tocopherol in sow serum or milk were not affected by dietary inclusion of DDGS for sows ($P > 0.05$; Table 5-4) in the current study. However, pigs from sows fed DDGS had reduced α-tocopherol ($P = 0.07$; Table 5-4) concentration in serum during lactation compared with those from sows fed CON. The calculated linoleic acid content was 11% (gestation) and 6% (lactation) greater for DDGS diets compared to CON, indicating that PUFA content was greater in DDGS diets. Furthermore, sow diets containing DDGS had 3 to 8% less analyzed vitamin E than CON. Research results reported by Mahan et al. (2000) suggest that dietary vitamin E levels for sows affects the α-tocopherol content of pig serum. Therefore, in the current study, the effects of DDGS in sow diets on the vitamin E status of pigs was likely related to the greater dietary PUFA and reduced dietary vitamin E content compared with those fed CON. Others have reported no effect of feeding DDGS on the vitamin E content of sow milk and plasma (Crowder and Johnston, 2011).

Selenium is a component of GPx which is an integral enzyme for metabolic antioxidant defense. Sows fed DDGS had reduced ($P = 0.05$) concentrations of Se in serum compared to CON. The Se content of colostrum from sows fed DDGS was reduced ($P < 0.05$) compared to sows fed CON, and this difference existed regardless of sampling day ($P < 0.05$). However, analyzed Se content of sow diets was not equal across

treatments in the present study. Gestation and lactation diets containing DDGS had 18% and 19% lower analyzed Se concentrations, respectively, compared to C-SBM diets. Mahan (2000) reported that adding dietary Se for sows at 6 d pre-partum elevated content of Se in colostrum, milk, and serum, indicating that in late gestation and lactation, elevated dietary Se has significant effects. Perhaps the reduced dietary Se content in gestation contributed to the 25% reduction in the Se content of colostrum. Similarly, during lactation, the Se content of serum from pigs from sows fed DDGS was reduced ($P < 0.001$) relative to pigs nursing sows fed C-SBM (Table 5-4), and this may be related to milk concentration of Se. Sow diet did not impact ($P > 0.05$) GPx activity in serum of nursing pigs (data not shown). Therefore, differences in the content of Se in milk or serum from sows in this study were likely affected by daily consumption of Se during gestation rather than dietary inclusion of DDGS. In support of this hypothesis, others have reported no effect of feeding DDGS on the Se content of sow milk and plasma (Crowder and Johnston, 2011).

The concentrations of α-tocopherol and Se increased ($P < 0.05$) in serum and decreased ($P < 0.05$) in milk after parturition. Similar results have been reported by others for Se (Mahan, 1994, 2000) and vitamin E (Loudenslager et al., 1986; Mahan, 2000; Mahan et al., 2000). In the current study, diminished demand for vitamin E and Se for lactogenesis in late lactation may have led to increased concentrations of these nutrients in sow serum. Despite declining concentrations in milk, α-tocopherol and Se concentrations increased ($P < 0.05$) in pig serum after farrowing, and this response is consistent with other reports (Loudenslager et al., 1986; Mahan, 1991) where this

increase is likely related to low reserves of vitamin E of pigs at birth (Mahan and Vallet, 1997).

The TBARS assay measures MDA, a highly reactive product of lipid peroxidation and commonly used as an indicator of metabolic oxidative stress (Griffiths, 2005). Interestingly, we found no influence ($P > 0.05$) of sow diet on TBARS in serum from nursing pigs despite a 17% reduction in the α-tocopherol content of pig serum when feeding DDGS diets to sows. Therefore, our results suggest adding DDGS to sow diets does not induce oxidative stress in nursing pigs.

Oxidative stress can be of great concern in nursery pigs because they may develop antioxidant deficiency signs, such as those observed for MHD, during the initial weeks post-weaning (Ullrey, 1981). However, no signs of MHD were observed in this experiment. Pallarés et al. (2002) suggested that several factors predispose pigs to developing MHD such as stress, genetics, and pathogenic infection. However, there were no known pathogenic challenges, and environmental stressors were minimal in the current study. Serum concentrations of α-tocopherol declined by 82% in pigs after the first month post-weaning (effect of time, $P < 0.05$; Table 5-5), which is consistent with results reported by others showing reduced circulating vitamin E levels within the first few weeks post-weaning (Chung et al., 1992; Moreira and Mahan, 2002; Lauridsen, 2010). These changes are likely related to metabolic and environmental changes associated with weaning. For example, dl-α-tocopheryl acetate is commonly supplemented in nursery diets, but d-α-tocopherol is the predominant form of vitamin E in sow milk (Mahan, 1994; Lauridsen and Jensen, 2007). Chung et al. (1992) reported that supplementing d-α-tocopherol improved retention of serum vitamin E post-weaning

compared to dl-α-tocopheryl acetate, suggesting greater bioavailability of the alcohol form. Varying bioavailability among vitamin E sources is related to inefficient removal of the acetate group by carboxyl ester hydrolase (**CEH**) before absorption in the intestinal lumen (Chung et al., 1992) because the activity of CEH declines after weaning (Jensen et al., 1997). Furthermore, stressors at weaning may contribute to the post-weaning decline in vitamin E status. Recently, Zhu et al. (2012) reported greater MDA content of serum from pigs 2 wks after weaning compared with pigs that were not weaned, but vitamin E concentrations of serum were not reported. Therefore, a combination of factors (i.e. reduced bioavailability and metabolic and environmental changes) may contribute to declining levels of vitamin E in pig serum post-weaning.

Dietary peroxidized lipids have been shown to reduce serum vitamin E (Eder, 1999; Boler et al., 2012; Liu et al., 2012a) and increase TBARS content (Yuan et al., 2007; Boler et al., 2012; Liu et al., 2012a) compared with feeding diets containing unperoxidized lipids. At weaning, pigs from sows fed DDGS had reduced ($P < 0.05$) α-tocopherol concentrations in serum compared to those from sows fed C-SBM (5.6 vs. 6.7 ± 0.12 μg/mL), but not when pigs were fed Ox-D (maternal diet × nursery diet × day; $P < 0.08$). The effect of sow diet on the α-tocopherol status of the pig at weaning is likely related to its concentration in milk. At d 47 and d 68 of age, pigs fed Ox-D+5VE had greater ($P < 0.05$) α-tocopherol concentration in serum than those fed other nursery diets (Table 5-5). Furthermore, at d 68 of age, pigs fed Ox-D tended to have greater ($P = 0.08$) α-tocopherol concentration in serum compared than those fed C. Regardless of sampling day, concentrations of α-tocopherol in serum differed ($P < 0.05$) for each nursery diet (2.5, 2.8, and 3.4 ± 0.09 μg/mL for CON, Ox-D, and Ox-D+5VE, respectively). Our

results agree with those of others who have reported increased serum α-tocopherol with increased dietary vitamin E content (Chung et al., 1992). Among pigs from sows fed DDGS, pigs fed the Ox-D+5VE nursery diets had reduced ($P < 0.05$) serum Se concentrations compared with those fed Ox-D (maternal diet × nursery diet; $P = 0.09$). Regardless of sow diet, pigs fed Ox-D+5VE had reduced ($P < 0.05$) Se concentrations in serum compared with those fed other nursery diets. Inclusion of DDGS in sow diets increased ($P < 0.05$) the concentration of serum TBARS in pigs fed CON nursery diets compared with pigs from sows fed C-SBM (maternal diet × nursery diet interaction; $P = 0.05$). Therefore, feeding diets with Ox-D did not induce metabolic oxidative stress in nursery pigs in the current study. Other researchers have reported that feeding diets containing DDGS did not increase circulating levels of TBARS in pigs (Weber and Kerr, 2011; Song et al., 2013) or broilers (Heincinger et al., 2011), which is in agreement with results of the current study. In fact, our results agree with those reported by Song et al. (2013) who reported that feeding a DDGS source with high concentrations of peroxidized lipids and S increased vitamin E status of pigs. They concluded that sulfur-containing compounds present in DDGS may have provided antioxidant benefits to overcome the potential negative effects contributed from peroxidized lipids.

Sulfur-containing AA have metabolic antioxidant properties. Methionine is a precursor of Cys which can be used to synthesize GSH or Tau. Sulfur-containing amino acids counteract oxidative stress by acting as reducing agents (Atmaca, 2004). Pigs fed Ox-D or Ox-D+5VE had increased ($P < 0.05$) concentrations of sulfur containing amino acids (**SAA**; sum of cystathione, cystine, Met, and Tau) in serum compared with those fed CON (Table 5-6). Pigs fed diets containing Ox-D or Ox-D+5VE had 30% greater (*P*

< 0.05) intake of Met+Cys (calculated using ADFI x mean dietary concentration of Met+Cys across phases) compared with those fed CON (4.4, 5.6, 5.6 ± 0.6 g/d for C, Ox-D, and Ox-D+5VE, respectively). In addition to SAA, corn contains relatively high levels of phenolic compounds (e.g. ferulic acid) that have significant antioxidant properties (Adom and Liu, 2002), and are likely concentrated by 3-fold in DDGS. Furthermore, the Ox-D nursery diet in the current study contained 22% higher concentrations of vitamin E than CON (mean weighted by duration of phase), allowing for increased vitamin E intake. Consequently, we suggest that greater intake of SAA, vitamin E, or other antioxidant compounds present in DDGS counteracted the negative effects of peroxidized lipids in DDGS.

Sow diet did not affect α-tocopherol, Se, or total GSH content of pig liver (Table 5-6). Pigs fed Ox-D+5VE had greater ($P < 0.05$) α-tocopherol concentration in liver than those fed other nursery diets. Dietary vitamin E levels correspond with α-tocopherol content of liver (Chung et al., 1992). The concentration of Se in liver was reduced ($P < 0.05$) by 22% in pigs fed Ox-D compared to C, but this effect was mitigated by feeding Ox-D+5VE. However, this effect may not be entirely explained by the dietary Se content, which was only 12% less (mean weighted by duration of phase) for the Ox-D diets compared to CON. The unequal magnitude of differences suggest that some nutrients in Ox-D may have impaired Se availability. Total S concentration of diets containing Ox-D was twice that of C. Sulfuric acid is used during the ethanol and DDGS production process and contributes to the total S content in DDGS (Rosentrater et al., 2012). Dietary sodium and potassium sulfate can partially alleviate depressed growth associated with feeding high (≥ 5 ppm) concentrations of Se, suggesting that excess S can reduce Se

absorption and utilization in rats (Halverson and Monty, 1960; Ganther and Baumann, 1962; Ardüser et al., 1985). This antagonistic relationship exists only with selenate (Halverson et al., 1962; Ardüser et al., 1985), but others have shown it to occur with selenite (Halverson and Monty, 1960; Ganther and Baumann, 1962). Sodium selenite was added at the same concentration (0.3 ppm) to all experimental diets in the current study, and it is unknown if antagonism exists between S and Se in swine when Se is supplemented at this concentration.

In summary, the antioxidant status of pigs during the post-weaning period was not reduced with the inclusion of DDGS in diets fed to nursery pigs or sows. In our experiment, feeding moderately peroxidized DDGS to sows and highly peroxidized, high S DDGS to nursery pigs did not contribute to MHD. These findings imply that sulfur containing AA or other inherent antioxidant compounds present in DDGS masked any negative effects of peroxidation products and eliminated the need for supplemental vitamin E in nursery pig diets.

Table 5-1. Composition of sow diets (as-fed basis)

	Gestation[1]		Lactation	
Ingredient,%	C-SBM	DDGS	C-SBM	DDGS
Corn	74.45	54.35	61.55	51.90
DDGS	0.00	40.00	0.00	20.00
Soybean meal (46.5% CP)	18.80	0.00	30.00	20.00
Choice white grease	2.00	0.50	3.70	3.00
Dicalcium phosphate	1.90	0.80	2.40	1.90
Limestone	1.40	2.30	1.30	1.80
Salt	0.35	0.35	0.35	0.35
Vitamin mineral premix[2]	0.50	0.50	0.50	0.50
Biotin[3]	0.20	0.20	0.20	0.20
Choline chloride (50%)	0.10	0.20	0.00	0.10
L-Lys	0.00	0.40	0.00	0.20
L-Trp	0.05	0.10	0.00	0.05
L-Thr	0.15	0.20	0.00	0.00
DL-Met	0.10	0.10	0.00	0.00
Total	100.00	100.00	100.00	100.00
Analyzed nutrient composition				
ME, kcal/kg[4]	3,341	3,351	3,413	3,417
CP,%	15.7	14.9	17.3	19.3
Crude fat,%	4.9	6.6	6.3	7.5
NDF,%	7.2	13.6	5.3	10.5
ADF,%	3.0	5.0	3.3	4.1
Ca,%	1.3	1.2	1.2	1.3
P,%	0.7	0.6	0.8	0.8
Lys,%	0.8	0.9	0.9	1.0
Met+Cys,%	0.4	0.5	0.5	0.5
Thr,%	0.6	0.6	0.7	0.7
Trp,%	0.2	0.2	0.2	0.3
S,%	0.2	0.3	0.2	0.3
Se, mg/kg	0.6	0.5	0.6	0.7
Vitamin E, IU/kg	69.0	67.0	65.0	60.0

[1]C-SBM = corn-soybean meal diet, DDGS = dried distillers grains with solubles diet.

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

[3]Supplied 0.51 mg of biotin (JBS United Inc., Sheridan, IN) per kg of diet.

[4]ME values were calculated using NRC (1998) values.

Table 5-2. Composition of nursery diets (as-fed basis)

	Phase 1 (d 19 - d 25)[1]			Phase 2 (d 26 - d 40)			Phase 3 (d 41 - d 68)		
Item	CON[2]	Ox-D[3]	Ox-D +5VE[4]	CON	Ox-D	Ox-D +5VE	CON	Ox-D	Ox-D +5VE
Ingredient,%									
Corn	43.44	26.42	26.29	63.48	42.67	42.56	67.86	47.52	47.41
DDGS	0.00	30.00	30.00	0.00	30.00	30.00	0.00	30.00	30.00
Soybean meal (46.5% CP)	23.48	10.40	10.40	32.00	23.00	23.00	29.00	19.51	19.51
Fish meal, menhaden	10.00	10.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00
Whey powder	20.00	20.00	20.00	0.00	0.00	0.00	0.00	0.00	0.00
Limestone	0.96	1.37	1.37	1.20	1.59	1.59	0.94	1.33	1.33
Monocalcium phosphate	0.68	0.00	0.00	1.44	0.73	0.73	0.96	0.26	0.26
Salt	0.25	0.25	0.25	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin mineral premix[5]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Antibiotic[6]	0.50	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00
Zinc oxide	0.05	0.05	0.05	0.00	0.00	0.00	0.00	0.00	0.00
α-tocopheryl acetate[7]	0.03	0.03	0.16	0.03	0.03	0.15	0.03	0.03	0.13
L-Lys HCl	0.03	0.32	0.32	0.31	0.47	0.47	0.24	0.42	0.42
DL-Met	0.06	0.04	0.04	0.09	0.03	0.03	0.06	0.00	0.00
L-Trp	0.00	0.05	0.05	0.01	0.03	0.03	0.00	0.03	0.03
L-Thr	0.02	0.07	0.07	0.10	0.09	0.09	0.07	0.07	0.07
Total	100.00	100.00	100.00	100.00	100.00	100.01	100.01	100.01	100.00
Analyzed nutrient composition									
ME, kcal/kg[8]	3,274	3,322	3,318	3,241	3,296	3,292	3,291	3,345	3,342
CP,%	23.3	23.9	24.6	20.6	22.8	23.3	19.3	20.4	21.5
Crude fat,%	2.7	3.7	3.7	2.4	3.5	3.6	2.2	3.9	3.9
NDF,%	4.7	10.6	11.7	8.0	12.2	10.4	6.5	10.3	13.0
ADF,%	2.8	5.2	5.5	2.8	5.7	5.6	2.9	5.5	6.0
Ca,%	1.6	1.5	1.4	1.0	1.1	1.2	0.8	0.6	0.6
P,%	1.0	0.9	0.9	0.7	0.7	0.8	0.6	0.6	0.6
Lys,%	1.4	1.6	1.5	1.2	1.3	1.4	1.3	1.1	1.3
Met+Cys,%	0.7	0.8	0.8	0.6	0.7	0.6	0.5	0.7	0.8
Thr,%	0.9	1.0	1.0	0.8	0.8	0.8	0.8	0.8	0.8
Trp,%	0.3	0.3	0.3	0.2	0.3	0.3	0.2	0.2	0.2
S,%	0.3	0.6	0.7	0.2	0.5	0.6	0.2	0.4	0.5
Se, mg/kg	0.7	0.7	0.8	0.6	0.6	0.5	0.5	0.4	0.4
Vitamin E, IU/kg	14.0	19.0	60.0	11.0	15.0	67.0	12.0	13.0	44.0

[1]19 d of age = weaning.

[2]CON = corn-soybean meal diets.
[3]Ox-D = diet containing peroxidized dried distillers grains with solubles (thiobarbituric acid reactive substances = 5.2 ng MDA eq./mg oil and peroxide value = 84.1 meq O_2/kg oil).
[4]Ox-D+5VE = diet containing peroxidized dried distillers grains with solubles (thiobarbituric acid reactive substances = 5.2 ng MDA eq./mg oil and peroxide value = 84.1 meq O_2/kg oil) and 5 times the NRC (1998) recommended level of vitamin E as dl-α-tocopheryl acetate.
[5]Premix supplied the following nutrients per kilogram of diet: 11,023 IU of vitamin A (retinyl acetate); 2,756 IU of vitamin D3; 4.41 mg of vitamin K (menadione dimethylpyrimidinol bisulfite); 9.92 mg of riboflavin; 55.11 mg of niacin; 33.07 mg of pantothenic acid as D-calcium pantothenate; 496.03 mg of choline as choline chloride; 0.06 mg of vitamin B12; 2.20 mg of pyridoxine; 1.65 mg of folic acid; 1.10 mg of thiamine; 0.22 mg of biotin; 2.20 mg of iodine (ethylenediamine dihydroiodide); 0.30 mg of selenium (sodium selenite); 90.39 mg of zinc (zinc oxide, SQM); 55.11 mg of iron (ferrous sulfate, SQM); 5.51 mg of copper (copper sulfate, SQM); and 17.64 mg of manganese (manganese oxide, SQM).
[6]Mecadox® 2.5 (carbadox 5.51 g/kg; Phibro Animal Health, Teaneck, NJ) provided 27.5 ppm carbadox.
[7]Concentration: 44,090 IU vitamin E/kg.
[8]ME values were calculated using NRC (1998) values for corn and soybean meal and 3,559 kcal/kg was used for DDGS (Pedersen et al., 2007).

Table 5-3. Main effects of maternal diet (MD) and nursery diet (ND) on growth performance of pigs

	Maternal Diet[1]		Nursery Diet[2]				P-values		
Item	C-SBM	DDGS	CON	Ox-D	Ox-D +5VE	PSEM[3]	MD	ND	MD x ND
Overall[4]									
Weaning wt., kg	6.6	6.7	6.7	6.6	6.6	0.36	0.91	0.64	0.25
Final BW, kg	28.7	29.1	28.2	29.2	29.3	1.39	0.65	0.33	0.07
ADFI, g	794	793	734^{ax}	830^{by}	816^{y}	82.4	0.97	0.03	0.44
ADG, g	456	469	456	463	468	28.8	0.47	0.81	0.28
Gain:Feed	0.58	0.60	0.63^{x}	0.57^{y}	0.58^{xy}	0.03	0.37	0.06	0.75

[1]C-SBM = corn-soybean meal sow gestation and lactation diets; DDGS = sow gestation and lactation diets contained 20 and 40% dried distillers grains with solubles, respectively.

[2]CON = corn-soybean meal nursery diets; Ox-D = nursery diets containing 30% peroxidized DDGS; Ox-D+5VE = nursery diets containing 30% peroxidized DDGS and 5 times the recommended (NRC, 1998) level of vitamin E as dl-α-tocopheryl acetate.

[3]Pooled standard error of the mean.

[4]Over 7 wk nursery period.

[a,b]Within a row and main effect, means without a common superscript differ ($P < 0.05$).

[x,y]Within a row and main effect, means without a common superscript differ ($P < 0.1$).

Table 5-4. The effect of maternal diet (MD) and MD × day interactions on metabolites in serum and milk from sows and serum from nursing pigs

	Maternal Diet[1]			P-values	
Item	C-SBM	DDGS	SEM[2]	MD	MD x d
Sow serum α-tocopherol, ug/mL[3]					
Farrowing[a]	1.51	1.35	0.258		
d 7[b]	2.53	2.55	0.258		
Weaning[b]	2.95	2.87	0.258		
Mean[4]	2.27	2.17	0.149	0.62	0.87
Milk α-tocopherol, ug/mL[3]					
Farrowing[a]	5.13	5.01	0.999		
d 7[b]	3.25	1.75	0.999		
Weaning[b]	1.76	1.63	0.999		
Mean	3.23	2.60	0.577	0.41	0.66
Pig serum α-tocopherol, ug/mL[3]					
Farrowing[a]	0.41	0.36	0.492		
d 7[b]	6.39	5.28	0.492		
Weaning[b]	6.47	5.43	0.492		
Mean	4.42	3.69	0.307	0.07	0.46
Sow serum Se, ppm[3]					
Farrowing[a]	0.21	0.19	0.009		
d 7[b]	0.24	0.22	0.009		
Weaning[b]	0.24	0.23	0.009		
Mean	0.23	0.21	0.005	0.05	0.86
Milk Se, ppm[3]					
Farrowing[a]	0.40[d]	0.30[e]	0.008		
d 7[b]	0.11	0.09	0.008		
Weaning[b]	0.11	0.09	0.008		
Mean	0.20	0.16	0.004	< 0.01	< 0.01
Pig serum Se, ppm[3]					
Farrowing[a]	0.07	0.06	0.003		
d 7[b]	0.10	0.09	0.003		
Weaning[c]	0.12	0.11	0.003		
Mean	0.094	0.09	0.002	< 0.01	0.85
Pig serum thiobarbituric acid reactive substances, µM malondialdehyde eq.[3]					
Farrowing[a]	6.17	7.52	0.779		
d 7[b]	1.22	1.39	0.744		
Weaning[b]	2.25	2.04	0.779		
Mean	3.21	3.65	0.440	0.49	0.58

[1]C-SBM = corn-soybean meal sow diets; DDGS = sow gestation and lactation diets containing 40 and 20% DDGS, respectively.

[2]Pooled standard error of the mean.

[3]Time effect ($P < 0.05$).

[4]Main effect of maternal diet, regardless of day.

[a,b,c]Within each variable, means across sampling times without a common superscript differ ($P < 0.05$).

[d,e]Within a row, means without a common superscript differ ($P < 0.05$).

Table 5-5. Effect of maternal diet (MD) × nursery diet (ND) interactions over time on Se and thiobarbituric acid reactive substances (TBARS) in serum from nursery pigs

Maternal diet[1]:	C-SBM			DDGS				P-values				
Nursery Diet[2]:	CON	Ox-D	Ox-D +5VE	CON	Ox-D	Ox-D +5VE	SEM[3]	MD	ND	MD x day	ND x day	MD x ND
Item												
Pig serum α-tocopherol, ug/mL[4,5]												
Wean, d 19[a]	6.48[d]	6.52[d]	6.96[d]	5.42[e]	6.08[de]	5.37[e]	0.194					
d 47[b]	0.96	0.90	1.35	0.81	0.95	1.49	0.176					
d 68[c]	0.91	1.29	2.57	0.69	1.28	2.58	0.176					
Mean[6]	2.78[d]	2.90[e]	3.62[f]	2.30[d]	2.77[e]	3.15[f]	0.123	0.02	< 0.01	< 0.01[7]	< 0.01[8]	0.12
Pig serum Se, ppm[4]												
Wean, d 19[a]	0.115	0.115	0.105	0.105	0.108	0.103	0.006					
d 47[b]	0.149	0.148	0.136	0.135	0.156	0.133	0.006					
d 68[c]	0.163	0.151	0.156	0.164	0.168	0.156	0.006					
Mean	0.142[de]	0.138[de]	0.132[de]	0.135[de]	0.144[d]	0.131[e]	0.005	0.84	< 0.01	0.10	0.27	0.09
Pig serum TBARS, μM malondialdehyde eq.[4]												
Wean, d 19[x]	0.68	0.84	0.74	0.79	0.74	0.74	0.080					
d 47[y]	0.55	0.71	0.59	0.76	0.69	0.71	0.080					
d 68[x]	0.65	0.66	0.74	0.95	0.79	0.79	0.080					
Mean	0.62[d]	0.74[de]	0.69[de]	0.83[e]	0.74[de]	0.75[de]	0.050	0.09	0.88	0.16	0.69	0.05

[1]C-SBM = corn-soybean meal sow diets; DDGS = sow gestation and lactation diets containing 40 and 20% DDGS, respectively.

[2]CON = corn-soybean meal nursery diets; Ox-D = nursery diets containing 30% peroxidized DDGS; Ox-D+5VE = nursery diets containing 30% peroxidized DDGS and 5 times the recommended (NRC, 1998) level of vitamin E as dl-α-tocopheryl acetate.

[3]Pooled standard error of the mean.

[4]Time effect ($P < 0.05$).

[5]MD x ND x day ($P = 0.08$).

[6]Interactive mean of MD x ND.
[7]C-SBM > DDGS on d 19 ($P < 0.05$).
[8]Ox-D+5xE > Ox-D and CON on d 47 and d 68 ($P < 0.05$). Ox-D > CON on d 68 ($P < 0.1$).
[a,b,c]Within a variable, time means without a common superscript differ ($P < 0.05$).
[x,y]Within a variable, time means without a common superscript differ ($P < 0.1$).
[d,e,f]Within a row, means without a common superscript differ ($P < 0.05$).

Table 5-6. Effect of maternal diet (MD) and nursery diet (ND) on the concentration of α-tocopherol, Se, and glutathione (GSH) in liver, and the concentration of amino acids in serum from nursery pigs

	Maternal Diet[1]		Nursery Diet[2]				P-values		
Item	C-SBM	DDGS	CON	Ox-D	Ox-D+5VE	PSEM[3]	MD	ND	MD x ND
Serum amino acids, umol/L[4]									
Cystathione	5.4	5.8	5.2^{a}	5.6^{ab}	6.2^{b}	0.39	0.23	0.03	0.31
Cystine	2.5	2.9	2.4	2.8	2.8	0.86	0.13	0.10	0.08
Met	58.8	54.6	48.3^{x}	59.1^{xy}	62.6^{y}	5.50	0.49	0.09	0.27
Tau	234.4	278.6	198.6^{a}	256.2^{ab}	314.6^{b}	56.98	0.23	< 0.01	0.10
Total sulfur amino acids[5]	315.3	343.0	247.6^{a}	343.3^{b}	396.6^{b}	49.55	0.50	< 0.01	0.02
Liver[4,6]									
α-Tocopherol, ug/g	3.5	3.2	2.1^{a}	2.6^{a}	5.2^{b}	0.21	0.27	< 0.01	0.53
Se, ug/g	0.6	0.6	0.64^{a}	0.50^{b}	0.58^{ab}	0.05	0.84	0.02	0.20
Total GSH, nmol/g	97.8	99.6	94.4^{xy}	87.7^{x}	114.1^{y}	8.19	0.86	0.09	0.50

[1]C-SBM = corn-soybean meal sow diets; DDGS = sow gestation and lactation diets containing 40 and 20% dried distillers grains with solubles, respectively.

[2]CON = corn-soybean meal nursery diets; Ox-D = nursery diets containing 30% peroxidized DDGS; Ox-D+5VE = nursery diets containing 30% peroxidized DDGS and 5 times the recommended (NRC, 1998) level of vitamin E as dl-α-tocopheryl acetate.

[3]Pooled standard error of the mean.

[4]Final sample collected at 68 d of age.

[5]Sum of Cystathione, Cystine, Met, and Tau.

[6]Liver data are on "as-is" basis.

[a,b]Within a row and main effect, means without a common superscript differ ($P < 0.05$).

[x,y]Within a row and main effect, means without a common superscript differ ($P < 0.1$).

CHAPTER 6. DIETARY PEROXIDIZED CORN OIL AFFECTS THE GROWTH PERFORMANCE AND ANTIOXIDANT STATUS OF NURSERY PIGS

SUMMARY

Two experiments were conducted to assess the impact of increasing dietary levels of peroxidized corn oil on the growth performance and antioxidant status of nursery pigs. In experiment one, 249 weanling barrows were blocked by initial body BW and assigned to 32 pens. Pens were randomly assigned to 1 of 4 dietary treatments in a 3-phase feeding program and consisted of control diets containing no supplemental corn oil, or similar diets containing 2, 4, and 6% of slowly peroxidized corn oil (SO). Diets were formulated to similar SID Lys:ME ratios. Corn oil was heated for 72 h at 95°C (air flow rate = 12 L/min) to yield SO (peroxide value [PV] = 134.9 meq O_2/kg; thiobarbituric acid reactive substances [TBARS] = 19.0 mg malondialdehyde [MDA] eq/kg. In Exp. 2, 128 weanling barrows were blocked by initial BW and randomly assigned to 1 of 32 pens. Pens were assigned to 1 of 4 dietary treatments in a 3-phase feeding program and contained 9% unheated oil + 0% rapidly peroxidized corn oil (RO), 6% unheated oil + 3% RO, 3% unheated oil + 6% RO, or 0% unheated oil + 9% RO. Therefore, diets were formulated to be isocaloric and contain equal levels of SID Lys:ME. Corn oil was heated for 12 h at 185°C (air flow rate = 12 L/min) to yield RO (PV = 5.7 meq O_2/kg; TBARS = 26.7 mg MDA eq/kg). In both experiments, diets were fed for 35 d, and ADG, ADFI, G:F, and caloric efficiency (ADG/ME intake) were determined. Serum was collected on d 0, 14, and 35 from 1 pig per pen that was subsequently harvested to obtain liver and heart tissue. Serum and liver samples were analyzed for concentrations of α-tocopherol and Se.

In Exp. 1, feeding SO resulted in a linear reduction in ADFI ($P = 0.05$), no effect on ADG, and thus, resulted in improved G:F (linear and quadratic; $P < 0.01$) with increased dietary SO. However, these responses were likely a result of increasing dietary energy density with increasing dietary concentrations of SO. Conversely, caloric efficiency declined by 1.2 to 2.4% (linear; $P < 0.04$) in pigs fed diets with 2 to 6% SO relative to those fed diets without SO. In Experiment 2, increasing dietary RO tended to linearly reduce final (d 35) BW ($P = 0.11$), ADG ($P = 0.10$), and reduced G:F ($P = 0.03$) and caloric efficiency ($P = 0.03$) without affecting ADFI. When pigs were fed diets with 3 to 9% RO, caloric efficiency declined by 2.4 to 4% relative to pigs fed diets without RO (linear; $P < 0.03$). The α-tocopherol content of serum declined with increasing dietary concentrations of SO (linear and quadratic; $P < 0.03$) and RO (linear and cubic; $P < 0.01$). These data suggest that SO and RO negatively affect the efficiency of energy utilization and serum α-tocopherol content of nursery pigs.

KEYWORDS: growth, lipid, pigs, nursery, swine, peroxidation

INTRODUCTION

Energy is the most expensive component of swine diets. Supplemental sources of concentrated lipids, corn, or dried distillers grains with solubles (**DDGS**) provide a substantial portion of energy in U.S. swine diets. Distiller's corn oil (**DCO**) is extracted prior to manufacturing DDGS at more than 85% of ethanol plants in the United States, generating more than 105,000 tonnes of DCO in 2013 for use in the production of biodiesel and animal feeds (Renewable Fuels Association, 2014).

Polyunsaturated fatty acids are highly susceptible to peroxidation. Lipid peroxidation is accelerated by exposure to heat, air, moisture, and pro-oxidant metals

which may be introduced during feed ingredient processing and storage. Animal fats, vegetable oils, and other lipid rich feed ingredients may be peroxidized to varying amounts depending on the temperature and duration of thermal exposure (Dibner et al., 2011; Song and Shurson, 2013).

Peroxidation degrades fatty acids into numerous secondary and tertiary peroxidation compounds (Spiteller et al., 2001; Seppanen and Csallany, 2002; Belitz et al., 2009), and degrades indigenous vitamin E (Seppanen and Csallany, 2002; Liu, 2012). Feeding peroxidized lipids reduces gain efficiency (McGill et al., 2011a,b; Tavárez et al., 2011), growth (Boler et al., 2012; Liu, 2012), and antioxidant status (Boler et al., 2012; Liu, 2012) of swine and broilers. However, specific mechanisms underlying the negative effects of peroxidized lipids are unclear.

Maximal dietary thresholds for inclusion of peroxidized lipids to avoid reductions in growth performance have not been established, and little information exists on the effects of increasing dietary concentrations of peroxidized lipids on growth performance of pigs. Therefore, the objective of these experiments was to investigate the effect of increasing dietary levels of peroxidized corn oil in non-isocaloric and iso-caloric diets on the growth and antioxidant status of nursery pigs. Corn oil was selected as the lipid source because of its high concentration of PUFA (NRC, 2012), and the increasing use of DCO in nursery pig diets.

MATERIALS AND METHODS

Experimental designs and procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Minnesota.

Exp. 1

This experiment was conducted at the University of Minnesota West Central Research and Outreach Center (**WCROC**) Swine Research Facility in Morris, and utilized 249 weaned (BW = 6.3 ± 1.4 kg) pigs (Genetiporc [Landrace x French Large White] x Norsvin Landrace). Pigs were stratified by BW into 8 blocks, assigned to 1 of 4 pens within block, and pens were randomly assigned to 1 of 4 dietary treatments within each block. Experimental diets were fed in 3-phases (phase 1 = d 0 to 4, phase 2 = d 4 to 14, and phase 3 = d 14 to 35) and included a control with 0% added corn oil, and diets containing 2, 4, or 6% slowly peroxidized corn oil (**SO**; Table 6-1). Diets were formulated to contain similar ratios of standardized ileal digestible AA and ATTD P to ME. All diets were formulated to meet or exceed NRC (2012) requirements for nursery pigs and were provided in meal form.

Refined, deodorized, and bleached corn oil (Stratas Foods, Memphis, TN) was heated at 95°C for 72 h with a constant forced air flow rate of 12 L/min to yield SO. After cooking, corn oil was stored in barrels for 12 d prior to mixing phase 1 diets. Mean daily temperature at the WCROC during the experiment was -4.7 ± 7.4°C. Oil peroxide value (**PV**) was 1.98 and 134.9 meq O_2/kg and thiobarbituric acid reactive substances (**TBARS**) content was 9.0 and 19.0 mg malondialdehyde (**MDA**) eq/kg for unheated oil and SO, respectively (Table 6-2). Pigs were housed in pens of 7 to 8 (2.4 × 1.2 m), and each pen contained a feeder (4 feeder spaces) and a cup drinker. Pigs were provided *ad libitum* access to water and experimental diets for 5 wks.

Exp. 2

This experiment was conducted at the University of Minnesota Southern Research and Outreach Center (**SROC**) Swine Research Facility in Waseca, and utilized 128 weaned (BW = 6.3 ± 1.4 kg) pigs (Topigs, [Winnipeg, Manitoba; Landrace x Yorkshire] x Duroc [Compart's Boar Store, Nicollet, MN]). Pigs were stratified by BW into 8 blocks, assigned to one of 4 pens within block, and pens were assigned randomly to 1 of 4 dietary treatments within each block. Dietary treatments were fed in 3 phases (phase 1 = d 0 to 4, phase 2 = d 4 to 14, and phase 3 = d 14 to 35) and included: 9% unheated oil + 0% rapidly oxidized oil (RO), 6% unheated oil + 3% RO, 3% unheated oil + 6% RO, and 0% unheated oil + 9% RO (**Table 6-3**). Therefore, within each phase, diets were formulated to be identical except for the ratio of RO to unperoxidized oil. All diets were formulated to meet or exceed NRC (2012) requirements for nursery pigs and were provided in meal form.

Refined, deodorized, and bleached corn oil (Stratas Foods, Memphis, TN) was heated at 185°C for 12 h with a constant forced air flow rate of 12 L/min to yield RO. After heating, corn oil was stored in barrels for 2 d before making phase 1 diets. Mean daily temperature at the SROC during the experiment was 23.3 ± 4.1°C. Oil PV was 1.7 and 5.7 meq/kg and TBARS content was 27.7 and 46.3 mg MDA eq/kg for unheated and RO, respectively (**Table 6-2**).

Pigs were housed in pens of 4 pigs (1.2 × 1.2 m), and each pen contained a feeder (3 feeder spaces) and a nipple drinker. Pigs were provided *ad libitum* access to water and experimental diets for 5 wks.

Data and Sample Collection

In each experiment, pigs were weighed individually on d 0, 4, 14, and 35, and pen feed disappearance was recorded at the end of each dietary phase. These data were used to calculate ADFI, ADG, and G:F of each pen.

In each pen, the pig closest to mean pen BW at d 0 was selected as the focal pig, and 20 to 30 mL blood was collected via jugular venipuncture into vacutainer tubes coated with silicone (Becton Dickson, Franklin Lakes, NJ) on d 0, 14, and 35. Blood samples were allowed to clot at room temperature (5 to 10 minutes), stored at 4°C (≤ 6 h), and centrifuged (1,400 x *g* for 10 min). Serum was transferred into microcentrifuge tubes and frozen at -80°C until further analysis.

On d 35, focal pigs (n = 32 in each experiment) were stunned with captive bolt and exsanguinated (Exp. 1) or injected intravenously with sodium pentobarbital (> 100 mg/kg BW; Exp. 2). Intact livers and hearts (without pericardium) were weighed individually and sampled. The heart somatic index and hepatosomatic index were calculated for each focal animal by the following formula: [(wet organ weight, g/BW, g) x 100]. Liver samples were snap frozen in liquid N, placed on dry ice, and frozen at -80°C until further analysis. Hearts were evaluated visually by a veterinarian for pathological signs of Mulberry Heart Disease (MHD). In Exp. 2, heart tissue was sampled and placed in 10% neutral buffered formalin. Heart sections were trimmed, embedded in paraffin, mounted onto slides, and stained with haematoxylin and eosin according to procedures described by Carson and Hladik (2005). A veterinary pathologist, blinded to treatments, evaluated heart sections histologically for lesions characteristic of MHD.

Laboratory Analysis

Quality and peroxidation characteristics of corn oil

Oil samples were retained when mixing phase 1 diets, frozen at -20°C, and analyzed for PV (method Cd 8-53; AOCS, 1992), TBARS, hexanal (Elisia and Kitts, 2011), moisture (method Ca 2c-25; AOCS, 2009), impurities (method Ca 3a-46; AOCS, 2011), and unsaponifiables (method Ca 6a-40; AOCS, 2011), free fatty acids (method Ca 5a-40; AOCS, 2012) and fatty acid profile (methods Ce 2-66; AOCS, 1997 and 996.06; AOAC, 2001) at the University of Missouri Agricultural Experiment Station Chemistry Laboratory (UMO-AESCL, Columbia). The TBARS assay was a modified version of the AOCS procedure (Cd 19-90; AOCS, 2001) with malonaldehyde used as a standard as described by Pegg (2001). Oxidative stability index at 110°C (method Cd 12b-92; AOCS, 2009) and *p*-anisidine value (method Cd 18-90; AOCS, 2011) were determined at Barrow-Agee Laboratory (Memphis, TN).

Diet nutrient composition

Feed samples were retained, frozen at -20°C, and analyzed for DM (method 930.15; AOAC, 2005), crude fat (method 920.39; AOAC, 2005), NDF (method 2002.04; AOAC, 2005, and Methods 5.1 and 5.2; NFTA, 1993), ADF, (method 973.18; AOAC, 2005 modified according to Tecator Application Note 3429), ash (method 942.05; AOAC , 2008), N (method 990.03; AOAC, 2005), Ca (method 985.01; AOAC, 2005), P (method 985.01; AOAC, 2005), S (method D4239; ASTM, 2011), Se, and α-tocopherol at Minnesota Valley Testing Laboratories (New Ulm). After digestion in nitric acid, Se was analyzed according to procedures described by Wahlen et al. (2005) using an Agilent

7500ce Inductively Coupled Plasma Mass Spectrometer (Agilent Technologies Inc, Santa Clara, CA). The α-tocopherol content of feeds and corn oil was measured using a modified AOAC method (971.30; AOAC, 2006) with high-performance liquid chromatography (**HPLC**) and a fluorescence detector. Amino acids (method 982.30; AOAC , 2006) were analyzed at the UMO-AESCL.

Liver and serum Se and α-tocopherol concentrations

In both experiments, Se and α-tocopherol concentrations in serum and liver samples were analyzed at the Michigan State University Diagnostic Center for Population and Animal Health (East Lansing). One gram of liver tissue was digested overnight in 2 mL nitric acid, and Se concentrations were determined according to the procedure of Wahlen et al. (2005) using an Agilent 7500ce Inductively Coupled Plasma Mass Spectrometer (Agilent Technologies Inc., Santa Clara, CA). For α-tocopherol analysis, liver samples were weighed and homogenized in distilled, deionized water (1:4 w/v). Serum samples and liver homogenates were mixed with equal volumes of hexane and a solution of butylated hydroxytoluene in ethanol (10% w/v). Mixtures were centrifuged at 1,900 × *g* for 10 min, and a known aliquot of the hexane layer was removed and dried under vacuum. Samples were dissolved in a chromatographic mobile phase (7:2:1, acetonitrile, methylene chloride, methanol) and analyzed by HPLC (Separation Module 2690) using a Waters Symmetry C18, 3.5 mm, 4.6 x 75 mm analytical column with detection by UV absorbency at 292 nm (Waters, Milford, MA). Trans-β-APO-8"-carotenal was used as an internal standard.

Serum TBARS

For experiment 2, serum was analyzed for TBARS concentration according to methods adapted from the Animal Models of Diabetic Complications Consortium (Feldman, 2004). Briefly, 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 12,000 × *g* for 15 min at 4º C. Two hundred microliters 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 dry block heater maintained at 100°C. Each vial was then cooled in an ice bath, and an aliquot was read at 532 nm using a spectrometer (SpectraMax 250, Molecular Devices, Sunnyvale, CA).

Concentration of serum triglycerides and cholesterol

In both experiments, commercial kits were used to evaluate serum samples for cholesterol (catalog number: C7509, Ponte Scientific, Inc., Canton, MI) and triglyceride content (catalog number: T7531, Ponte Scientific, Inc., Canton, MI). Two-microliters of serum or standards were combined in duplicate with 200 μL cholesterol or triglyceride reagents, and incubated at 37°C for 5 minutes. Cholesterol and triglycerides were measured indirectly using a spectrophotometer (SpectraMax 250, Molecular Devices, Sunnyvale, CA) at 520 nm and 540 nm, respectively, and concentrations were determined from standard curves.

Serum tryptophan

Concentration of Trp was determined in serum from d 35 of age by liquid chromatography–mass spectrometry (LC-MS). Briefly, 5 μL of samples and standards were mixed with 5 μL of 100 μM *p*-chlorophenylalanine (internal standard), 50 μL of 10 mM sodium carbonate, and 100 μL of dansyl chloride (3 mg/mL in acetone). The mixture was incubated at 25°C for 15 min and centrifuged (18,000 × *g*) for 10 min. Five microliters of supernatant were injected and separated in an Acquity BEH C18 column (Waters, Milford, MA) by a gradient of mobile phase ranging from water to 95% aqueous acetonitrile containing 0.1% formic acid for 10 min. The eluent was introduced into a SYNAPT quadrupole time-of-flight mass spectrometer (Waters, Milford, MA) for mass detection. Mass chromatograms and spectral data were acquired and processed by MassLynx^{TM} software (Waters, Milford, MA) in centroid format. The concentration of Trp in serum was determined by calculating the ratio between Trp peak area and the peak area of *p*-chlorophenylalanine using QuanLynx^{TM} software (Waters, Milford, MA).

Statistical Analysis

The MIXED procedure of SAS (v9.3; SAS Inst. Inc., Cary, NC) was used to evaluate the effect of diet as a main effect and block as a random effect. Linear, quadratic, and cubic orthogonal polynomial contrasts were used to compare dietary treatment means. For serum data, the repeated measures option was used to evaluate the effect of time and its interaction with diet, and weaning values were used as covariates. Because ADFI varied across treatments, average daily intake of analyzed vitamin E or Se (overall daily feed intake × mean dietary concentration weighted by phase) was included

as a covariate when evaluating the concentration of α-tocopherol or Se, respectively, in serum and liver. The sum of triglyceride and cholesterol content of serum was a covariate for serum α-tocopherol analysis because concentrations of α-tocopherol are influenced by total serum lipids (Thurnham et al., 1986; Traber and Jialal, 2000). Pen was used as the experimental unit. Normality of model residuals was evaluated using the UNIVARIATE procedure of SAS. Results are reported as least squares means. Effects were significant at $P < 0.05$, whereas values between $0.05 \leq P \leq 0.10$ were considered statistical trends.

RESULTS

Experiment 1

Six pigs were removed from the experiment for reasons unrelated to treatment: one pig died with a twisted gut, one pig died with signs of streptococcus, one pig died with signs of MHD (4% SO treatment), and three pigs were euthanized after failing to thrive. No other pigs exhibited pathological signs of MHD. Of the 6 pigs removed from the experiment, 1 pig was fed 0% SO, 3 pigs were fed 2% SO, and 2 pigs were fed 4% SO.

Growth performance

There was no effect of dietary treatment on ADG and BW at weaning, d 14, and d 35 (**Table 6-4**). However, feeding dietary SO resulted in a cubic ($P = 0.04$) effect on d 4 BW (mean = 7.0, 7.3, 7.1, and 7.2 ± 0.6 kg for 0, 2, 4, and 6% SO, respectively). The ADFI declined linearly with increased SO during phases 2 and 3, and over the entire 5-wk trial ($P \leq 0.05$), but not within phase 1. Improved G:F was observed with increasing dietary concentration of SO during phase 1 (quadratic, $P = 0.01$; cubic, $P = 0.04$), phase 3 (linear, $P < 0.01$), and over the entire trial (linear, $P < 0.01$). Calculated average daily ME

intake increased linearly with SO in phase 1 ($P = 0.07$). Caloric efficiency (g ADG/Mcal ME) changed with SO in phase 1 (quadratic and cubic, $P < 0.04$) and declined linearly in phase 3 ($P < 0.01$) and overall ($P = 0.04$). Vitamin E intake increased (linear and cubic, $P < 0.01$) and Se intake declined (linear, $P < 0.01$; cubic, $P = 0.02$) with increasing dietary SO (**Table 6-5**).

Metabolic peroxidation indicators

The hepatosomatic index increased linearly ($P < 0.05$) with increasing SO (**Table 6-5**). The α-tocopherol concentration of liver was not affected ($P > 0.1$), but Se content tended to decline linearly ($P = 0.06$) with increased dietary SO (**Table 6-5**). Over the entire experiment, the α-tocopherol concentration declined (linear, $P = 0.03$; quadratic, $P = 0.01$) with increased dietary SO, but there was a day × diet interaction ($P < 0.05$; **Table 6-6**). On d 14, α-tocopherol concentration of serum declined (linear, $P < 0.01$; quadratic, $P = 0.04$) with SO, but not on d 35 ($P > 0.1$). Over the entire experiment, Se concentration of serum changed cubically ($P < 0.01$) with increased SO. The serum concentration of α-tocopherol declined ($P = 0.06$) and serum Se concentration increased from d 14 to 35 ($P < 0.01$).

Experiment 2

One pig (0% RO) died with signs of clostridium. Regardless of treatment, all pens developed loose, grey stools after 1 wk of treatment. This condition remained in the majority of pens throughout the experiment, and may be related to the high fat content of the experimental diets. No other signs of apparent ill health developed, and no pigs displayed pathological or histopathological lesions of MHD.

Growth performance

During phase 1, ADG, G:F, and caloric efficiency declined linearly ($P < 0.05$) with increasing RO (**Table 6-8**). Consequently, BW on d 4 declined linearly ($P= 0.01$) with increased RO (mean = 6.6, 6.5, 6.4, and 6.3 ± 0.2 kg, for diets with increasing concentrations of peroxidized corn oil. During phase 3, ADG ($P = 0.07$), ADFI ($P = 0.10$), and caloric intake ($P = 0.1$) tended to decline linearly with increasing RO, but G:F was not affected. Final BW measured at 35- d post weaning tended to decline linearly with increasing RO ($P = 0.11$), coinciding with a linear reduction in ADG ($P = 0.10$), G:F ($P = 0.03$), and caloric efficiency ($P = 0.03$) measured over the 35-d feeding period. Intake of vitamin E declined (linear, quadratic, and cubic; $P < 0.01$) and Se changed quadratically ($P = 0.05$) with increased dietary RO.

Metabolic peroxidation indicators

The heart somatic index and hepatosomatic index were not affected by dietary treatment (**Table 6-9**). The α-tocopherol and Se concentrations of liver were not affected by dietary treatment. The Trp concentration of serum tended to decline linearly with increased dietary RO ($P = 0.08$). Over the entire experiment, the concentration of α-tocopherol in serum declined (linear, cubic; $P < 0.01$) with increased RO, but there was a diet × day interaction ($P < 0.05$; **Table 6-10**). The α-tocopherol concentration of serum declined on d 14 (linear, $P < 0.01$) and d 35 (linear and cubic $P \leq 0.01$). Over the 35-d experiment, Se concentration of serum tended to decline linearly ($P = 0.1$). The serum concentration of α-tocopherol and Se declined ($P < 0.01$) from d 14 to 35. The concentration of TBARS in serum tended to increase ($P = 0.06$) linearly with RO.

DISCUSSION

Growth Performance

Understanding the peroxidative status of dietary lipids is of critical importance for swine nutritionists to minimize any negative consequences to animal health and growth performance. Dietary inclusion of peroxidized lipids reduces growth performance of pigs (Takahashi and Akiba, 1999; DeRouchey et al., 2004; Harrell et al., 2010; Tavárez et al., 2011; Boler et al., 2012; Liu, 2012), which may be caused partially by a reduction in feed intake that often accompanies this response. Some researchers have also shown that lipid peroxidation contributes to cellular inefficiencies and damage which can affect growth and health of animals negatively (Lykkesfeldt and Svendsen, 2007). Lipids present in cellular and subcellular membranes can peroxidize, and secondary and tertiary compounds produced during peroxidation can alter enzymes, protein, and DNA, which affects their functionality (Yu, 1994).

Peroxidized lipids often reduce ADFI when replacing fresh lipids for swine (DeRouchey et al., 2004; Yuan et al., 2007; Harrell et al., 2010; Boler et al., 2012; Liu, 2012). The decrease in ADFI with increasing peroxidized corn oil in Exp. 1 may be attributed to increased dietary energy density with increasing concentration of SO because diets were not formulated to be isocaloric. This response is consistent with those reported by others (Smith et al., 1999; Engel et al., 2001) who indicate feed intake is reduced to maintain energy intake as the caloric density of the diet increases. In Exp. 1, the gain efficiency improvement (7.6%) with increasing concentrations of peroxidized corn oil was of similar magnitude to the reduction in ADFI (7.3%) in pigs fed diets containing 6% SO relative to controls. However, over the entire 35 d trial, caloric

efficiency (ADG/ME intake) declined from 1.2% to 2.0% in pigs fed 3 or 6% SO relative to controls, respectively, indicating that feeding SO reduced the efficiency of energy utilization. In Exp. 2, growth rate and efficiency declined with increasing concentrations of dietary RO, particularly during the initial post-weaning period. When measured over the entire 35 d experiment, caloric efficiency declined by 2.4 to 4% for pigs fed diets with 3 to 9% RO, respectively, relative to those fed 0% RO. We did not measure the effects of these responses during the nursery phase on subsequent growth rate and gain efficiency responses during the growing-finishing period. However, other research suggests that growth performance observed in the nursery phase can affect growth and efficiency of pigs in the finishing period (Tokach et al., 1992; Mahan et al., 1998).

Results from these experiments suggest that feeding peroxidized lipids reduces the efficiency of energy utilization for nursery pigs, but the effect may be dependent on the temperature and duration of thermal exposure imposed on dietary lipids. For example, the magnitude of reduction in caloric efficiency was greater for RO compared to SO, and this difference may relate to amount and characteristics of secondary and tertiary peroxidation compounds produced during peroxidation in each of these processes. However, directly connecting specific products of peroxidation with reductions in animal growth and efficiency is difficult because of the lack of a comprehensive measure of all peroxidation compounds.

Lipid peroxidation is a complex process that produces a wide variety of compounds which degrade antioxidants, alter diet flavor, and influence health as well as metabolic oxidative status of animals (Lykkesfeldt and Svendsen, 2007; Belitz et al., 2009). In addition, the relative concentrations of peroxidation compounds are influenced

by lipid composition as well as temperature and duration of thermal conditions of production (Liu, 2012). Historically, PV has been the most commonly used indicator of lipid peroxidation. However, the peroxides and aldehydes that are initially produced during the peroxidation process are subsequently degraded with continual exposure, resulting in a bell shaped curve of PV concentration over time (Fitch Haumann, 1993; DeRouchey et al., 2004; Danowska-Oziewicz and Karpińska-Tymoszczyk, 2005). Therefore, measures such as PV, anisidine value, and TBARS may be misleadingly low in lipids subjected to high temperatures for an extended period of time(Danowska-Oziewicz and Karpińska-Tymoszczyk, 2005). As a result, some researchers have suggested that PV has limited utility for lipids subjected to temperatures exceeding 150˚C (Shahidi and Zhong, 2005). Our results support these observations and suggest that PV is an unreliable indicator of lipid quality. In our 2 experiments, the PV of SO was 23 fold that of RO, but caloric efficiency of pigs was more substantially affected by feeding RO than SO.

Although PV is not a reliable indicator of peroxidation, it has been used extensively as a common indicator of lipid quality, and some researchers have used it to suggest maximum tolerable levels of peroxidized lipids in swine diets. DeRouchey et al. (2004), Azain (2001), and Gray and Robinson (1941) suggested maximal threshold levels for PV of 2.4 meq O_2/kg diet, 5 meq O_2/kg lipid and 20 meq O_2/kg lipid, respectively. In experiment 1, feeding diets with a PV $\leq$ 8.09 meq O_2/kg diet (134.9 meq O_2/kg oil $\times$ 6%) adversely affected caloric efficiency but not ADG. However, PV concentrations of both the diet and corn oil far exceed the current suggested threshold levels (Gray and Robinson, 1941; Azain, 2001; DeRouchey et al., 2004). In Exp. 2, feeding diets with

relatively low PV (≤ 0.51 meq O_2/kg diet) reduced ADG by 8.1% without impacting ADFI, leading to a 2.7% reduction in gain efficiency relative to pigs fed fresh lipids. These results further indicate that PV is a poor predictor of lipid quality for pigs fed diets with peroxidized lipids.

Consequently, more appropriate measures of lipid peroxidation or combinations thereof must be identified to facilitate determination of the maximum tolerable limits of peroxidized lipids in diets for swine. Liu (2012) reported that ADG tended to be negatively associated (r = -0.29, P = 0.09) with TBARS content of dietary lipid. Interestingly, at the greatest inclusion level of peroxidized oil, the TBARS content of diets in Exp. 1 were approximately 75% lower than diets used in Exp. 2 (≤ 1.1 and 4.2mg MDA eq/kg diet for Exp. 1 and 2, respectively). Furthermore, the hexanal concentration of diets in Exp. 2 were approximately double that of diets in Exp. 1 (≤ 0.23 and 0.53 μg/g diet for Exp. 1 and 2, respectively). Perhaps TBARS or hexanal are superior predictors of lipid quality for pigs fed diets with peroxidized lipids.

Metabolic Measures

The hepatosomatic index serves as a biological indicator of toxicity (Juberg et al., 2006). We observed increased hepatosomatic index with increasing dietary peroxidized corn oil in experiment 1, but not in experiment 2. Others demonstrated feeding diets containing peroxidized lipids increased liver size (Huang et al., 1988; Eder, 1999; Liu, 2012), and this phenomenon may relate to increased synthesis of microsomal enzymes to mitigate toxicity (Huang et al., 1988). Therefore, results of experiment 1 are in agreement with those of others indicating that peroxidized corn oil contributes to increased liver weight. Conversely, hepatosomatic index was not affected by dietary RO, and the reason

for this discrepancy is unclear. Feeding diets containing increasing levels of peroxidized corn oil had no effect on heart weight, which is consistent with results reported by Eder (1999).

Mulberry Heart Disease is associated with a deficiency of vitamin E and Se for nursery pigs (AASV, 2009) . Vitamin E and Se have important roles in the antioxidant system (NRC, 2012). Generally, the dietary inclusion of peroxidized lipids reduces antioxidant status (Liu and Huang, 1995; Boler et al., 2012; Liu, 2012), which could lead to the development of MHD. However, across both experiments, only one pig developed MHD. Pallarés et al. (2002) suggested that multiple factors may predispose pigs to develop MHD (e.g. stress, genetics, pathogenic infection). Stress was minimal and there were no known pathological infections in the pigs used in the current study. Lipid peroxidation partially degraded the indigenous vitamin E in corn oil, and this phenomenon has been reported by others (Seppanen and Csallany, 2002; Liu, 2012). Therefore, daily intake of vitamin E was dissimilar across dietary treatments which partially contributed to reduced α-tocopherol concentration in serum of pigs fed peroxidized lipids. Others reported that peroxidized lipids reduce the vitamin E content of serum or liver (Liu and Huang, 1995; Tavárez et al., 2011; Boler et al., 2012; Liu, 2012). However, we did not observe substantial effects of peroxidized corn oil on the concentration of vitamin E or Se in liver. Perhaps if diets were fed for an extended duration (i.e. > 5 wks), changes in vitamin E or Se concentrations would have been detected in the liver. The concentration of TBARS in serum is indicative of metabolic oxidative stress, and these concentrations increased with dietary RO in Exp. 2. Therefore, our data suggest that metabolic oxidative stress occurs when feeding

peroxidized corn oil to nursery pigs, but not to the extent that they develop MHD. Generally, liver concentrations < 2 μg α-tocopherol/g are considered deficient (Rice and Kennedy, 1989), and mean values did not fall below this threshold in the current experiments. However, others reported MHD in pigs with reserves exceeding that threshold (Pallarés et al., 2002; Desrosiers, 2003; Shen et al., 2011), indicating that additional factors contribute to the onset of MHD. Based on the current results, feeding peroxidized corn oil to nursery pigs does not contribute to MHD.

We observed a cubic effect of peroxidized lipids on the Se content of serum in Exp. 1. However, the practical significance of this finding is unclear because the direction and magnitude of difference varied among dietary treatments. However, the linear reduction in the Se concentration of liver suggests a negative effect of peroxidized lipids, but comparable data in the literature are lacking. Instead, limited reports have evaluated the activity of glutathione peroxidase (**GPx**), a Se dependent enzyme with an antioxidant role. Some research results suggest that peroxidized lipids stimulate GPx activity of broilers fed peroxidized lipids (Upton et al., 2009), but little research has evaluated this connection in swine.

In Exp. 2, we discovered that RO reduced the concentration of Trp in serum, and this finding has been reported in other studies in pigs (Liu, 2012) and rodents (Chen et al., unpublished data). Recent work in rodents suggests that metabolites of the kynurenine pathway (e.g. kyneurenic acid and nicotinamide N-oxide) are up-regulated in response to peroxidized soybean oil (Chen et al., unpublished data). However, the practical significance of this finding is not immediately apparent, and it is unclear if elevated catabolism of Trp reduces the amount available for protein synthesis. In addition to roles

in the synthesis of muscle protein and neurotransmitters (Wu, 2009), Trp is incorporated into acute phase proteins (Reeds et al., 1994). Consequently, some researchers have suggested that pigs benefit from increased dietary Trp in response to stress (Kim et al., 2010; Shen et al., 2012), and suggest Trp requirements may depend on stressors such as cleanliness of the rearing environment (Le Floc'h et al., 2009). However, no research has been conducted to determine if animals fed dietary peroxidized lipids respond to increased dietary concentrations of Trp. Many metabolites of Trp in both the kynurenine and indole pathways act as antioxidants (Goda et al., 1999; Wu, 2009) which could potentially be used in response to metabolic oxidative stress.

In summary, PV is a poor indicator of lipid peroxidation, and feeding peroxidized corn oil reduces caloric efficiency of nursery pigs. Over a 35 d experiment, reductions in caloric efficiency were reduced for SO (1.2 to 2%) compared to RO (2.4 to 4%), indicating that conditions of temperature and duration of heating to create peroxidation affect the subsequent feeding value of peroxidized lipids and growth performance responses of nursery pigs. In both experiments, feeding peroxidized corn oil reduced the vitamin E content of serum, but Mulberry Heart Disease was not detected. Further studies need to evaluate whether adding antioxidants can alleviate some of the negative growth performance responses when feeding peroxidized corn oil to nursery pigs.

Table 6-1. Composition of nursery diets (as-fed basis), Exp. 1

	Phase 1 (weaning - d 4)[1]				Phase 2 (d 4 - d 14)				Phase 3 (d 14 - d 35)			
Item	0[2]	2[2]	4[2]	6[2]	0	2	4	6	0	2	4	6
Ingredient,*%*												
Corn	40.24	36.48	32.87	28.85	46.05	42.54	38.73	34.92	64.42	60.95	57.46	53.94
Soybean meal, 47.5% CP	15.40	17.04	18.53	20.40	22.94	24.32	26.00	27.68	31.28	32.66	34.04	35.42
Peroxidized corn oil[2]	0.00	2.00	4.00	6.00	0.00	2.00	4.00	6.00	0.00	2.00	4.00	6.00
Fish meal	6.00	6.00	6.00	6.00	8.00	8.00	8.00	8.00	0.00	0.00	0.00	0.00
Dried whey	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	0.00	0.00	0.00	0.00
Lactose	10.00	10.00	10.00	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Spray dried porcine plasma	5.00	5.00	5.00	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Antibiotic[3]	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.00	0.00	0.00	0.00
Zinc oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00
Vitamin/mineral premix[4]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Lys	0.25	0.26	0.27	0.29	0.19	0.21	0.22	0.24	0.48	0.49	0.50	0.52
DL-Met	0.14	0.16	0.17	0.19	0.12	0.14	0.16	0.18	0.16	0.18	0.20	0.21
L-Thr	0.05	0.06	0.07	0.08	0.06	0.07	0.08	0.09	0.15	0.16	0.17	0.18
Monocalcium phosphate	0.02	0.07	0.13	0.20	0.07	0.12	0.18	0.23	1.08	1.10	1.15	1.20
Limestone	1.03	1.06	1.09	1.12	0.71	0.74	0.77	0.80	1.20	1.24	1.26	1.30
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.70	0.70	0.70	0.70
α-tocopheryl acetate[5]	0.04	0.04	0.04	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Analyzed composition												
ME, kcal/kg[6]	3,367	3,462	3,559	3,655	3,367	3,462	3,559	3,655	3,253	3,350	3,448	3,544
Crude protein,%	21.00	21.50	21.80	21.70	21.50	22.20	27.70	22.60	20.30	20.30	20.40	20.80
Crude fat,%	2.17	3.98	5.95	7.31	2.51	4.52	6.29	7.90	2.04	3.91	6.03	7.56
NDF%	4.20	2.90	4.10	3.60	5.10	4.00	4.70	4.80	4.70	5.30	5.20	4.70
ADF,%	1.75	1.87	1.94	2.10	2.57	3.86	2.30	4.35	3.02	3.84	2.33	2.66
Ca,%	0.98	1.02	1.07	1.06	0.91	1.02	1.10	1.10	0.93	0.85	0.85	0.93
P,%	0.54	0.53	0.54	0.57	0.55	0.58	0.59	0.61	0.57	0.55	0.56	0.56
Se mg/kg	1.21	0.90	1.10	0.89	0.85	0.83	0.88	0.79	0.72	0.68	0.72	0.69
Vitamin E, IU/kg	12.62	10.63	13.07	11.74	12.11	12.11	11.15	16.09	8.19	13.58	11.30	12.40
Lys,%	1.38	1.39	1.55	1.53	1.41	1.49	1.46	1.48	1.41	1.40	1.56	1.34
Met + Cys,%	0.74	0.78	0.92	0.77	0.76	0.80	0.81	0.81	0.83	0.88	0.71	0.78
Thr,%	0.85	0.86	0.96	0.96	0.87	0.91	0.92	0.92	0.85	0.82	0.84	0.83
Trp,%	0.30	0.31	0.30	0.31	0.30	0.27	0.29	0.29	0.25	0.26	0.26	0.26

[1]Initial weaning weight was 6.3 ± 1.4 kg.

[2]Diets contained either 0, 2, 4, or 6% of corn oil that had been heated for 72 h at 95°C with a constant forced air flow rate of 12 L/min. Peroxide value = 134.9 meq O_2/kg and TBARS = 19.0 mg MDA eq /kg.

[3]Supplied 38.6 mg of tiamulin per kilogram of diet as 0.175% Denagard (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) premix and 440 mg of chlortetracycline per kilogram of diet as 0.4% Aureomycin 50G (Zoetis, Inc., Florham Park, NJ).

[4]Premix supplied the following nutrients per kilogram of diet: 11,023 IU of vitamin A (retinyl acetate); 2,756 IU of vitamin D3; 4.4 mg of vitamin K (menadione dimethylpyrimidinol bisulfite); 9.9 mg of riboflavin; 55.1 mg of niacin; 33.1 mg of pantothenic acid as D-calcium pantothenate; 496.0 mg of choline as choline chloride; 0.06 mg of vitamin B12; 2.2 mg of pyridoxine; 1.7 mg of folic acid; 1.1 mg of thiamine; 0.2 mg of biotin; 2.2 mg of iodine (ethylenediamine dihydroiodide); 0.3 mg of selenium (sodium selenite); 90.4 mg of zinc (zinc oxide, Sea Questra Mineral [SQM®; Qualitech, Lakeville, MN]); 55.1 mg of iron (ferrous sulfate, SQM); 5.5 mg of copper (copper sulfate, SQM); and 17.6 mg of manganese (manganese oxide, SQM).

[5]Concentration: 44,090 IU vitamin E / kg.

[6]ME values were calculated using NRC (2012) values.

Table 6-2. Characteristics of corn oil used in Exp. 1 and Exp 2

	Exp.1[1]		Exp. 2[2]	
Item	Un	SO	Un	RO
Peroxide value, meq/kg	1.98	134.88	1.66	5.71
TBARS, mg MDA eq/kg	9.02	19.04	27.7	46.3
p-Anisidine value	3.86	150	5.32	138
Hexanal, μg/g	1.23	3.87	1.49	5.93
OSI, h	10.55	1.65	10.75	2.15
Vitamin E, IU/100 g	35.7	0.98	27.7	23.3
Free fatty acids,%	0.49	0.99	0.26	0.53
Moisture,%	0.39	0.53	0.19	0.17
Impurities,%	0.14	0.05	< 0.05	< 0.05
Unsaponifiables,%	0.01	0.00	1.72	1.49
Fatty acids,%				
C14:0	0	0	0.03	0
C14:1	0	0	0	0
C15:0	0	0	0	0
C16:0	11.51	12.53	11.49	11.22
C16:1	0.11	0.11	0.1	0.1
C18:0	2	2	1	2
C18:1	30.27	32.08	29.78	27.42
C18:2	53.83	50.63	53.93	48.68
C18:3	0.88	0.75	0.96	0.66
Unsaturated/saturated ratio	6.3	5.7	6.7	6.0

[1]Un = corn oil was not subjected to heat treatment and SO = corn oil heated at 95°C for 72 h with air flow rate of 12 L/min.

[2]Un = corn oil was not subjected to heat treatment and SO = corn oil heated at 185°C for 12 h with air flow rate of 12 L/min.

Table 6-3. Composition of nursery diets (as-fed basis), Exp. 2

	Phase 1 (weaning - d 4)[1]				Phase 2 (d 4 - d 14)				Phase 3 (d 14 - d 35)			
Item	0[2]	3[2]	6[2]	9[2]	0	3	6	9	0	3	6	9
Ingredient,*%*												
Corn	27.20	27.20	27.20	27.20	36.01	36.01	36.01	36.01	48.82	48.82	48.82	48.82
Soybean meal, 47.5% CP	22.91	22.91	22.91	22.91	31.89	31.89	31.89	31.89	37.01	37.01	37.01	37.01
Peroxidized corn oil[2]	0.00	3.00	6.00	9.00	0.00	3.00	6.00	9.00	0.00	3.00	6.00	9.00
Unperoxidized corn oil[3]	9.00	6.00	3.00	0.00	9.00	6.00	3.00	0.00	9.00	6.00	3.00	0.00
Fish meal	9.00	9.00	9.00	9.00	8.00	8.00	8.00	8.00	0.00	0.00	0.00	0.00
Dried whey	10.00	10.00	10.00	10.00	10.98	10.98	10.98	10.98	0.00	0.00	0.00	0.00
Lactose	12.91	12.91	12.91	12.91	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Spray dried porcine plasma	5.00	5.00	5.00	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Antibiotic[4]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Zinc oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.00	0.00	0.00	0.00
Vitamin/mineral premix[5]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
L-Lys	0.38	0.38	0.38	0.38	0.40	0.40	0.40	0.40	0.54	0.54	0.54	0.54
DL-Met	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.24	0.24	0.24	0.24
L-Thr	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.18	0.18	0.18	0.18
Dicalcium phosphate	0.21	0.21	0.21	0.21	0.35	0.35	0.35	0.35	1.51	1.51	1.51	1.51
Limestone	1.02	1.02	1.02	1.02	0.87	0.87	0.87	0.87	0.89	0.89	0.89	0.89
Salt	0.51	0.51	0.51	0.51	0.65	0.65	0.65	0.65	0.82	0.82	0.82	0.82
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Analyzed composition												
ME, kcal/kg[6]	3,786	3,786	3,786	3,786	3,737	3,737	3,737	3,737	3,692	3,692	3,692	3,692
Crude protein,%	23.10	22.40	23.40	22.70	24.90	24.90	24.20	24.80	21.50	20.10	22.60	22.60
Crude fat,%	10.30	11.25	10.56	11.11	10.86	12.24	10.89	10.56	11.59	9.76	12.09	10.36
NDF%	3.10	3.40	2.80	1.40	4.80	3.00	4.80	4.00	5.00	7.30	5.70	7.40
ADF,%	3.68	2.36	2.61	3.29	2.92	2.48	2.87	5.76	2.96	3.08	3.82	3.19
Ca,%	1.17	1.14	1.04	1.10	1.08	1.07	1.22	1.08	0.85	0.85	1.12	0.95
P,%	0.71	0.69	0.68	0.69	0.70	0.68	0.73	0.70	0.66	0.67	0.76	0.70
Se mg/kg	0.73	0.69	0.72	0.68	0.63	0.79	0.77	0.68	0.63	0.57	0.64	0.63
Vitamin E, IU/kg	47.40	42.01	41.49	40.23	44.30	42.97	38.32	27.91	50.72	26.87	29.31	26.36
Lys,%	1.83	1.77	1.80	1.85	1.77	1.73	1.80	1.74	1.70	1.83	1.77	1.71
Met + Cys,%	0.98	0.95	0.97	0.97	1.00	0.82	0.89	0.89	0.86	0.89	0.82	0.87
Thr,%	1.04	1.05	1.05	1.05	1.07	0.98	0.96	0.96	1.01	0.91	0.95	0.91
Trp,%	0.30	0.30	0.30	0.31	0.30	0.29	0.30	0.32	0.27	0.26	0.30	0.28

[1]Pigs were weaned at approximately 19 d of age; BW = 6.3 ± 1.4 kg.

[2]Diets contained either 0, 3, 6, or 9% of corn oil that had been heated for 12 h at 185°C with a constant forced air flow rate of 12 L/min. Peroxide value = 5.7 meq O2/kg and TBARS = 26.7 µg MDA eq/g.

[3]Unperoxidized corn oil was not subjected to heat treatment. Peroxide value = 1.7 meq O2/kg and TBARS = 48.3 mg MDA eq /kg.

[4]Mecadox® 2.5 (carbadox 5.51 g / kg; Phibro Animal Health, Teaneck, NJ) provided 27.5 ppm carbadox.

[5]Premix supplied the following nutrients per kilogram of diet: 11,023 IU of vitamin A as retinyl acetate; 2,756 IU of vitamin D3; 22 IU of vitamin E as dl-alpha tocopheryl acetate; 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; 992 mg of choline as choline chloride; 0.06 mg of vitamin B12; 14.3 mg of pyridoxine; 1.65 mg of folic acid; 2.20 mg of thiamine; 0.33 mg of biotin; 2.20 mg of iodine as ethylenediamine dihydroiodide; 0.30 mg of selenium as sodium selenite; 299 mg of zinc as zinc sulfate; 299 mg of iron as ferrous sulfate; 19.8 mg of copper as copper sulfate; and 17.6 mg of manganese as manganese oxide.

[6]ME values were calculated using NRC (2012) values.

Table 6-4. Growth performance of pigs fed increasing dietary concentrations of peroxidized corn oil, Exp. 1

	Level of peroxidized corn oil[1],%					P-values		
Item	0	2	4	6	PSEM[2]	Linear	Quadratic	Cubic
Phase 1								
ADG, g	158.8	231.3	192.7	204.1	22.38	0.27	0.13	0.07
ADFI, g	203.4	248.2	220.5	236.8	25.13	0.31	0.37	0.11
Gain:feed	0.78	0.94	0.87	0.87	0.04	0.16	0.01	0.04
Energy intake, Mcal ME/d	684.9	859.4	784.6	865.6	90.00	0.07	0.41	0.12
Caloric efficiency, g ADG/Mcal ME	231.0	272.2	245.2	237.6	10.26	0.86	0.01	0.04
Phase 2								
ADG, g	292.8	310.2	298.5	287.9	24.69	0.53	0.14	0.47
ADFI, g	407.6	427.1	391.7	378.4	29.07	0.01	0.12	0.11
Gain:feed	0.72	0.72	0.75	0.76	0.02	0.13	0.53	0.56
Energy intake, Mcal ME/d	1359.4	1466.5	1382.6	1372.0	100.00	0.78	0.12	0.12
Caloric efficiency, g ADG/Mcal ME	216.2	210.0	212.0	208.3	5.41	0.35	0.80	0.56
Phase 3								
ADG, g	490.2	487.2	490.7	474.4	27.86	0.35	0.52	0.57
ADFI, g	772.29	761.46	754.95	710.75	46.16	0.02	0.31	0.57
Gain:feed	0.63	0.64	0.65	0.67	0.01	< 0.01	0.21	0.94
ME intake, Mcal/d[3]	2.51	2.55	2.60	2.52	0.16	0.78	0.29	0.56
Caloric efficiency, g ADG/Mcal ME[4]	195.6	191.42	189.04	188.71	1.91	< 0.01	0.17	0.97
Overall (d 0 to 35)								
Initial wt., kg	6.3	6.4	6.3	6.4	0.51	0.54	0.68	0.12
Final BW, kg	20.2	20.6	20.4	20.0	1.38	0.61	0.24	0.71
ADG, g	393.2	398.7	396.1	390.2	25.94	0.78	0.55	0.91
ADFI, g	597.91	602.49	578.95	561.64	39.74	0.05	0.44	0.59
Gain:feed	0.65	0.66	0.67	0.69	0.005	< 0.01	0.23	0.99
Energy intake, Mcal ME/d	1.97	2.06	2.02	1.99	0.14	0.96	0.23	0.52
Caloric efficiency, g ADG/Mcal ME	198.59	196.15	193.84	194.67	1.57	0.04	0.27	0.65

[1]Corn oil was heated for 72 h at 95°C with a constant forced air flow rate of 12 L/min.

[2]PSEM = pooled standard error of means.

[3]ME = metabolizable energy.
[4]Mcal = ME intake, Mcal/d.

Table 6-5. Selected tissue and serum parameters of pigs fed increasing dietary concentrations of peroxidized corn oil, Exp. 1

	Level of peroxidized corn oil[1],%					P-values		
Item	0	2	4	6	PSEM[2]	Linear	Quadratic	Cubic
Average daily vitamin E intake, IU[3]	5.84	7.73	6.62	7.76	0.49	< 0.01	0.07	< 0.01
Average daily Se intake, mg[3]	0.48	0.45	0.47	0.42	0.03	< 0.01	0.42	0.02
Heart								
Heart somatic index[4],%	0.54	0.56	0.55	0.58	0.02	0.13	0.41	0.36
Liver								
Hepatosomatic index[5],%	2.73	3.10	3.08	3.05	0.10	0.04	0.05	0.38
α-Tocopherol, ug/g, wet wt.[6]	2.53	2.20	2.20	2.12	0.29	0.30	0.64	0.71
Selenium, ug/g, wet wt.[6]	0.75	0.72	0.67	0.70	0.02	0.06	0.21	0.46
Serum								
Trp, uM[6,7]	58.13	55.57	59.21	54.37	4.31	0.70	0.80	0.44

[1] Corn oil was heated for 72 h at 95°C with a constant forced air flow rate of 12 L/min.
[2]PSEM = pooled standard error of means.
[3]Mean analyzed dietary content across phases x ADFI.
[4][(Heart weight, g/BW, g) x 100].
[5][(Liver weight, g/BW, g) x 100].
[6]Covariate adjusted for average daily intake of vitamin E, Se, or Trp, respectively.
[7]Serum collected at d 35 of experiment.

Table 6-6. Metabolites in serum from pigs fed increasing dietary concentrations of peroxidized corn oil, Exp. 1

	Level of peroxidized corn oil[1],%					P-values				
Item	0	2	4	6	PSEM[2]	Day	Diet x day	Linear	Quadratic	Cubic
Serum α-tocopherol, ug/mL[3]										
d 14	0.43	0.21	0.24	0.19	0.04			< 0.01	0.04	0.08
d 35	0.23	0.18	0.18	0.26	0.04			0.69	0.13	0.81
Mean (d 14 and 35)	0.33	0.20	0.21	0.22	0.03	0.06	0.02	0.03	0.01	0.29
Serum selenium, ng/mL[4]										
d 14	119.73	124.89	114.65	122.57	4.06			0.92	0.71	0.05
d 35	142.58	141.89	132.65	146.82	4.06			0.84	0.05	0.06
Mean (d 14 and 35)	131.15	133.39	123.65	134.70	3.18	< 0.01	0.68	0.95	0.10	0.01
Serum triglycerides, ug/mL[5]										
d 14	356.11	430.79	384.00	364.26	48.90			0.63	0.19	0.49
d 35	377.94	411.71	383.32	349.98	48.90			0.93	0.55	0.29
Mean (d 14 and 35)	366.82	420.73	383.32	356.72	34.63	0.94	0.96	0.43	0.48	0.79
Serum cholesterol, ug/mL[6]										
d 14	531.12	558.66	628.09	602.65	61.04			0.56	0.88	0.57
d 35	767.2	686.71	662.28	610.7	61.02			0.27	0.06	0.65
Mean (d 14 and 35)	649.16	622.68	645.19	606.68	45.45	0.01	0.19	0.80	0.61	0.75

[1]Corn oil was heated for 72 h at 95°C with a constant forced air flow rate of 12 L/min.

[2]PSEM = pooled standard error of means.

[3]Data were covariate adjusted for concentration at weaning (mean = 2.2 ± 0.4 ug/mL), intake of vitamin E, and cholesterol content.

[4]Data were covariate adjusted for concentration at weaning (mean =125.8 ± 5.0 ng/mL) and intake of Se.

[5]Data were covariate adjusted for concentration at weaning (mean = 536.8 ± 77.4 ug/mL).

[6]Data were covariate adjusted for concentration at weaning (mean = 960.7 ± 111.44 ug/mL).

Table 6-7. Concentration of amino acids in serum from pigs fed increasing dietary concentrations of peroxidized corn oil, Exp. 1

	Level of peroxidized corn oil[1],%					P-values		
Item	0	2	4	6	PSEM[2]	Linear	Quadratic	Cubic
d 35 serum concentration, uM								
Alanine	397.93	364.93	358.03	278.35	35.63	0.05	0.01	0.44
Arginine	213.29	214.80	236.51	218.71	17.76	0.78	0.64	0.59
Asparagine	133.49	137.51	164.86	145.44	19.83	0.62	0.44	0.52
Citrulline	53.08	65.06	58.50	51.29	5.33	0.28	0.62	0.08
Glutamate	138.10	143.38	129.10	100.46	12.35	0.07	0.02	0.15
Glutamine	955.36	1,030.06	1,074.70	1,001.54	63.70	0.60	0.52	0.25
Glycine	1,215.40	1,203.51	1,220.80	1,215.31	79.28	1.00	0.96	0.97
Histidine	75.63	72.01	84.08	89.64	6.97	0.16	0.05	0.44
Isoleucine	188.04	178.60	206.65	174.10	15.17	0.38	0.83	0.41
Lysine	211.10	193.26	246.01	206.41	19.99	0.28	0.66	0.58
Methionine	66.18	58.73	73.28	72.18	8.75	0.63	0.41	0.72
Ornithine	176.18	180.74	185.39	158.93	20.15	0.80	0.61	0.45
Phenylalanine	86.09	80.91	87.40	89.96	6.31	0.71	0.48	0.50
Proline	294.79	275.46	284.48	265.21	20.86	0.75	0.38	1.00
Serine	134.73	134.79	134.78	131.20	11.02	0.99	0.82	0.86
Taurine	149.20	147.95	163.40	171.26	13.31	0.51	0.18	0.74
Threonine	193.05	195.99	230.31	229.65	18.95	0.19	0.05	0.91
Tryptophan	55.31	55.40	57.19	54.55	4.28	0.97	0.98	0.75
Tyrosine	142.11	127.98	118.90	121.73	10.97	0.37	0.13	0.40
Valine	184.21	161.94	191.84	185.30	19.07	0.63	0.67	0.65

[1]Corn oil was heated for 72 h at 95°C with a constant forced air flow rate of 12 L/min.

[2]PSEM = pooled standard error of means.

Table 6-8. Growth performance of pigs fed increasing dietary concentrations of peroxidized corn oil, Exp. 2

	Level of peroxidized corn oil[1],%					P-values		
Item	0	3	6	9	PSEM[2]	Linear	Quadratic	Cubic
Phase 1								
ADG, g	71.6	56.7	13.8	-5.3	23.41	0.01	0.92	0.58
ADFI, g	87.9	88.2	83.3	70.2	10.59	0.20	0.50	0.95
Gain:feed	0.80	0.50	0.04	-1.17	0.63	0.03	0.50	0.90
Energy intake, Mcal ME/d	0.33	0.33	0.32	0.27	0.04	0.20	0.50	0.95
Caloric efficiency, g ADG/Mcal ME	210.51	131.39	-10.43	-308.07	165.24	0.03	0.50	0.90
Phase 2								
ADG, g	330.1	355.9	360.6	344.7	20.11	0.52	0.22	0.99
ADFI, g	405.0	438.0	447.1	429.4	23.40	0.38	0.23	0.98
Gain:feed	0.82	0.81	0.81	0.80	0.03	0.64	0.82	0.91
Energy intake, Mcal ME/d	1.51	1.64	1.67	1.60	0.09	0.38	0.23	0.98
Caloric efficiency, g ADG/Mcal ME	220.71	216.41	215.95	215.30	7.41	0.64	0.82	0.91
Phase 3								
ADG, g	458.5	446.0	441.3	415.2	17.17	0.07	0.67	0.68
ADFI, g	653.7	647.2	645.0	601.5	20.95	0.10	0.38	0.63
Gain:feed	0.70	0.69	0.68	0.69	0.01	0.43	0.41	0.97
Energy intake, Mcal ME/d	2.41	2.39	2.38	2.22	0.08	0.10	0.38	0.63
Caloric efficiency, g ADG/Mcal ME	190.13	186.46	185.14	186.79	3.38	0.43	0.41	0.97
Overall (d 0 to 35)								
Initial wt., kg	6.3	6.3	6.3	6.3	0.23	0.32	0.91	0.87
Final BW, kg	19.5	19.5	19.2	18.5	0.59	0.11	0.44	0.85
ADG, g	377.5	375.8	369.4	347.0	13.62	0.10	0.43	0.84
ADFI, g	515.3	523.6	524.3	491.6	16.81	0.35	0.23	0.73
Gain:feed	0.73	0.72	0.70	0.71	0.01	0.03	0.34	0.79
Energy intake, Mcal ME/d	1.95	1.98	1.99	1.86	0.06	0.35	0.23	0.73
Caloric efficiency, g ADG/Mcal ME	193.76	189.30	185.82	186.34	2.60	0.03	0.34	0.79

[1]Peroxidized corn oil was heated for 12 h at 185°C with a constant forced air flow rate of 12 L/min. Unperoxidized corn oil was added so all

[2]PSEM = pooled standard error of means.

Table 6-9. Selected tissue parameters of pigs fed increasing dietary concentrations of peroxidized corn oil, Exp. 2

	Level of peroxidized corn oil[1],%					P-values		
Item	0	3	6	9	PSEM[2]	Linear	Quadratic	Cubic
Average daily vitamin E intake, IU[3]	24.99	17.38	17.44	13.95	0.65	< 0.01	< 0.01	< 0.01
Average daily Se intake, mg[3]	0.33	0.34	0.36	0.32	0.01	0.80	0.05	0.13
Heart								
Heart somatic index[4],%	0.53	0.53	0.52	0.59	0.03	0.24	0.24	0.40
Liver								
Hepatosomatic index[5],%	3.33	3.44	3.41	3.51	0.14	0.38	0.97	0.63
α-Tocopherol, ug/g, wet wt.[6]	2.34	2.65	2.70	3.14	0.49	0.50	0.87	0.73
Selenium, ug/g, wet wt.[6]	0.57	0.57	0.59	0.58	0.02	0.58	0.80	0.48
Serum								
Trp, uM[6,7]	53.08	39.45	44.18	40.95	0.05	0.08	0.20	0.14

[1] Corn oil was heated for 12 h at 185°C with a constant forced air flow rate of 12 L/min. Unperoxidized corn oil was added so all diets contained 9% supplemental corn oil.

[2]PSEM = pooled standard error of means.

[3]Mean analyzed dietary content weighted by phase length x overall ADFI.

[4][(Heart weight, g/BW, g) x 100].

[5][(Liver weight, g/BW, g) x 100].

[6]Covariate adjusted for average daily intake of vitamin E, Se, or Trp, respectively.

[7]Serum collected at d 35 of experiment.

Table 6-10. Metabolites in serum from pigs fed increasing dietary concentrations of peroxidized corn oil, Exp. 2

	Level of peroxidized corn oil[1],%					P-values				
Item	0	3	6	9	PSEM[1]	Day	Diet x day	Linear	Quadratic	Cubic
Serum α-tocopherol, ug/mL[3]										
d 14	1.40	0.91	0.89	0.49	0.06			< 0.01	0.64	0.07
d 35	0.58	0.28	0.30	0.13	0.08			< 0.01	0.07	0.01
Mean (d 14 and 35)	0.99	0.59	0.59	0.31	0.08	< 0.01	0.03	< 0.01	0.32	0.01
Serum selenium, ng/mL[4]										
d 14	114.38	113.62	103.06	106.19	7.05			0.19	0.76	0.39
d 35	130.38	131.37	130.93	118.06	7.05			0.28	0.39	0.76
Mean (d 14 and 35)	122.38	122.50	117.00	112.13	5.10	0.37	< 0.01	0.10	0.64	0.74
Serum TBARS, uM[5]										
d 14	0.76	0.83	0.81	0.88	0.05			0.13	1.00	0.36
d 35	0.82	0.83	0.82	0.83	0.05			0.89	1.00	0.86
Mean (d 14 and 35)	0.79	0.83	0.81	0.85	0.02	0.99	0.85	0.06	1.00	0.22
Serum cholesterol, ug/dL[6]										
d 14	1070.02	1086.21	970.42	689.52	74.07			0.19	0.76	0.39
d 35	837.22	684.56	880.28	541.14	74.07			0.28	0.39	0.76
Mean (d 14 and 35)	953.62	885.38	925.35	615.33	65.71	0.37	< 0.01	0.10	0.64	0.74
Serum triglycerides, ug/dL[7]										
d 14	269.19	234.37	211.88	220.00	44.71			0.13	1.00	0.36
d 35	329.38	273.30	271.68	173.72	44.71			0.89	1.00	0.86
Mean (d 14 and 35)	297.06	252.67	239.03	194.79	37.85	0.99	0.85	0.06	1.00	0.22

[1]Corn oil was heated for 12 h at 185°C with a constant forced air flow rate of 12 L/min. Unperoxidized corn oil was added so all diets contained 9% supplemental corn oil.

[2]PSEM = pooled standard error of means.

[3]Data were covariate adjusted for concentration at weaning (mean = 5.9 ± 0.5 ug/mL), intake of vitamin E, and cholesterol content.

[4]Data were covariate adjusted for concentration at weaning, mean = 128.1 ± 3.7 ng/mL and intake of Se.

[5]Data were covariate adjusted for concentration at weaning, mean = 0.83 ± 0.07 uM.
[6]Data were covariate adjusted for concentration at weaning, mean = 2,734.8 ± 305.6 ug/dL.
[7]Data were covariate adjusted for concentration at weaning, mean = 373.3 ± 67.2 ug/dL.

Table 6-11. Concentration of amino acids in serum from pigs fed increasing dietary concentrations of peroxidized corn oil, Exp. 2

	Level of peroxidized corn oil[1],%					P-values		
Item	0	3	6	9	PSEM[2]	Linear	Quadratic	Cubic
d 35 serum concentration, uM								
Alanine	591.87	513.33	591.14	501.44	42.95	0.19	0.26	0.88
Arginine	186.04	189.93	174.99	182.90	18.47	0.94	0.77	0.91
Asparagine	182.05	163.54	151.50	161.86	16.31	0.65	0.35	0.40
Aspartic Acid	48.35	86.16	72.14	53.36	14.52	0.18	0.99	0.04
Citrulline	73.07	70.34	76.93	75.10	7.33	0.93	0.71	0.95
Glutamate	718.35	720.01	716.43	568.33	84.60	0.42	0.21	0.35
Glutamine	743.03	650.40	658.61	654.80	61.37	0.52	0.26	0.38
Glycine	1,409.18	1,406.37	1,246.74	1,388.09	96.49	0.45	0.56	0.40
Histidine	78.00	75.11	76.00	75.71	6.80	0.99	0.85	0.85
Isoleucine	111.46	103.44	97.91	99.49	9.80	0.81	0.39	0.66
Leucine	170.42	136.34	151.04	133.43	11.57	0.22	0.12	0.54
Lysine	183.10	168.76	166.37	213.49	26.75	0.58	0.47	0.27
Methionine	24.42	17.45	20.17	16.09	1.21	0.24	0.11	0.71
Ornithine	96.77	102.81	94.91	109.33	7.33	0.57	0.42	0.61
Phenylalanine	86.52	82.26	82.36	79.64	4.46	0.80	0.36	0.88
Proline	285.30	245.88	259.44	260.17	13.89	0.29	0.32	0.16
Serine	206.99	241.15	200.34	225.51	17.56	0.36	0.85	0.80
Taurine	255.30	266.45	217.80	247.76	34.06	0.67	0.59	0.75
Threonine	245.02	278.57	291.21	312.80	23.26	0.40	0.08	0.82
Tryptophan	53.12	39.52	44.33	40.69	3.85	0.09	0.07	0.20
Tyrosine	94.07	79.79	89.36	92.31	7.98	0.55	0.89	0.25
Valine	209.94	191.11	203.49	185.66	18.09	0.83	0.52	0.98

[1]Peroxidized corn oil was heated for 12 h at 185°C with a constant forced air flow rate of 12 L/min. Unperoxidized corn oil was added so all diets contained 9% supplemental corn oil.

[2]PSEM = pooled standard error of means.

CHAPTER 7. IMPLICATIONS

Maximizing the efficiency of energy utilization is important because energy is the most expensive dietary component. Energy is provided to animal diets in the form of grains such as corn or dried distillers grains with solubles (**DDGS**), but concentrated sources of dietary lipid such as corn oil may be added, as well. Over 85% of U.S. ethanol plants currently extract corn oil prior to manufacturing DDGS, generating more than 105,000 tonnes of distiller's corn oil (**DCO**) in 2013 for use in the production of biodiesel and animal feeds (Renewable Fuels Association, 2014).

Lipids high in PUFA, such as corn oil, are prone to peroxidation, particularly upon exposure to heat, light, oxygen, and metal (Belitz et al., 2009). Therefore, lipids in DDGS (Song and Shurson, 2013) and concentrated lipid sources (Dibner et al., 2011) may become peroxidized depending on storage or processing conditions.

Peroxidation generates numerous compounds which have been associated with negative effects on physiology (Yu, 1994), growth (Tavárez et al., 2011; Boler et al., 2012; Liu, 2012), and antioxidant status (Inoue et al., 1984; Engberg et al., 1996; Takahashi and Akiba, 1999; Tavárez et al., 2011). Lipid hydroperoxides are primary products of lipid peroxidation which subsequently react to form numerous products (Belitz et al., 2009). Therefore, a wide variety of primary, secondary, and tertiary products can be measured to assess the extent of peroxidation. Peroxide value (**PV**), thiobarbituric acid reactive substances (**TBARS**), and *p*-anisidine value (**AnV**) may be assessed, but these compounds are liable and usually degraded throughout peroxidation (DeRouchey et al., 2004; Danowska-Oziewicz and Karpińska-Tymoszczyk, 2005; Liu, 2012).

Identifying the optimal dietary measures to assess the extent of lipid peroxidation and connecting these markers with changes in animal health and growth has been a historic challenge for nutritionists because peroxidation is a dynamic process. The information contained in this dissertation provides some initial information to build on knowledge in this field.

In chapter 2, a summary of experimental evidence revealed that feeding peroxidized lipids to broilers and pigs reduced gain efficiency by 4.3%. Negative relationships were exposed for dietary concentration of TBARS or PV and ADG for pigs and broilers, respectively. However, others have established that PV and TBARS fluctuate during peroxidation, particularly in lipids exposed to temperatures exceeding 150^0C (Danowska-Oziewicz and Karpińska-Tymoszczyk, 2005; Liu, 2012). Unfortunately, PV is the sole measure used to estimate the extent of lipid peroxidation in some studies (Inoue et al., 1984; Cabel et al., 1988; Lin et al., 1989; Takahashi and Akiba, 1999; Anjum et al., 2002, 2004; Upton et al., 2009; McGill et al., 2011a,b; Tavárez et al., 2011) which prohibits assessment of the utility of other measures of lipid peroxidation.

Two experiments were conducted to identify the optimal markers for lipid peroxidation in corn oil exposed to varying thermal conditions. Based on the results presented in chapter 3, TBARS, hexanal, and 4-hydroxynonenol consistently indicated the extent of peroxidation in corn oil exposed to high (185°C) or low (95°C) temperatures for 12 or 72 h, respectively. Interestingly, PV and AnV effectively indicated peroxidation in oil exposed to low temperatures, but not at high temperatures. Therefore, assessment of secondary and tertiary products of peroxidation is more appropriate for lipids exposed to

high temperatures. Results from chapter 4 suggest that PV, AnV, and TBARS of DCO and DDGS continue to increase throughout 28 d of storage at 38°C with 90% relative humidity. These data suggest that PV, AnV, and TBARS are reliable markers under these conditions (≤ 38°C). Together our results indicate that suitability of markers for peroxidation depends on thermal conditions of storage and processing. However, information on historical processing, storage, and handling conditions is generally not available to feed formulators and nutritionists. Ultimately, the concentration of secondary products of peroxidation, such as hexanal, 2,4-hydroxynonenol, and TBARS, may indicate peroxidation in lipids subjected to either high or low temperatures. In chapter 4, peroxidation was impeded by 2 antioxidant compounds, but adding antioxidants did not completely stop peroxidation. Furthermore, the value of impeding peroxidation is not apparent, because direct relationships between growth and levels of peroxidation products have not been established conclusively. Ultimately, it is unclear how compounds produced during peroxidation relate to efficiency and growth of animals.

To investigate the effects of peroxidized lipids on the growth and antioxidant status of pigs, 3 experiments were conducted. A highly peroxidized DDGS was evaluated in diets for nursery pigs in chapter 5. Feeding highly peroxidized DDGS increased the vitamin E status of nursery pigs, and did not affect growth performance. We speculate that high levels of dietary sulfur from DDGS increased levels of sulfur-containing antioxidants *in vivo*, resulting in a sparing effect on vitamin E. These findings suggest that feeding highly peroxidized DDGS is not detrimental for nursery pigs provided high levels of dietary sulfur. Feeding DDGS to sows reduced the vitamin E status of pigs at weaning, but this effect did not persist post-weaning when antioxidant deficiency

diseases can be an issue. These results indicate that feeding DDGS is not a concern for the development of metabolic oxidative stress.

To eliminate the confounding influence of dietary sulfur concentrations, nursery pigs were fed increasing levels of peroxidized corn oil. Results from chapter 6 confirm findings reported by others regarding the negative effects of peroxidized lipids for animal growth, efficiency, and antioxidant status. Feeding slowly heated corn oil reduced the caloric efficiency of nursery pigs from 1.2 to 2.4% with 2 to 6% dietary inclusion. However, feeding rapidly heated corn oil reduced caloric efficiency from 2.4 to 4% with 3 to 9% dietary inclusion. Reduced growth performance during the nursery phase may have lasting effects on the performance of pigs to harvest (Tokach et al., 1992; Mahan et al., 1998).

The divergence in magnitude of response across the 2 experiments suggests the effects of peroxidized lipids depend on the temperature and duration of thermal exposure. Interestingly, the concentration of TBARS in diets containing the rapidly heated corn oil was 4-fold that of the slowly heated corn oil. Furthermore, the concentration of hexanal in diets containing the rapidly heated corn oil was approximately double that of diets with slowly heated corn oil. These findings indicate that TBARS or hexanal may be associated negatively with efficiency of nutrient utilization when pigs are fed diets with peroxidized lipids.

These experiments also uncovered a potential connection between endogenous catabolism of Trp and peroxidized corn oil that must be further explored. Our preliminary results suggest that Trp catabolism is increased in response to rapidly peroxidized corn oil, but the practical implications on the availability of Trp for protein synthesis are

unclear. Feeding peroxidized corn oil resulted in metabolic oxidative stress, suggesting that a metabolic antioxidant imbalance contributes to reduced growth and efficiency. Additional research will evaluate if the negative effects of peroxidized lipids are ameliorated by adding dietary antioxidants.

Mechanisms underlying the negative effects of peroxidized lipids are not well established. Inefficiencies in growth performance may result from consequences of altered metabolic oxidative status (Cabel et al., 1988; McGill et al., 2011b; Tavárez et al., 2011) or altered energy value and nutrient digestibility (Inoue et al., 1984; Liu and Huang, 1995; Engberg et al., 1996; Yuan et al., 2007). The information contained in this dissertation will facilitate future research to better understand specific mechanisms and develop predictive models of effects of peroxidized lipids on growth performance and efficiency of animals.

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