BIOLOGICAL ASSESSMENT AND METHODS TO EVALUATE LIPID PEROXIDATION WHEN FEEDING THERMALLY-OXIDIZED LIPIDS TO YOUNG PIGS

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CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

Energy is the most expensive component of swine diets. Lipids are commonly added to swine diets as concentrated energy sources to improve feed efficiency, but they also reduce feed dust, supply fat soluble vitamins and essential fatty acids, and improve diet palatability (Pettigrew and Moser, 1991). However, lipids are highly sensitive to peroxidation (Frankel et al., 1984, Linfield et al., 1985; Mayes 1996), especially when subjected to heat during processing or cooking, which leads to depletion of endogenous antioxidants (Seppanen and Csallany, 2002) and production of an assortment of peroxidation products (Lin et al., 1989; Adam et al., 2008). Thus, lipids used in animal production not only vary in fatty acid composition due to their origin, but may also contain various concentrations of primary and secondary lipid peroxidation products depending upon fatty acid composition, storage length and conditions, and processing conditions (Dibner et al., 1996a; Cabel et al., 1998; DeRouchey et al., 2004; Canakci, 2007).

Negative effects of feeding oxidized lipid to animals have been reported in several previous studies. Kimura et al. (1984) noted that feeding oxidized soybean oil not only impaired growth performance, but caused diarrhea in rats. Alexander et al. (1987) and Behniwal et al. (1993) also reported that rats fed diets containing oxidized corn or peanut oil had impaired growth rate. Similarly in broiler studies, ingestion of oxidized poultry fat led to impaired growth compared with birds fed fresh poultry fat

(Cabel et al., 1988; Dibner et al., 1996a). DeRouchey et al. (2004) observed reduced growth performance when pigs consumed rancid choice white grease. However, there is very little information regarding the mechanism responsible for the poor performance when pigs are fed peroxidized lipids. Furthermore, although measurements of lipid peroxidation provide useful information to evaluate the degree of peroxidation, this information may not be valid due to the drawbacks of method used for characterizing the lipid peroxidation and the time at which the analysis occurred. Therefore, a better knowledge of the comparative benefits and limitations of methods used to evaluation lipid peroxidation and their relationships to the physiological effects and the energy value of oxidized lipids when added to swine diets is needed. Therefore, the objectives of this thesis are:

1. To assess various measurements commonly used for evaluation of lipid peroxidation.

2. To evaluate the effects of feeding thermally-oxidized vegetable oils and animal fats on growth performance, liver gene expression, and liver and serum fatty acid composition in young pigs.

3. To determine the effect of lipid source and peroxidation level on DE and ME content and on apparent total tract digestibility of DM, GE, ether extract, nitrogen, and carbon in diets fed to young pigs.

4. To investigate the effect of thermally-oxidized vegetable oils and animal fats on metabolic oxidation status, gut barrier function, and immune response of young pigs.

DEFINITION AND CHARACTERISTICS OF LIPIDS

Lipids

Lipids are a broad category of substances that are sparingly soluble in water, but show variable solubility in a number of organic solvents (e.g., acetone, ethanol, ethyl ether, petroleum ether, and methanol), and include a wide variety of chemical compounds (Fahy et al., 2005). Lipids can be divided into eight well-defined categories of a comprehensive system (Table 1.1). Dietary lipids refer to lipid substances in the diet that can originate from multiple feed ingredients or can be added as a relatively pure lipid extracted from different sources. Triglycerides are the major component of dietary lipids while minor components of dietary lipids include free fatty acid (**FFA**), phospholipids, sterols, fat-soluble vitamins, tocopherols, pigments, waxes, and fatty alcohols. Most of the time, dietary lipids are in the form of triglycerides. In practice, lipids that remain solid at room temperature are referred to as "fats," and when in liquid form at room temperature, they are called "oils". However, there is little chemical distinction between fats and oils since the substances are all composed predominantly of esters (triglycerides).

Table 1.1. Lipid categories of the comprehensive classification system and the number of structures[1]

Category	Structures
Fatty acyls	2,678
Glycerolipids	3,009
Glycerophospholipids	1,970
Sphingolipids	620
Sterol lipids	1,744
Prenol lipids	610
Saccharolipids	11
Polyketides	132

[1]Adapted from Fahy et al. (2009).

Triglycerides

Triglycerides consist of 3 fatty acids attached to one glycerol molecule by an ester bond (Figure 1.1). Most of the time, all 3 fatty acids are "mixed" triglycerides in which two or three different fatty acids are present in the molecule. The fatty acid profile of a triglyceride dictates the physical and chemical characteristics of the lipid. Depending on the lipid origin, the fatty acid composition of lipids can vary substantially as shown in Table 1.2. For example, corn oil and canola oil are vegetable oils and have high concentrations of unsaturated fatty acids. The major fatty acid in corn oil is linoleic acid (C18:2) which accounts for approximately 59% of the total fatty acids, whereas the most abundant fatty acid in canola oil is oleic acid (C18:1) making up about 56% of the total fatty acids.

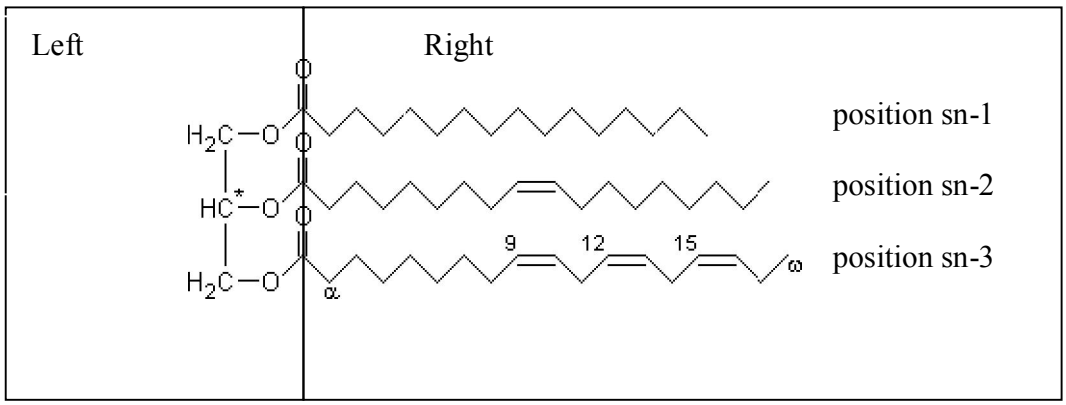

Figure 1.1. Chemical structure of an unsaturated triglyceride (adapted from http://en.wikipedia.org/wiki/Triglyceride). The left portion of the structure is glycerol, whereas the right portion of the structure from top to bottom are palmitic acid, oleic acid, alpha-linolenic acid. The chemical formula is $C_{55}H_{98}O_6$. In a natural L-glycerol derivative as shown above, the secondary hydroxyl group is shown as C-2; the carbon atom above this then becomes C-1 while that below becomes C-3, and the prefix *sn* is placed before the stem name of the compound. The term "triacyl-*sn*-glycerol" should then be used to designate the molecule rather than "triglyceride".

Table 1.2. Fatty acid composition of different lipid sources[1]

	Fatty acid composition, %									S[3]	U[4]	IV[5]
	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	≥20[2]			
Animal fats												
Beef tallow	0.9	2.7	24.9	4.2	18.9	36.0	3.1	0.6	0.3	52.1	47.9	44
Choice white grease	0.2	1.9	21.5	5.7	14.9	41.1	11.6	0.4	1.8	40.8	59.2	60
Lard	0.2	1.3	23.8	2.7	13.5	41.2	10.2	1.0	1.0	41.1	58.9	64
Poultry fat	0.1	0.9	21.6	5.7	6.0	37.3	19.5	1.0	1.2	31.2	68.8	78
Restaurant grease	--	1.9	16.2	2.5	10.5	47.5	17.5	1.9	1.0	29.9	70.1	75
Vegetable oils												
Canola	--	--	4	0.2	1.8	56.1	20.3	9.3	3.6	7.4	92.6	118
Coconut	44.6	16.8	8.2	--	2.8	5.8	1.8	--	--	91.9	8.1	10
Corn	--	--	10.9	--	1.8	24.2	59.0	0.7	--	13.3	86.7	125
Cottonseed	--	0.8	22.7	0.8	2.3	17.0	51.5	0.2	0.1	27.1	72.9	105
Olive	--	--	11.0	0.8	2.2	72.5	7.9	0.6	0.3	14.1	85.9	86
Palm	0.1	1.0	43.5	0.3	4.3	36.6	9.1	0.2	0.1	51.6	48.4	50
Peanut	--	0.1	9.5	0.1	2.2	44.8	32.0	--	6.4	17.8	82.2	92
Safflower	--	0.1	6.2	0.4	2.3	11.7	74.1	0.4	--	9.5	90.5	140
Sesame	--	--	8.9	0.2	4.8	39.3	41.3	0.3	0.2	14.8	85.2	110
Soybean	--	0.1	10.3	0.2	3.8	22.8	51.0	6.8	0.2	15.1	84.9	130
Sunflower	--	--	5.4	0.2	3.5	45.3	39.8	0.2	--	10.6	89.4	133

[1]Adapted from NRC (1998).
[2]> 20 = fatty acid with carbon chain length equal or greater than 20.
[3]S = total saturated fatty acids (%).
[4]U = total unsaturated fatty acids (%).
[5]IV = Iodine Value.

Fatty acids

The most abundant fatty acids in lipids contain an even number of carbon atoms and a single carboxyl group as shown in Table 1.3. Fatty acids have both trivial and chemical names. Fatty acids occurring in fats and oils can also be classified into saturated and unsaturated fatty acids according to their degree of saturation. Saturated fatty acids contain only single carbon-to-carbon bonds and are the least reactive chemically (Ratnayake and Galli, 2009). Moussavi et al. (2008) reported that saturated lipid is preferentially incorporated into adipose tissue stores due to the absence of double bonds. Unsaturated fatty acids contain one or more carbon-to-carbon double bonds and include mono-unsaturated fatty acids (**MUFA**) which have one double bond, and poly-unsaturated fatty acids (**PUFA**) which have two or more double bonds (Ratnayake and Galli, 2009). Since animals have limited ability to produce certain unsaturated fatty acids through *de novo* fatty acid synthesis, these unsaturated fatty acids are essential precursors for synthesis of biologically active compounds such as cell membrane lipids and steroid hormones (Moussavi et al., 2008). As a result, some fatty acids, primarily linoleic acid and linolenic acid, are considered to be essential fatty acids which must be supplied in adequate amounts in the diet. Either the methyl or the carboxyl end of the fatty acid chain can be used to designate the position of the double bonds. The Δ system numbers from the carboxyl end. For example, α-linolenic acid is named C18:3 Δ9, 12, 15, while in ω or n nomenclature, the α-linolenic acid is named as C18:3 ω- or n-6 counting from the methyl end the fatty acid. Although animals have the ability to desaturate fatty acids to some extent, they all lack specific enzymes to add double bonds in the correct position to produce n-3 or n-6 fatty acids. Therefore, linoleic and arachidonic members of the n-6

family, and linolenic, an n-3 fatty acid, are collectively referred to as the essential fatty acids because these fatty acids are precursors for the eicosanoids involved in endocrine functions (Kliewer et al., 1997; Bhathena, 2000). Fatty acids can also be classified as short-chain fatty acids containing 2-4 carbons, medium-chain fatty acids with carbon chain length from 6-12, and long-chain fatty acids composed of 14-20 carbons.

The characteristics and properties of specific lipids are highly dependent on the degree of unsaturation as well as the carbon chain length of its fatty acid constituents. Generally speaking, lipids containing a high level of unsaturated fatty acids tend to have a lower melting point and tend to be more liquid at room temperature than those have a lower degree of unsaturation (Table 1.3). And as the chain length of a saturated fatty acid decreases, the melting point also decreases (Table 1.3). Thus, saturated fatty acids with longer chains have higher melting points than short-chain saturated fatty acids.

Chemical reactions of triglycerides and fatty acids

Hydrolysis of lipids

Glycerides can be readily hydrolyzed. Partial hydrolysis of triglycerides will produce mono- and diglycerides and FFA. Presence of an acid catalyst will fully hydrolyze mono-, di-, and triglycerides to yield glycerol and FFA. In industrial production, ‘soap’ or fatty acid salts and glycerol can be obtained through a saponification reaction, in which triglycerides are reacted with sodium or potassium hydroxide to produce glycerol and a fatty acid salt. In addition to chemical hydrolysis, glycerides also can be hydrolyzed by enzymes (lipases) in the digestive tract of humans and animals as well as in bacteria.

Table 1.3. Major fatty acids found in triglycerides[1]

	Common Name	Chemical Name	Carbon No.	No. of Double Bonds	Melting Point, °C
Saturated fatty acids	butyric	butanoic	4	0	-7.9
	caproic	hecanoic	6	0	-3.4
	caprylic acid	octanoic acid	8	0	16.5
	capric acid	decanoic acid	10	0	31.4
	lauric acid	dodecanoic acid	12	0	44.0
	myristic acid	tetradecanoic acid	14	0	58.0
	palmitic acid	hexadecanoic acid	16	0	63.0
	stearic acid	octadecanoic acid	18	0	71.5
	arachidic acid	eicosanoic acid	20	0	75.4
Mono-unsaturated fatty acids (MUFA)	palmitoleic acid	hexadecenoic acid	16	1	1.5
	oleic acid	octadecenoic acid	18	1	16.3
Poly-unsaturated fatty acids (PUFA)	linoleic acid	octadecadienoic acid	18	8	-5.0
	alpha-linolenic acid	octadecatrienoic acid	18	3	-11.3
	arachidonic acid	eicosatetraenoic acid	20	4	-49.5
	timnodionic acid	eicosapentaenoic acid (EPA)	20	5	-51.0
	cervonic acid	docosahexaenoic acid (DHA)	22	6	-45.0

[1]Adapted from Azain (2001).

Lipid Peroxidation

Increasing attention has been focused on lipids because of the remarkable implications of their peroxidative damage. Lipid peroxidation plays an important role in determining the flavor, aroma, nutritional quality, and, in some cases, even the texture of a food or feed product. The chemicals produced from oxidation of lipids are not only responsible for rancid flavors, but are also involved in destroying vitamins and other nutrients. The addition of peroxidized lipids to foods negatively affects food consumption as a result of rancidity flavor (DeRouchey et al., 2004). Furthermore, many toxic secondary lipid peroxidation products have the potential to interact with proteins and carbohydrates and can be absorbed directly (Seppanen and Csallany, 2006). Thus, knowing the peroxidative condition of lipids is extremely important in biochemical and nutritional studies with animals.

Lipid oxidation of fatty acids, also known as lipid peroxidation, is an autocatalytic process caused by free radicals in the presence of molecular oxygen. Free radicals are substances which have unpaired electrons that are highly reactive and which initiate the lipid oxidation processes. The processes of lipid peroxidation are considered to occur in three phases: an initiation or induction phase, propagation phase, and a termination phase (Khayat and Schwall, 1983; Frankel, 1985; Fernindez et al., 1997) and are shown in Table 1.4.

The initiation phase can be caused by many factors including heat, light, metal ions and irradiation. During the initiation phase, lipid free radicals are formed by hydrogen atom abstraction (Table 1.4), and the reaction of these free radicals with oxygen forms

hydroperoxides (•OOH). Both the free radicals and the hydroperoxides are highly reactive. During the propagation stage, the peroxyl radicals react with other lipid molecules to form hydroperoxides and generate new free radicals by hydrogen abstraction (Hultin, 1994; Fraser and Sumar, 1998). In the final termination phase of lipid peroxidation, the free radicals generated interact to form relatively unreactive compounds including hydrocarbons, aldehydes, and ketones (Hultin, 1994).

Table 1.4. Summary of lipid peroxidation phases

Phase of lipid peroxidation	Reactions		
Initiation	$RH + O_2$	$\longrightarrow$	$R\cdot + \cdot OOH$
Propagation	$R\cdot + O_2$	$\longrightarrow$	$ROO\cdot$
	$RH + ROO\cdot$	$\longrightarrow$	$R\cdot + ROOH$
	$ROOH$	$\longrightarrow$	$RO\cdot + \cdot OH$
Termination	$R\cdot + R\cdot$	$\longrightarrow$	$R - R$
	$R\cdot + ROO\cdot$	$\longrightarrow$	$ROOR$
	$ROO\cdot + ROO\cdot$	$\longrightarrow$	$ROOR + O_2$

RH represents an unsaturated fatty acid
R· represents all potential lipid-derived radicals after hydrogen abstraction.

The rate of lipid peroxidation depends on several factors including: fatty acid composition, environmental temperature, the level of antioxidants (e.g. α-tocopherol) and pro-oxidants such as heavy metals and undissociated metal salts (AOCS, 2005). In general, unsaturated lipids are more susceptible to oxidation than saturated lipids (Shahidi, 1998). As temperature, oxygen pressure, and pro-oxidant substances such as heavy metals increase, the rate of lipid peroxidation will be accelerated significantly.

Hydroperoxides, which are produced primarily in the initial phase of lipid peroxidation, are susceptible to further oxidation/ decomposition (Enser, 2001). Their

breakage leads to the generation of many secondary lipid peroxidation products such as pentanal, hexanal, 4-hydroxynonenal and malondialdehyde (MDA) as well as other oxygenated compounds such as aldehydes, acids and ketones (Raharjo and Sofos, 1993; Shahidi, 1994; Fernindez et al., 1997). Both primary and secondary peroxidation products are responsible for various qualities of lipids and possibly, are directly related to poor performance of animals when fed oxidized lipids.

DIGESTION, ABSORPTION, AND METABOLISM OF LIPIDS

Digestion

Like other nutrients, digestion of lipids in the gastrointestinal tract primarily includes physicochemical and enzymatic activities. However, unlike other nutrients, lipids are minimally soluble in water, which requires emulsification for digestion of lipids to occur in an aqueous medium. The emulsification processes facilitate the enzymatic digestion of lipids by organizing the dietary lipids in the form of droplets in the aqueous digestive system (Carey et al., 1983; Overland et al., 1993). Lipases hydrolyze lipids primarily at the lipid-water interface (Entressangles and Desnuelle, 1968; Brockman, 1984), and as a result, lipid emulsification directly affects the lipid-water interface area, modulates the binding of lipase onto the droplet surface, and consequently influences the activity of lipid digestion (Armand et al., 1992; Borel et al., 1994).

The first step of lipid digestion starts in the stomach with the action of gastric lipase at the lipid-water interface. The major digestion products in the gastric phase are diacylglycerol and FFA (Thomsom et al., 1989), and all of these digested lipids play an important role in the intestinal phase of digestion by acting as emulsifying agents (Lieu,

1994). Clark et al. (1969) demonstrated that gastric lipase primarily catalyzes the short- and medium-chain fatty acids on the sn-3 position of triacylglycerol rather than long-chain fatty acids and medium-chain length fatty acids. Therefore, many researchers have suggested that gastric lipase for suckling animals is particularly important due to the high proportions of medium-chain fatty acids in the milk of many species including pigs (Drackley, 2000).

In human studies, the contribution of gastric lipase to the hydrolysis of lipids is about 25% (Carriere et al. 1993). Similarly, studies by Newport and Howarth (1985) and Chiang et al. (1989) found that approximately 25 to 50% of dietary lipid in newborn pigs could be hydrolyzed in the stomach into diacylglycerols, monoacylglycerols and FFA. In addition, Liu et al. (2001) demonstrated that the activity of gastric lipase developed slowly before nursing piglets reached 3 weeks of age, and the total activity of gastric lipase at day 28 was much higher than that at day 21.

The entry of lipids in the small intestine stimulates the release of the gastrointestinal hormone cholecystokinin, which stimulates the release of bile from the gall bladder into the intestinal lumen. Bile salts, mainly composed of cholic acid conjugated with taurine, are synthesized from oxidation of cholesterol in hepatocytes and are essential for lipid digestion by synergizing with pancreatic lipase during lipid digestion due to its lipid emulsion function (Gaull and Wright, 1987).

Lipid digestion is completed in the small intestine and most of the lipid digestion takes place in the proximal small intestine by pancreatic enzymes, which are synthesized and secreted from the pancreatic acinar cells (Williams et al., 2009). Pancreatic lipase is

the most important enzyme for catalyzing lipid droplets passing from the stomach into the small intestine (Lowe, 1994), and has a strong preference for acylglycerols over phospholipids, cholesterol esters, and galactolipids (Verger, 1984). Pancreatic triglyceride lipase cleaves triglyceride at the sn-1 and sn-3 position yielding 2-monoglyceride and two FFA. Dietary phospholipids are lipid derivatives of glycerol and are similar to triglycerides. Phospholipids are hydrolyzed by activated pancreatic phospholipase producing 1-lysophospholipids and FFA. Pancreatic cholesterol esters hydrolase completely hydrolyzes cholesterol esters into FFA and free cholesterol (Bauer et al., 2005). Corring et al. (1978) investigated the activity of pancreatic digestive enzyme activity in the piglet from 0 to 8 weeks of age found that activity increased as the piglet grew. A study by Liu et al. (2001) found that synthesis of pancreatic lipase is low until piglets receive nourishment by suckling, and the amount of pancreatic lipase increases greatly especially from 2 to 4 weeks of age (Figure 1.2). Similarly, Cera et al. (1990) noted that the activity of pancreatic lipase in suckling piglets increased significantly from day 2 to 35 and decreased to a minimum level 3 d post-weaning when pigs were weaned at day 21, and then increased linearly.

Absorption

After pancreatic digestion, the cleaved FFA and ionized 2-monoacyglycerides enter into the bile micelles, combine with phospholipids and cholesterol, and are synthesized into mixed micelles. Mixed micelles allow the digested lipid to cross the unstirred water layer and transport the digested lipid products to the microvillus membrane of enterocyte

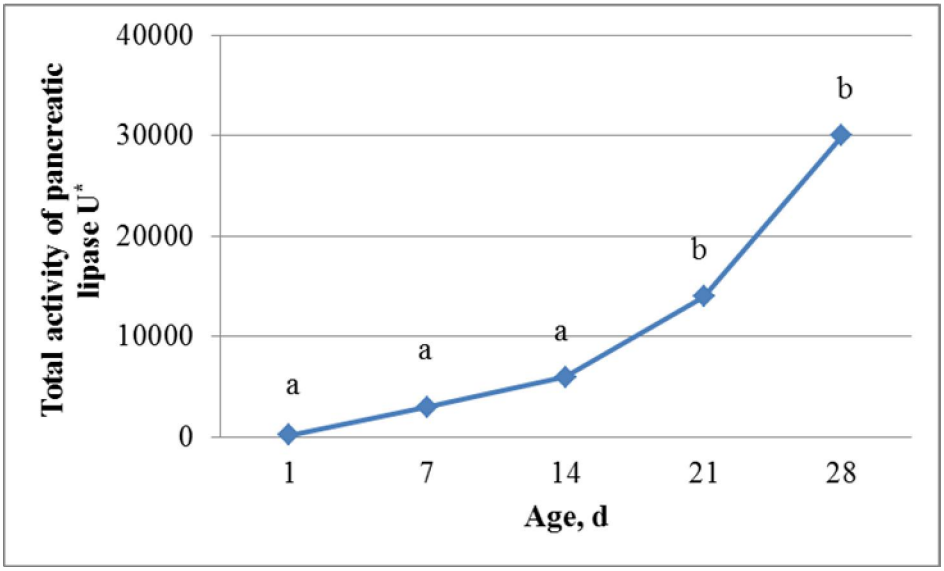

Figure 1.2. Development of pancreatic lipase activity in nursing piglets (adapted from Liu et al., 2001).
***1 U is defined as 1 µm butyric acid released from tributyrin in one min at 25°C**
[ab]Means without common letters differ ($P < 0.05$)

where the FFA, ionized 2-monoacyglyceride, 1-lysophospholipids, and ionized cholesterol are absorbed (Bauer, et al., 2005). Absorbed lipids are resynthesized to form triglycerides and phospholipids in the smooth endoplasmic reticulum of enterocytes through esterification. Triglycerides can be synthesized via the 2-monoacylglyceride or via 3-glycerol-phosphate and the 2-monoacylglyceride pathway, which is the primary route in the fed state (Hernell and Blakberg, 1982). Triglycerides, phospholipids, and cholesterol are combined with apolipoproteins to synthesize chylomicrons. Chylomicrons are the most important complex made by the intestine to transport dietary triglycerides to cells for energy use. Chylomicrons are secreted directly to the lymph circulation, and then to the general blood stream through the thoracic duct. In extrahepatic tissues, chylomicrons are cleaved by lipoprotein lipase releasing triglycerides and yielding

chylomicron remnants. These remnants interchange components with other plasma lipoproteins and finally are taken up by the liver. After absorption by the liver, some triglycerides are used directly to produce energy through lipid oxidation or ketone genesis, some are stored in the liver, and the remainder of the triglycerides are transported attached to lipoproteins, primarily in the form of very low density lipoproteins, to peripheral tissues and directly incorporated into adipose tissue. Triglycerides in adipose tissue are mainly stored energy and can later release fatty acids for lipid oxidation.

Metabolism

After digestion and absorption, most lipids are incorporated directly into adipose tissues or, to a lesser extent, oxidized to produce energy through lipid oxidation yielding ATP (Hems et al., 1975). The amount of fat stored in adipose tissue depends on the energy balance of the whole organism (Hems et al., 1975). Excess calories, regardless of whether they are in the form of lipid, carbohydrate, or protein, are all stored as lipid in the body. Consequently, appreciable amounts of adipose tissue are derived from dietary carbohydrate and some protein. Through *de novo* fatty acid synthesis from carbohydrate and protein, pigs have the ability to make saturated and monounsaturated fatty acids. *De novo* lipogenesis occurs in the cellular cytosol, and usually starts from acetyl-CoA or 3-hydroxybutyrate, and then acetyl units (malonyl-CoA) are added repeatedly to an initial starting molecule after a sequential cyclical elongation process (Drackley, 2000). Carbohydrates, proteins, or other fatty acids are used as substrates for the synthesis of acetyl-CoA both inside and outside the mitochondria (Lassiter and Edwards, 1982). In adipose tissue, the predominant product of de novo fatty acid synthesis is palmitic acid

(C16:0). Once palmitic acid is synthesized, it can be directly incorporated into acylglycerides and stored in adipose tissue or go through further processing to produce long-chain fatty acids and unsaturated fatty acids. In adipose tissue, palmitic acid can be converted into stearic acid (C18:0) in the microsomal fraction through the chain-elongation reaction. Through fatty acid desaturation, C18:0 and C16:0 can be converted into C18:1 and of C16:1 respectively (Pond et al., 1995). However, because pigs only have Δ-9 desaturase and lack a desaturase enzyme that can introduce double bonds beyond the 9th and 10th carbon in the fatty acids, the essential fatty acids including n-3 and n-6 polyunsaturated fatty acids, cannot be made by the body and must be supplied in the diet. Although the digestibility of dietary lipids varies greatly, the efficiency of using metabolizable energy for fat deposition is generally greater than 90% in pigs (Stahly, 1984). For pigs, adipose tissue is the primary anatomical site for *de novo* lipogenesis, with the liver being a less important contributor.

Energy produced from the oxidation of FFA is a primary source of energy for the body. Lipids are mobilized by hormone sensitive lipase from adipose tissue into the blood as FFA when the body needs energy fuels (Crabtree and Newsholme, 1972). The released FFA combine with serum albumins forming complexes, through which the fatty acids are distributed throughout the organism (Brody, 1999). Once serum protein and fatty acid complexes reach the target tissue, the fatty acids are released from serum albumin and cross into the cytosol. Most of the fatty acid oxidation occurs in the mitochondrial matrix where enzymes used for the fatty acid oxidation are located (Kennedy and Lehninger, 1949). Membrane transporters (carnitine palmitoyltransferase I) are required for the fatty

acids with a chain-length more than 12 carbons to cross the cell membrane into the mitochondria, where they undergo fatty acid degradation (Reddy and Hashimoto, 2001). During the β-oxidation process, two-carbon molecules (acetyl-CoA) are gradually cleaved from the fatty acid. Acetyl-CoA enters the citric acid cycle for production of NADH and $FADH_2$. Both of the NADH and $FADH_2$ are subsequently transported into the electron transport chain for the generation of ATP (Alberts et al., 2008).

Insulin is one of the most important hormones involved in regulation of lipid metabolism (Saltiel and Kahn, 2001; Koch et al., 2008). In response to an increase in blood glucose, a large amount of insulin is synthesized and secreted to increase the rate of storage pathways, including the *de novo* lipogenesis pathways. A high concentration of insulin causes the activation of the rate-limiting enzymes (acetyl-CoA carboxylase and pyruvate dehydrogenase) and increased production of acetyl-CoA and malonyl-CoA, both of which are substrates required for fatty acid synthesis (Brody, 1999). Compared to the promoting effect on lipogenesis, an increased concentration of insulin inhibits lipolysis by inactivation of hormone sensitive lipase. Insulin deactivates hormone sensitive lipase by activating protein phosphatase 2A and consequently, prevents the hydrolysis of FFA from triacylglycerides in adipose tissue (Brody, 1999). In addition to insulin, glucagon is another hormone that plays an important role in lipid metabolism (Schade et al., 1979). Generally, the secretion of glucagon is associated with low blood glucose and increases metabolic demand when energy is needed. Oxidation of fatty acids is required to meet the metabolic demand. In contrast to insulin's effects, a high

concentration of glucagon increases the release of FFA from adipose tissue by activating hormone sensitive lipase.

Factors affecting lipid digestion, absorption, and metabolism

Age of pigs

The apparent digestibility of various lipids in nursery pigs increases with age (Hamilton and McDonald, 1969; Frobish et al., 1970). Cera et al. (1988) also found that the apparent digestibility of various lipids in nursery pigs increased with age after weaning (Table 1.5), and became stabilized around 4 wk of age.

Table 1.5. Apparent total tract digestbility of different fat in pigs weaned at 21 d of age[1]

Weeks after weaning	Corn oil	Lard	Tallow
1	0.79	0.68	0.65
2	0.80	0.72	0.72
3	0.89	0.84	0.82
4	0.89	0.85	0.82

[1]Adapted from Cera et al. (1988)

Research by Mourot et al. (1995) demonstrated that the synthesis of lipogenic enzymes increases in various adipose tissues as the pig grows, with the greatest proportion of lipid deposited in adipose tissue as pigs approach the finishing phase. Specifically, lipid deposition is around 30 to 50 g per day in the nursery, while lipid deposition increases to 250 to 450 g per day in the finishing phase. In addition, their study also found that for a typical corn-soybean meal diet supplemented with 3 to 5% lipids, at least 50% of lipid accumulation is derived from *de novo* lipogenesis, which is

presumably synthesized by using glucose hydrolysis from carbohydrate as a substrate for lipogenesis.

Chain-length of fatty acids

Chain length of a fatty acid is a key factor determining fat digestion and absorption due to various characteristics of different chain lengths of fatty acids (NRC, 1998). Short-chain fatty acids and glycerol are more soluble in water than long-chain fatty acids, and as a result, they can diffuse directly into the enterocyte. The rate of digestion and absorption of fatty acids is negatively related to chain length (Braude and Newport, 1973). Lipids rich in medium-chain fatty acids are more digestible than lipids concentrated with long-chain fatty acids (Cera et al., 1989; Jorgensen et al., 1993; Overland et al., 1994; Jorgensen and Fernandez, 2000; Straarup et al., 2006). Cera et al. (1989) found that nursery pigs had higher apparent total tract digestibility of coconut oil than the digestibility of lipids from corn oil or tallow, and the apparent total tract digestibility of short chain fatty acids of was reported above 90%.

Degree of unsaturation

Since unsaturated fatty acids are easier to form micelles for absorption compared to saturated fatty acids, the level of various fatty acids and the ratio of unsaturated to saturated fatty acids are important factors in predicting lipid digestibility (Freeman et al., 1968; Stahly, 1984; Powles et al. 1995). In addition, unsaturated fatty acids may provide assistance in digestion of saturated fatty acid digestion because digestibility of saturated fatty acids is improved if unsaturated fatty acids are mixed with saturated fatty acids (Powles et al., 1993). Stahly (1984) reported the apparent digestibility of lipids in pigs is

between 70% to 80% when the unsaturated to saturated fatty acid ratio is above 1.5, but when the unsaturated to saturated fatty acids ratio less than 1.3, apparent digestibility of lipids declined significantly. However, studies by Wiseman et al. (1990) and Powles et al. (1993) demonstrated that a significant increase in lipid digestibility was associated with changes in unsaturated to saturated fatty acid ratio when it increased from 0.93 to 2.08.

Configuration of fatty acids in the lipids

The position and distribution of fatty acids in triglycerides also plays an important role in lipid digestion and absorption (Small, 1991; Bracco, 1994). Gastric and pancreatic lipases hydrolyze fatty acids from the 1- and 3-positions of dietary triacylglycerols yielding FFA and 2-monoacylglycerols (Small, 1991). Ramirez et al. (2001) investigated the absorption and distribution of dietary fatty acids from different sources and found that the 2-monoglycerides have a greater potential than FFA for micellar incorporation. Therefore, they suggested that the animal favors the digestion of fatty acids attached to the sn-2 position in triglycerides compared to fatty acids bound to sn-1 or sn-3 position.

Free fatty acid composition

Free fatty acid concentrations may also affect lipid digestibility (NRC, 1998). Free fatty acids are less water soluble than monoglycerides or diglycerides, and lipids with a high FFA concentration have a lower incorporation rate into micelles leading to a reduced absorption efficiency (Sklan, 1979; Tso et al., 1981). However, a recent study by DeRouchey et al. (2004) showed that FFA concentrations of at least 53% does not adversely affect utilization of CWG in nursery pigs, which suggests that the amount of

FFA in the lipid may play an important role in whether the FFA of a lipid will negatively affect the lipid digestibility.

Dietary ingredient composition

Lipid digestibility is also affected by dietary lipid and fiber concentration. In general, inclusion of supplemental lipids in the diet improves lipid digestibility, while the addition of fiber into the diet decreases lipid digestibility (Dierick et al., 1989; Noblet and Shi, 1993; Jorgensen et al., 2000). Results from previous studies consistently showed that lipid digestibility of pigs increased linearly with increasing inclusion of dietary lipid, and reached a plateau when inclusion rate was above 20% (Wiseman and Cole., 1987; Jorgensen and Fernandez, 2000). Increased digestibility caused by the addition lipids to the diet was possibly due to the fact that lipids assist in reducing digesta passage rate (Li and Sauer, 1994; Azain, 2001). In contrast to the effect of lipid addition to the diet, Just (1982 a,b,c) reported that for each 1 percentage unit increase in crude fiber in the diet, apparent fat digestibility decreased by 1.3 to 1.5 percent. Shi and Noblet (1993) also noted that the ileal lipid digestibility decreased as the dietary acid detergent fiber (ADF) level increased.

Inclusion of lipids in the diet increases fatty acid absorption, and consequently enhances the flux of circulating chylomicrons and very low density lipoproteins. As in other species, increased incorporation of exogenous fatty acids into adipocytes leads to a decreased rate of de novo fat synthesis in pigs (Allee et al., 1971a,b; Farnworth and Kramer, 1987), which helps explain that dietary lipid does not always result in increased lipid deposition. In nonruminant species, fatty acids from dietary lipids can be

incorporated directly into adipose tissue, and the composition of adipose tissue fatty acids is closely related to that of dietary fatty acids (Azain, 2001). Content of essential fatty acids, such as linoleic acid, in different adipose tissue sites is proportional to the consumption of essential fatty acids (Wood et al. 1986). Thus, modification of carcass lipid composition can be achieved by manipulation of dietary fatty acid composition (Wood, 1984; Wiseman and Agunbiade, 1998; Eggert et al., 2001).

FUNCTION OF DIETARY LIPIDS

Lipids are commonly added to swine diets primary to serve as a concentrated form of energy because lipids supply approximately 2.25 times more energy than carbohydrates and proteins (Azain, 2001). In addition to energy, lipids also provide other benefits including supplying fat soluble vitamins and essential fatty acids, reducing dust in facilities, attenuating growth reduction during heat stress, improving pellet quality, and improving the palatability of diets (Pettigrew and Moser, 1991). Fatty acids may be oxidized for energy, incorporated into cell membranes, utilized for synthesis of biologically active compounds, or deposited into adipose tissue to provide an energy source (Ratnayake and Galli, 2009).

Energy source

Lipids added to animal diets are primarily used as an energy source to improve gain efficiency. Lipids have high caloric density because 1 gram of lipid can supply about 9 calories of gross energy, whereas 1 gram of protein or carbohydrate can each only supply about 4 calories of gross energy (Babatunde et al., 1968; Cera et al., 1988a,b; 1989a;

1990; Li et al., 1990; Jones et al., 1992; Jorgensen et al., 2000). However, DE and ME content varies greatly among different sources and qualities of lipids (Table 1.6). Digestibility of dietary fats can be influenced by the age of animals, ratio of unsaturated-to-saturated (U:S) fatty acids in the lipids, chain length of fatty acids comprising the lipids, and FFA concentration of lipids (NRC, 1998).

Improved feed efficiency

Supplementation of lipids to diets results in increased energy density, which is expected to reduce feed intake because pigs eat for a fairly constant DE intake (NRC, 1998). The improved growth performance is probably due to the effects of dietary lipids decreasing intestinal passage rate and(or) improving digestibility of other nutrients, which leads to an increase in availability of energy and other nutrients (Pettigrew and Moser, 1991).

Pettigrew and Moser (1991) analyzed data from 92 studies evaluating the effects of dietary fat on performance and carcass characteristics of pigs weighing from 20 to 100 kg (Table 1.7). They found that addition of lipids to swine diets increased ADG, reduced ADFI, and improved feed conversion in 78.2%, 81.5%, and 94.5% of the total observations, respectively. Azain (2004) suggested that the negative effect of reduced feed intake caused by increased dietary fat could be offset as the calorie:protein ratio was adjusted, and the extra-caloric and extra-metabolic effects of dietary lipids contribute to greater efficiency of digestion and energy retention, which most likely account for increased growth performance and carcass fat. Pettigrew and Moser (1991) concluded

Table 1.6. Energy values of various sources of lipids on an as-fed basis[1]

	Energy Content, kcal/kg		
Type of Lipid	DE[2]	ME[3]	NE[4]
Animal Fats			
Beef tallow	8,000	7,680	4,925
Choice white grease	8,290	7,955	5,095
Lard	8,285	7,950	5,100
Poultry fat	8,520	8,180	5,230
Restaurant grease	8,550	8,205	5,245
Vegetable Oils			
Canola (Rapeseed)	8,760	8,410	5,365
Coconut	8,405	8,070	5,160
Corn	8,755	8,405	5,360
Cottonseed	8,605	8,260	5,275
Olive	8,750	8,400	5,360
Palm	8,010	7,690	4,935
Peanut	8,735	8,385	5,350
Safflower	8,760	8,410	5,365
Sesame	8,750	8,400	5,360
Soybean	8,750	8,400	5,360
Sunflower	8,760	8,410	5,365
Fish Oils			
Anchovy	8,445	8,105	5,185
Herring	8,680	8,330	5,320
Menhaden	8,475	8,135	5,200

[1]Adapted from NRC (1998).
[2]Calculated by the following relationship (Powles et al. 1995): DE (kcal/kg) = (36.898 – (0.005 × FFA) – (7.330 × $e^{-0.906 \times U:S}$))/4.184 where FFA is the FFA content in g/kg and U:S is the ratio of unsaturated-to-saturated fatty acids. In calculating the DE, the FFA concentrations of all fats were assumed to be 50 g/kg (or 5%).
[3]Calculated as 96% of DE.
[4]Calculated from the equation (Ewan, 1989): NE = 328 + (0.599 × ME) – (15 × % Ash) – (30 × % ADF), $R^2 = 0.81$.

that the increase in carcass fat was independent of whether the calorie to protein ratio in the diet was maintained.

A common 'rule of thumb' is that feed conversion efficiency will improve approximately 2% for each 1% increase in lipid added to swine diets (Campbell, 2005). However, in practical production, the improvement in efficiency of growth must compensate for the likely increased dietary cost caused by addition of lipids. Therefore, the cost of lipid addition relative to expected economic value of improved growth performance must be considered for profitable pig production (Baudon et al., 2003; Collins et al., 2009).

Table 1.7. Summary of effects of supplemental dietary fat on performance and backfat depth in growing-finishing pig diets[1]

	No. of Responses			Total Comparisons
Category	Positive	Negative	No Response	
ADG, kg	72	10	10	92
No adjustment[2]	39	4	7	50
Constant protein:energy ratio	33	6	3	42
ADFI, kg	15	75	2	92
No adjustment	7	41	2	50
Constant protein:energy ratio	8	34	0	42
Gain: feed ratio	87	0	5	92
No adjustment	47	0	3	50
Constant protein:energy ratio	40	0	2	42
Average backfat, cm	61	16	13	90
No adjustment	32	11	7	50
Constant protein:energy ratio	29	5	6	40

[1]Adapted from Pettigrew and Moser (1991).
[2]No adjustment means that diets supplemented with lipids were formulated without keeping the essential amino acid to ME ratio constant based on NRC (1998) recommendations.

Decreased Carcass Leanness

Carcass leanness, commonly measured as backfat thickness, is an important characteristic for optimizing economic returns from any feeding strategy (Collins et al., 2009). Pettigrew and Moser (1991) analyzed data involving 92 studies where supplemental fat was added to growing-finishing (20- to 100- kg) pig diets (Table 1.7) and found that the backfat thickness increased with the addition of supplemental fat in 68% of the total experiments. Several experiments evaluating the addition of dietary lipids on pork fatness in grow-finish swine have been published since the Pettigrew and Moser (1991) review. A summary of 11 studies conducted after this review and published in the Journal of Animal Science are summarized in Table 1.8. For backfat thickness, only 27% of the experiments showed that addition of lipid (> 5%) to grower-finisher diets increased backfat thickness, which is contrast to the 68% of the studies summarized by Pettigrew and Moser (1991). Campbell (2005) suggested that the addition of fat to finisher diets in the USA has little impact on backfat thickness which is likely due to the use of improved lean genotypes of pigs. However, the type of fat, dietary protein and energy density, stress management, and environmental conditions may also play important roles in fat metabolism, and thus influence carcass backfat depth. In addition, differential effects of supplemental lipids on backfat thickness may also be influenced by the timing of feeding during the growing and finishing phases.

Table 1.8. Summary of effects of supplemental dietary fat on backfat depth in growing-finishing pigs[1].

Source	Backfat depth[1]
Azain et al., 1991	NS
Azain et al., 1992	NS
Myer et al., 1992	NS
Williams et al., 1994	NS
Smith et al., 1999	NS
De la Llata et al., 2001	NS
Engel et al., 2001	NS
Van de Ligt et al., 2002	+
Gatlin et al., 2002a	NS
Apple et al., 2004	+
Apple et al., 2009	+

[1]+ = an increase in backfat; NS = no significant difference.

Modifying Carcass Lipid Composition

Unlike ruminants, where dietary fatty acids are saturated in the rumen before absorption in the small intestine, fatty acids from the diet can be directly incorporated into adipose tissue in nonruminant species (Azain, 2001). Lipids in adipose tissue either originate from *de novo* fatty acid synthesis from dietary carbohydrate and lipids, or are made up by directly incorporating dietary lipids into adipocytes. With the long history of genetic selection for leanness, increased leanness of pigs has gradually decreased *de novo* lipogenesis causing dietary fatty acid composition to play an increasingly important role in determining fatty acid composition of carcass fat. In addition, because an increasing amount of dietary lipid decreases *de novo* synthesis of fatty acids (Farnworth and Kramer, 1987; Chilliard, 1993), pork fat composition will be largely influenced by contribution of dietary fat, particularly for the essential unsaturated fatty acids which

cannot be synthesized through *de novo* lipogenesis. In pigs, modification of carcass lipid composition can be achieved by manipulation of dietary triacylglycerol composition (Wood, 1984; Wiseman and Agunbiade, 1998; Eggert et al., 2001). Koch et al. (1968) investigated the effect of changing dietary fat source from unsaturated oil (safflower oil) to saturated fat (tallow) on fatty acid composition of pork fat. These researchers fed diets containing 10% safflower oil for 5 weeks, then switched to the diets containing 10% tallow, and alternated diets at each 2-week period from the 5^{th} to 11^{th} week. Results from this study suggested that the major change in the fatty acid composition of pork fat resulted from the alteration of the dietary lipid source that occurred during the first 4 weeks after the diet change. Wood et al. (1994) found that the bulk of the change in fatty acid composition of adipose tissue took place within 25 d after a dietary change. Warnants et al. (1999) investigated the incorporation and elimination rate of dietary PUFA in pork fat and found: 1) the incorporation and elimination rate of PUFA such as C18:2 were similar in backfat, and the increase or decrease in C18:2 concentration reached a plateau at 6 weeks following a dietary change-over (Figure 1.3); 2) the greatest changes (approximately 50% of changes in C18:2 and C18:1) in fatty acid composition occurred during the first 2 wk after the diet change (Figure 1.3 and Figure 1.4); and 3) the change in C18:2 appeared in a converse pattern compared to C18:1 and deposition of C18:2 of backfat was primarily at the expense of C18:1. A recent study by Xu et al. (2010) noted that the iodine value (**IV**) of belly fat was reduced 5% within 21 d after DDGS had been removed from diets for finishing pigs. In contrast to the studies in which major alterations in fatty acid composition occurred during in the first 2 to 4 weeks

following a dietary change, Gatlin et al. (2002a) found that the greatest rate of change for C18:2 concentration (approximately 2% per wk) in backfat was from 6 to 8 wk after the dietary concentration of C18:2 was switched to a lower level.

Modification of carcass lipid composition can be achieved by adjusting dietary triacylglycerol composition (Wood, 1984; Wiseman and Agunbiade, 1998; Eggert et al., 2001). Fatty acid composition of triglycerides dictates the physical and chemical characteristics of lipid, such as melting point, lipid firmness, lipid color, and rate of lipid oxidation. As a result, manipulation of dietary fatty acid composition by adding various sources of lipids will affect firmness of pork fat, color of fat and meat, flavor of pork products, and rate of pork spoilage (Dave and Ghaly, 2011).

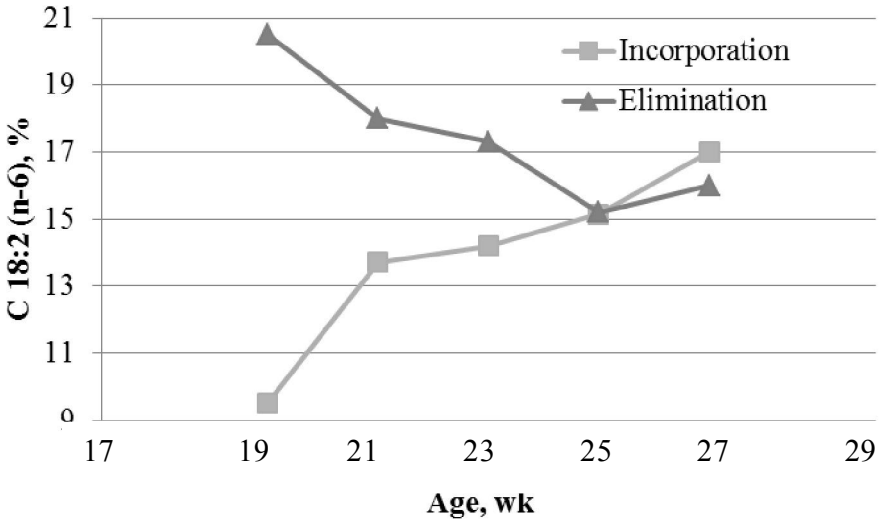

Figure 1.3. Incorporation and elimination rates of C18:2 (linoleic acid) from backfat biopsy samples (Warnants et al., 1999)

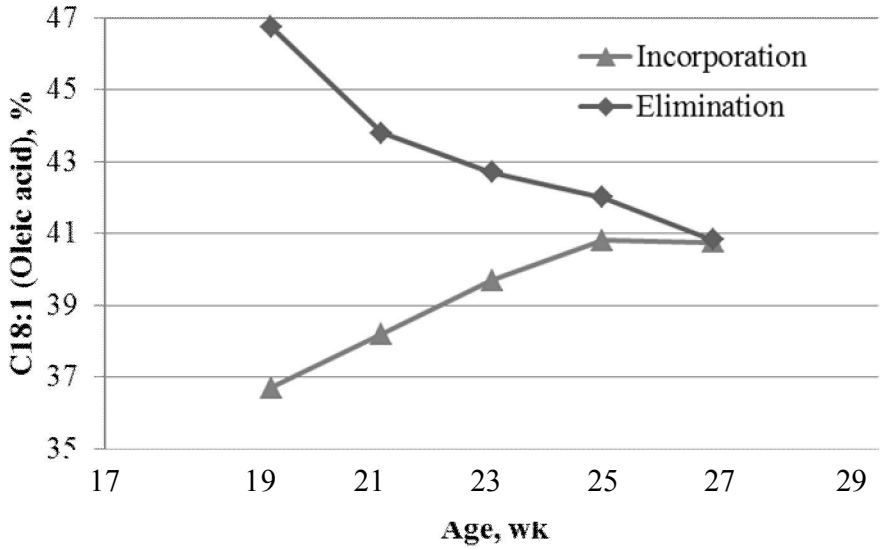

Figure 1.4. Incorporation and elimination rates of C18:1 (oleic acid) from backfat biopsy samples (Warnants et al., 1999).

Pork carcass fat firmness

Pork carcass fat firmness has received increasing attention during the past few years as feed prices have increased, resulting in least-cost formulated diets using ingredients that may compromise fat quality (Stein and Shurson, 2009). Fat firmness is one of the main contributors influencing pork fat quality (National Pork Producers Council, 2000). In general, soft fat is an undesirable property for further processing and affects the ability of pork products to meet product specifications which leads to a lower price (Irie, 1999). Soft fat reduces product "workability" and causes many problems including: 1) difficult fabrication, particular for producing bacon by slicing of bellies, 2) an oily/wet appearance which may often be transparent, and 3) reduced product shelf life, or increased susceptibility to oxidative damage (Enser et al., 1984; Wood and Enser, 1997; NPPC, 2000; Carr et al., 2005).

Iodine value (IV), is the most commonly used measurement of the degree unsaturation of lipid and has a high negative association with the pork fat firmness. Previous studies consistently found that pork fat softness tends to be increased with increasing content of unsaturated fatty acids or increasing IV of fat (Whittington et al., 1986; Nishioka and Irie., 2006). Whittington et al. (1986) investigated the relationship between fat softness and fatty acid concentrations and suggested C18:2 and C18:0 content were important factors in predicting pork fat firmness. In addition, results from numerous studies have shown that a significant increase of polyunsaturated fatty acid (e.g., C18:2) content of pork fat was observed in pigs fed diets supplemented with lipids containing high concentrations of polyunsaturated fatty acids, such as corn oil, soybean oil, safflower oil, and sunflower seed oil rich in C18:2. Wiseman et al. (1992) suggested that about 25 to 30 d are required to observe a shift in fatty acid profile of carcass fat. Skelley et al. (1975) found that feeding pigs diets containing 0 to 30% of full-fat soybeans resulted in backfat IV increasing from 61 to 69, C18:2 content of backfat increasing from 11.2 to 21.3%, and fat firmness score decreasing from 3.5 to 2.9 on a 5-point scale (lower value = softer). As the inclusion rate of sunflower seed increased from 0% to 20%, the linoleic acid (C18:2) content of belly fat increased from 15.8% to 33.3% and the IV increased from 56.9 to 71.6 (Hartman et al., 1985). Leszczynski et al. (1992a,b) also found that feeding diets containing increasing levels of full-fat soybeans to pigs for 6 weeks increased C18:2 content in loin and bacon. Gatlin et al. (2002a) investigated the effect of feeding 5% blended lipids containing different ratios of soy oil and tallow (1:0, 3:1, 1:1, 3:2, and 0:1) to growing-finishing pigs on pork fat quality and

found that after an 8-wk feeding program, as the proportion of soy oil increased in the diet the backfat C18:2 content and IV significantly increased, which resulted in a linear decrease in fat firmness. On the other hand, reduced pork carcass firmness is less noticeable when feeding pigs lipids containing reduced amounts of polyunsaturated fatty acids such as tallow, choice white grease, and poultry fat. Leszczynski et al. (1992b) found pigs fed diets containing 4% tallow for 6 weeks had similar levels of C18:2, SFA, and UFA in loin fat compared to loins from pigs fed a corn-soybean control diet without additional fat. In agreement with earlier findings, Engel et al. (2001) found that the inclusion of choice white grease or poultry fat up to 6% in growing-finishing diets slightly increased C18:2 content in loin fat, but the C18:2 content in belly fat was not different among various treatments. Firmness of 10th rib backfat and the belly, measured by an Instron tester were also not affected by dietary fat source (choice white grease or poultry fat) and level (Engel et al., 2001). In summary, results from these studies have shown that compared to vegetables oils with high degree of unsaturated fatty acids, dietary inclusion of animal fats are less of a concern for causing soft pork fat.

Pork fat and meat color

Color is one of the most important factors determining the appearance and attractiveness of meat to consumers (Tikk et al., 2006), and an opaque white color of pork fat is most preferred by consumers (Maw et al., 2003). The major fatty acids, including C16:0, C16:1, C18:2 and C18:3 that comprise most of the fatty acids in pork fat are colorless, however, pork fat color is associated with fatty acid composition of pork adipose tissue as the amount of fat soluble pigment changes as the fatty acid composition

varies (O'Connor, 1960; Cameron and Enser, 1991; Maw et al., 2003). Lipids with a high level of unsaturated fatty acids (e.g., C18:2 and C18:3) have a low melting point (O'Connor, 1960), and the low melting point allows such fat to contain more fat-soluble pigments (carotenoids). Therefore, pork fat with higher percentages of polyunsaturated fatty acids, such as C18:2 and C18:3, will have increased yellow color due to the higher concentration of fat-soluble pigments (carotenoids). Cameron and Enser (1991) suggested that increasing polyunsaturated fatty acids, such as C18:2, in the pork fat contributed to an increased fat coloration. Maw et al. (2003) confirmed this finding and also observed that fat color was associated with fatty acid composition of pork adipose tissue. As concentration of C18:2 and C18:3 increased in the fat, the color of backfat increased from white to yellow, and the increased amount of C18:2 and C18:3 was at the expense of decreased C16:0, C16:1, and C18:1 concentration. However, the study by Gatlin et al. (2002a) failed to find fat color differences among carcasses from pigs fed with various mixtures of lipids resulting in a difference in C18:2 content and IV of backfat.

The National Pork Producers Council (2000) reported that most consumers preferred a bright, reddish-pink color in fresh pork. Besides affecting the fat color, fatty acid composition of the intramuscular fats play an important role in influencing meat color when measuring meat color by reflectance (Karlsson et al., 1993). Skibsted et al. (1998) reported that as concentration of unsaturated fatty acids increase in the meat (lean tissue and adipose tissue), the likelihood of meat discoloration increased. Unsaturated fatty acids are more susceptible to lipid oxidation producing hydroperoxides and many secondary reaction lipid oxidation products (e.g. pentanal, hexanal, 4-hydroxynonenal

and malondialdehyde) and consequently, cause discoloration of the pigment in meat (Simitzis and Deligeorgis, 2010).

Flavor of meat products

The consumer's decision to purchase red meat, including pork, is generally determined by meat flavor (Theunissen 1979). Genetics and environment determine meat flavor. Animal species is the most important genetic aspect of meat flavor, and diet composition is the most important environmental factor (Shahidi and Rubin, 1986). The products from Maillard reactions during cooking and the degradation of lipids are primarily responsible for different meat flavors (Shahidi and Wanasundara, 2002). In addition, variations in fatty acid composition contribute to the various characteristic flavors of meat from different animal species (Mottram, 1998; Wood et al., 2004). To date, there is disagreement regarding the effect of dietary lipids on the meat flavors. Different observations reported in various studies related to the effects of dietary lipid on meat flavors can be explained by difference in dietary fatty acid composition and level of lipid, as well as the length of feeding period.

Polyunsaturated fatty acids are more susceptible to lipid peroxidation compared to saturated fatty acids, with monounsaturated fatty acids being the intermediate (AOCS, 2005). Therefore, increasing the proportion of unsaturated fatty acids in pork allows the production of more volatile aldehydes (Mottram, 1998; Wood et al., 2004), and higher yield of volatile lipid peroxidation products can increase off-flavors and consequently, reduce flavor intensity in pork rich in unsaturated fatty acids (Wood et al., 1999).

Many studies have been conducted to evaluate the effect of dietary lipids on pork flavor. St. John et al. (1987) evaluated the effect of feeding canola oil (high in C18:1) on various characteristics of pork and found an increased content of C18:1, and C18:2 at the expense of C18:0, C16:0 and C14:0 concentrations in adipose tissue, but no differences in flavor, juiciness, and overall tenderness were not noted. West and Myer (1987) reported that feeding peanuts instead of corn diets increased the unsaturation of backfat as indicated by the increased C18:1 and C18:2 and decreased C16:0 and 18:0, but the increased unsaturation had no effect on pork flavor. Similarly, Hartman et al. (1985) reported that substituting sunflower seed for soybean meal and corn increased the unsaturation of pork backfat, but had no adverse effect on pork flavor. Several other studies also found that feeding pigs different dietary source of lipids changed fatty acid composition of adipose tissue but not the pork flavor (Leszczynsk et al., 1992a,b; Sheard et al., 2000; Corino et al., 2002; and Teye et al., 2006a).

In contrast to studies in which fatty acid composition of adipose tissue was changed but flavor was not, many researchers have found that both pork fatty acid composition and flavor are influenced by dietary lipid source. Coxon et al (1986) found that pigs fed diets containing fish meal and(or) marine oil had increased levels of long-chain polyunsaturated fatty acids in pork, and the degree of off-flavor increased as the dietary fishmeal or fish oil increased. They also suggested that the minimum concentration of a total long chain n-3 PUFA (C20:4, C20:5, C22:5; C22:6) that caused detectable off-flavor is 0.5%. Castell and Cliplef (1988b) found an increased incidence of off-flavor in cooked pork as pigs were fed diets containing more than 13.5% of raw full-fat soybeans. Miller

et al. (1990) compared effects of adding 10% of different sources of lipids [animal fat (45.3% C18:1), safflower oil (72% C18:1), sunflower (80.9% C18:1), canola oil (50.7% C18:1)] in diets on various characteristics of pork in grow-finish pigs and found that bacon and loin samples from pigs fed canola oil containing diets had a reduced palatability score and increased off-flavor score as result of the a higher level of C18:3 in the bacon and loin. The higher level of C18:3 may explain the lower off-flavor score because polyunsaturated fatty acids are more prone to peroxidation and produce lipid peroxidation products that contribute to off-flavors. Similarly, Romans et al. (1995a,b), Leskanich et al. (1997), and Bryhni et al. (2002) found increasing rancid odors in pork as the long chain n-3 PUFA concentration increased due to higher dietary concentrations of polyunsaturated fatty acids.

Shelf life of meat products

A large quantity of meat is wasted annually at the consumer, retailer and food service levels which have a substantial economic and environmental impact (Kantor et al., 1997; Heller and Keolian, 2003). Many factors, including pre-harvest handling of livestock, post-harvest handling of meat, microbial spoilage, and autolytic enzymatic decomposition, are responsible for meat spoilage, but lipid peroxidation also plays an important part in deterioration of meat quality (Shahidi, 1998; Skibsted et al., 1998; Miller, 2002; Jay et al., 2005). After harvesting of animals, autoxidation of lipids in animal tissue starts to occur almost instantly after the blood circulation stops and metabolic processes are blocked (Gray and Pearson, 1994; Kohen and Nyska, 2002; Linares et al., 2007). Autoxidation of lipids and the production of free radicals and other

lipid peroxidation products are natural processes which affect fatty acids and contribute to oxidative deterioration of meat and off-flavor development, which shortens shelf-life (Gray, 1978; Pearson et al., 1983; Simitzis and Deligeorgis, 2010).

Shelf-life of pork, as commonly found with other meats, is primarily determined by the persistence of the bright red color of oxymyoglobin on the meat surface. This color changes from red to brown as the product ages, due to the appearance of metmyoglobin. Production of metmyoglobin can be accelerated by several factors, including free radicals and other lipid peroxidation products derived from peroxidation of unsaturated fatty acids. The level of the antioxidant vitamin E (α-tocopherol) and prooxidants, such as the presence of free iron in muscles, play a role in determining the rate of peroxidation of lipids in meat, but fatty acid composition is the key factor influencing lipid peroxidation in meat (Yamauchi et al. 1980, 1982; Skibsted et al., 1998). The susceptibility of fatty acids to peroxidation depends mainly on the degree of unsaturation. Shahidi (1998) found that the susceptibility of 18 carbon fatty acids to lipid peroxidation (from less reactive to strongly sensitive) were: C18:0 < C18:1 < C18:2 < C18:3. Hydroperoxides produced from lipid peroxidation are susceptible to further decomposition (Simitzis and Deligeorgis, 2010), which leads to the production of secondary lipid peroxidation products under the catalysis of transition metal ions and heme compounds in the meat (Fernindez et al., 1997; Shahidi, 1994; Raharjo and Sofos, 1993; Skibsted et al., 1998). These reactive secondary peroxidation products are the key compounds that not only cause undesirable rancid flavors, but also result in the loss of color and nutritive value of meat due to several effects on lipids, pigments, proteins, carbohydrates, and vitamins

(Simitzis and Deligeorgis, 2010). Therefore, meat products from pigs that consumed high amounts of polyunsaturated fatty acids are more likely to undergo oxidative damage. Romans et al. (1995 a,b) investigated the effect of feeding various levels (0 to 15%) of linseed and feeding duration (1-4 wk) on various characteristics of pork. They reported that bacon from pigs fed 10 or 15%, but not those fed 5% linseed, had obvious defects in sensory quality. Similarly, Leskanich et al. (1997) found that compared to pigs fed diets containing 3% tallow:soybean oil (4:1), meat from pigs fed diets containing 3% rapeseed oil:fish oil (2:1) had an increased unsaturated fatty acid content, an increase in odor, and a higher thiobarbituric acid reactive substances (**TBARS**, a measure of the potential for oxidative damage) value. In contrast, Sheard et al. (2000) compared effects of feeding pigs a linseed-rich diet with a control diet on sensory qualities and oxidative stability of pork chops, liver, bacon and sausages. These researchers found a significant increase of C18:3 and PUFA in these meat products, but no differences in sensory qualities and oxidative stability of pork chops, bacon, and sausage were observed.

Reduced heat stress

Heat stress is a major concern in pork production during the hot summer months. To improve heat loss and minimize heat production during heat stress, typical responses of pigs include reducing feed intake, increasing respiration rate and water consumption, and decreasing activity, which impair growth and reproductive performance (Neil et al., 1996; Spencer et al., 2003). Reduced feed consumption is caused by increased heat production due to digestion and absorption known as heat increment, and is the primary reason for reduced growth rate. The utilization of lipids as energy ingredients results in

a reduced heat increment compared to other energy sources (carbohydrates and proteins). So addition of dietary lipids creates less of a heat burden on pigs exposed to heat stress (Azain, 2001). Results from several studies have consistently shown that feeding diets supplemented with a high concentration of lipids to pigs exposed to heat stress improves growth performance compared with pigs fed diets without supplemental lipids (Stahly and Cromwell, 1979; Coffey et al., 1982; Neil et al., 1996; Spencer et al., 2003). Thus, one of the important nutritional practices to minimize the negative effects of heat stress on feed consumption is to supplement swine diets with lipids while maintaining a constant ratio of other nutrients to the dietary energy content.

Improved air quality

Dust in swine confinement facilities contributes to the rapid deterioration of buildings and equipment (Bundy et al., 1974). Ventilation ducts, fan motors, thermostats, timers and other accessories may become less reliable or malfunction and have a shortened life span due to dust-induced deterioration (Bundy and Hazen, 1975). Doig and Willoughby (1971) demonstrated that swine facilities with high ammonia and dust concentrations lead to an increase in both the incidence and severity of pneumonia in pigs. Furthermore, swine facility dust has been associated with increased incidence of acute or chronic malfunction of the respiratory tract producing various symptoms in humans (Cermdk and Ross, 1978). Dust in confinement swine housing consists of animal hair, skin, dried feces, and feed, with most dust derived from feed (Curtis et al., 1975 a,b; Honey and McQuitty, 1979). Lipids have the ability to bind minute particles together, and therefore, the addition of lipids to swine diets plays an important role in reducing swine

dust in swine facilities. This reduction in dust has major advantages for health of both pigs and people.

Chiba et al. (1985) investigated the effect of dietary lipid on dust levels in swine buildings as well as the integrity of the respiratory system of swine. They reported that addition of 2.5% animal fat to a growing-finishing swine diet resulted in a 21% reduction of aerial dust in swine confinement buildings, and the addition of 5.0% dietary fat led to a 50% reduction in dust. Furthermore, reduced dust concentration and decreased irritating gas resulting from dietary addition reduced lung lesion in pigs compared with control pigs (Chiba et al. 1985). Gore et al. (1986) demonstrated that adding 5% soybean oil to the diets of nursery pigs reduced settled dust for feeds by approximately 45%. In the same study, these researchers also found that the total aerial bacterial colony counts in the building were lower when pigs were fed with the oil-based feed. Similarly, Chiba et al. (1987) found that addition of 7% tallow to the diet not only improved pig performance and reduced aerial and settled dust concentrations, but also decreased aerial ammonia concentrations and the numbers of bacterial colony forming particles (60 and 75%, respectively) in the building. Mankell et al. (1995) investigated the effect of adding soybean oil to the diet on dust generation from swine feed and found that the total dust concentrations generated from swine feeds was markedly reduced when 1% soybean oil was added, and was further reduced by adding 3% soybean oil. However, another study by Welford et al. (1992) found that addition of oil to the feed did not result in a statistically significant decrease in airborne dust concentrations, which was possibly due to the different physical characteristics of the feeds. Feeds made from low-bulk density

corn can generate more total dust than those made from normal-bulk density corn and may require the addition of lipids for dust control.

Carriers of fat soluble vitamins

Vitamins are organic compounds that are different from amino acids, carbohydrates, and lipids, and are required in relatively small amounts for supporting life. Vitamins are generally classified as either fat-soluble or water-soluble. The fat soluble vitamins include vitamins A, D, E, and K.

Vitamin A is a general term describing several forms of retinoids, and among all isomers of vitamin A, retinol, retinal, and retinoic acid are the most prevalent forms found in the body (Johnson and Russell, 2010). Vitamin A is essential for the retina of the eye to maintain the normal function of vision (Ross, 2006). In addition, vitamin A functions in very different roles including the maintenance of reproduction, the growth and maintenance of differentiated epithelia, and routine of mucus secretions (Ross, 2006). The negative effects of vitamin A deficiency in swine including reduced weight gain, incoordination, posterior paralysis, blindness, increased cerebrospinal fluid pressure, decreased plasma levels, and reduced liver storage (NCR, 1998). Rough hair coat, scaly skin, sensitivity to touch, bleeding from cracks which appear in the skin above the hooves, blood in urine and feces, loss of control of the legs accompanied by an inability to rise and periodic tremors are the gross toxicity signs of excessive consumption of vitamin A in pigs (Anderson et al., 1966).

Vitamin D is a group of fat-soluble secosteroids. The two major forms of vitamin D are ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3), which are formed in

plants and in the skin of animals, respectively (NRC, 1998). Vitamin D and its hormonal metabolites, together with parathyroid hormone and calcitonin, play essential roles in promoting formation of bone and maintaining calcium and phosphorus homeostasis (Pittas et al., 2010). Thus, animals experience poor absorption and metabolism of calcium and phosphorus yielding insufficient bone calcification as a result of vitamin D deficiency. The typically sign of vitamin D deficiency in young pigs is rickets, whereas osteomalacia (diminished bone mineral content) are commonly observed in mature swine with a vitamin D deficiency. Ingestion of a high level of vitamin D (a daily oral dose of 6.25 μg) leads to toxicity in young pigs as indicated by reduced feed intake, growth rate, feed efficiency, and calcification of the aorta, heart, kidneys and lungs (Quaterman et al.,1964; Hancock et al., 1986). Furthermore, a higher daily administration of vitamin D_3 (11.825 ug) even can be lethal for young pigs (Long, 1984).

The main function of vitamin E is to act as an antioxidant of lipids, protecting cell membranes and preventing damage to membrane associated enzymes, and inclusion of high levels of vitamin E in the diet helps to improve the immune response (Peplowski et al., 1980; Wuryastuti et al., 1993; Kolb and Seehawer, 1998). There are eight isomers of vitamin E in nature including: α, β, γ, and δ tocopherols (Stern et al., 1947) and α, β, γ, and δ tocotrienols (Whittle et al., 1966). Tocopherols found in green plants and seeds can serve as sources of vitamin E in feed. A wide range of pathological conditions has been associated with vitamin E deficiency including skeletal and cardiac muscle degeneration, gastric ulcers, anemia, liver necrosis and sudden death (NRC, 1998). Furthermore, deficiency of vitamin E may play a role in causing mastitis-metritis-agalactia complex in

sows (Ullrey, 1981; Whitehair et a., 1984). No vitamin E toxicity have been reported in swine (NRC, 1998) and no toxicity was noted in growing pigs fed diets added of 550 mg/kg vitamin E (Bonnette et al., 1990).

The three basic forms of Vitamin K are phylloquinones (K_1), menaquinones (K_2), and menadiones (K_3). The K_1 are produced in plants; the K_2 are formed by microbial fermentation; and the K_3 are synthetic. But all three forms of vitamin K are biologically active (NRC, 1998). Vitamin K is essential for post-translational modification of certain proteins required for the normal coagulation of blood (Suttie, 1980). In addition, results from some studies suggest that vitamin K is also needed for maintaining calcium metabolism by forming some specific vitamin K dependent proteins and peptides (Suttie 1980; Kormann and Weiser, 1984). A dietary lack of vitamin K increases prothrombin and clotting times and can contribute to internal hemorrhage and death (NRC 1998). However, animals have the ability to tolerate high amounts of menadione (1000 times of animal's requirement), and no signs of toxicity have been noted (NRC, 1998).

Because of the important biological and chemical activities of fat soluble vitamins in supporting essential metabolism, and because pigs cannot synthesize sufficient quantities of these vitamins from other feed components, metabolic constituents, or by microorganisms in the intestine, all of these the fat soluble vitamins are required to be supplemented in the diet. Furthermore, the absorption of all of these fat soluble vitamins involves solubilization in bile salts, incorporation into micelles, and absorption by passive diffusion along with uptake of dietary lipids (Parker, 1996; Cohn, 1997). Thus, the presence of dietary lipids is thought to be important in affecting absorption of fat

soluble vitamins. Little information regarding the impact of dietary fat on fat soluble vitamin absorption in pigs is available. Dimitrov et al. (1991) reported that dietary fat enhances the absorption of vitamin E in humans. Similarly, Roodenburg et al. (2000) investigated the effect of dietary fat on the bioavailability of vitamin E and carotenoids and found that a limited amount of lipids are essential for optimal uptake of vitamin E and carotenes.

Many vitamins, including vitamin A, D, and E, are sensitive substances that can suffer loss of activity due to conditions encountered during processing or storage of premixes and feed. One of the important stress factors that negatively influence the biological activity of vitamins is rancid fat (NRC, 1998). The loss of biological activity of fat soluble vitamins caused by lipid peroxidation has been reported (Seppanen and Csallany, 2002; Adam et al., 2007). Furthermore, peroxidized lipids may also lead to a poor absorption of tocopherols (Liu and Huang, 1995).

Source of the essential fatty acids

Because mammals, including pigs, lack the enzymes to synthesize fatty acids with double bonds at the n-3 or n-6 position, some specific unsaturated fatty acids, have been generally regarded as essential fatty acids which are required by pigs and must be obtained through the diet (Simopoulos, 2008). Typically, the essential fatty acids include linoleic acid (C18:2) and arachidonic acid (C20:4), members of n-6 family, and linolenic acid (C18:3), an n-3 fatty acid. In addition to acting as an energy source, essential fatty acids serve as substrates for the production of polyunsaturated fatty acids used in cellular structures and as precursors for the production of regulatory substances such as

glycerolipids, long chain polyunsaturates and eicosanoids. The lack of alpha-linolenic acid contributes to the development of neurological abnormalities (Horrobin, 1998) and poor growth (Connor, 1999) in humans. A lack of linolenic acid has been associated with scaly dermatitis and poor growth (Innis, 1991). The NRC (1998) recommended 0.10% of linoleic acid (C18:2) in swine diets, which is approximately 0.5 g per day for nursery pigs and 3 g per day for finishing pigs. However, no specific recommendations for dietary concentrations of n-3 fatty acids have been established. In practical animal production, even though essential fatty acids are necessary for maintaining the normal function of the animal's body, the most important role of lipid in swine diets is as a concentrated energy source. As a result of the essential fatty acid composition in grains and grain by-products, there is little concern for poor animal performance or health caused by a deficiency of essential fatty acids when pigs are fed grain-based diets (NRC, 1998, Azain, 2001).

Conjugated linoleic acid

Conjugated linoleic acid (CLA) refers to a group of positional and geometric isomers of linoleic acid (Pettigrew and Esnaola, 2001). The main isomers in the synthetic form of CLA are the cis-9, trans-11 and trans-10, cis-12 versions which have been studied extensively because of their beneficial functions for improving growth performance, pork quality and immunity (Bassaganya-Riera et al., 2001; Thiel-Cooper et al., 2001; Weber et al., 2001).

Effect of CLA on growth performance

Dugan et al. (2004) analyzed data from 16 studies evaluating the effects of dietary CLA on performance and carcass characteristics in pigs (Table 1.9) and found that the addition of CLA in swine diets improved feed conversion in 44% of the total observations and the average improvement in feed conversion was about 6.5%. The variation in feed conversion efficiency might be explained by different CLA isomers and level in the diet as well as the duration of the feeding period.

Table 1.9. Summary of effects of dietary CLA on performance and carcass composition in pigs[1]

Reference	Feed efficiency	Reduction of carcass fat	Increases of carcass lean
Demaree et al., 2002	-	ND	ND
D'Souza and Mullan, 2002	-	+	ND
Dugan et al., 1997	+	+	+
Dugan et al., 2001	-	+	+
Dunshea et al., 2002	+	+	-
Eggert et al., 2001	-	ND	ND
Gatlin et al., 2002b	-	ND	ND
O'Quinn et al., 2000	-	+	+
Ostrowska et al., 1999	+	+	+
Ostrowska et al., 2003	+	+	+
Ramsay et al., 2001	-	ND	ND
Swan et al., 2001	-	-	+
Thiel-Cooper et al., 2001	+	+	+
Tischendorf et al., 2002	-	+	+
Wiegand et al., 2001	+	+	ND
Wiegand et al., 2002	+	+	+

[1]Adapted from Dugan et al. (2004).
+ = positive effect; NS = no significant difference; ND = no data reported.

Effects of CLA on carcass composition

Addition of CLA to swine diets improves leanness, which might be related to the inhibiting effect of CLA on lipogenesis (Heckart et al., 1999). Dugan et al. (2004) found that dietary CLA reduced the percentage of carcass fat in 11 of 12 studies reviewed

(Table 1.9). Furthermore, 9 of 10 experiments reported increased carcass leanness caused by addition of CLA to the diet. Although the changes in both fat and lean concentrations are quite variable in magnitude, the direction of responses was remarkably consistent. The decrease in the amount of carcass was from 6.2 to 25%, and the addition of carcass lean tissue was from 2.3 to 9.87%.

Interestingly, in contrast to changes in carcass composition, addition of dietary CLA may increase intramuscular fat deposition. Dugan et al. (1999) found that feeding 2% dietary CLA to pigs increased intramuscular fat as indicated by improvement of loin marbling scores and the amount of solvent extractable intramuscular fat. Improved loin quality has been consistently reported in 7 studies (Table 1.10), with 4 studies failing to show any changes.

Table 1.10. Effects of dietary CLA on intramuscular fat in pigs[1]

Reference	Increase of intramuscular fat
D'Souza and Mullan, 2002	NS
Dugan et al., 1999	+
Eggert et al., 1998	NS
Eggert et al., 1999a,b	+
Joo et al., 2002	+
Larsen et al., 1999	+
Sparks et al., 1999	+
Thiel-Cooper et al., 1999	NS
Tischendorf et al., 2002	NS
Wiegand et al., 1999	+
Wiegand et al., 2001	+
Wiegand et al., 2002	+

[1]Data are adapted from Dugan et al. (2004); + = positive effect; NS = no significant difference.

Another economically important function of dietary CLA is that it can lead to an improvement in fat firmness by increasing the content of saturated fatty acids of fat

caused by decreasing mRNA expression and(or) catalytic activity of Δ-9 desaturase (Lee et al., 1998; Smith et al., 2002). Delta-9-desaturase is responsible for converting palmitate (16:0) and stearate (18:0) to palmtoleic (16:1) and oleic acids (18:1), respectively. In addition to decreasing the amount of unsaturated fatty acids in adipose tissue, increased belly firmness by addition of CLA to swine diets had been consistently found by many researchers (O'Quinn et al., 2000; Thiel-Cooper et al., 2001; Eggert et al., 2001; Gatlin et al., 2002b; Weber et al., 2006). This response may help reduce the problem of soft carcass fat and improve bacon slicing and sausage quality when pigs are fed diets containing high concentrations of polyunsaturated fatty acids. Furthermore, Joo et al. (2002) and Wiegand et al. (2002) suggested that feeding CLA to pigs may increase loin muscle peroxidation stability.

MEASURES OF LIPID QUALITY

Lipids added to animal feeds vary in fatty acid composition and concentration of lipid peroxidation products depending upon storage length and conditions, and effects of processing (Canakci, 2007). The following methods are used to evaluate the quality of lipids: color, titration, moisture, insoluble, unsaponifiable matter, FFA, and the fatty acid profile.

Color

Fat color has little to do with nutritional quality directly, but may be an indicator of the composition or source of the lipid. Color ranges from pure white to very dark but is generally not a reflection of nutritional value.

Titer

Titer describes the hardness of the lipids and is determined by the fatty acid composition. Titer is measured by melting the fatty acids after a fat has been hydrolyzed and subsequently cooling the fat. The titer of a lipid is the congealing temperature in degrees centigrade (Meeker, 2006). Because saturated fatty acids have a higher melting point compared to unsaturated fatty acids, lipid from beef has a higher titer than that from swine or poultry as degree of unsaturation varies. Animal fats with a titer over 40 are termed tallows, whereas fats with a titer less than 40 are considered greases (Haas, 2005; Meeker, 2006).

Moisture

Moisture is determined by the weight percentage of the lipid after drying at 105°C for 4 hours. Moisture existing in the lipids not only interferes directly with their energy value but also accelerates corrosion of fat handling equipment and rancidity of lipid. Therefore, the maximum acceptable concentration of moisture in lipids is between 0.5 and 1.0% (Azain, 2001; Baiao and Lara, 2005).

Impurities

Impurities in lipids are determined in petroleum ether at temperatures between 40-60°C and are expressed as the percentage of the insoluble fraction of the lipid. Insoluble impurities commonly include small particles of hair, fiber, bone, and hide, soil, which not only decrease the energy content of lipids but also contribute to the production of fat clogging in fat handling screens and accumulating sludge in fat storage tanks (Azain,

2001). The standard for impurity content for feed lipids is less than 1% (Azain, 2001; Baiao and Lara, 2005).

Unsaponifiables

Unsaponifiables mainly refer to sterols, hydrocarbons, pigments, fatty alcohols, and vitamins which are soluble in lipids but cannot be hydrolyzed by alkaline saponification used to split triglycerides into glycerol and FFA (Azain, 2001). Some of these substances may have energy or nutritive value, whereas some are minimally digestible. Therefore, they are to be limited to 1% or less of the product because increasing their concentration decreases the energy value of the lipid (Azain, 2001; Baiao and Lara, 2005).

Free fatty acids (FFA)

Free fatty acid analysis measures the percentage of total FFA present in the lipid. Free fatty acid content can be determined by using NaOH or KOH to titrate oleic acids, which are the predominant FFA in lipids (AOAC, 1995). Results are expressed as mg of NaOH or KOH /g of diet or fat. Although FFA generally have the same energy value as the triglycerides, most feed-grade lipids have an upper limit for FFA content because the presence of FFA in the diets reduces palatability and accelerates the corrosion of feed handling equipment (Azain, 2001; Baiao and Lara, 2005). For example, an animal fat should have a maximum of 15% FFA and fat blends with a higher level of soap stock are allowed to have up to 20% FFA (Baiao and Lara, 2005). Low levels of FFA have minimal effect on the flavor of lipids, particularly if they are 16 or 18 carbon fatty acids (< 2%) as commonly found in soybeans, corn, or animal fat. However, the shorter carbon

chain fatty acids, such as lauric acid (C12:0), can possibly cause a strong objectionable flavor even at a low concentration (approximately 0.3%), and this objectionable flavor is readily detectable in rancid coconut and coconut oil (Robards et al., 1988).

Iodine value

Iodine value (IV), also called iodine number, is a measure of the proportion of unsaturated fatty acids relative to saturated fatty acids. Iodine value is determined based on the fact that each double bond in a fatty acid takes up two atoms of iodine when fatty acids react with iodine (Knothe, 2002). Iodine value is expressed as the grams of iodine absorbed per 100 g of sample. Vegetable oils generally contain more unsaturated fatty acids than saturated fatty acids, and as a result, oils have a higher IV than fats because there are more double bonds present in unsaturated fatty acids. For example, the iodine number for tallow, which consists of a high amount of saturated fatty acids ranges from 40 to 45, whereas the IV of soybean oil which is predominantly linoleic acid (50%) is greater than 100. Because the fatty acid profile of a lipid and its IV are highly related, and the fatty acid composition of a lipid determines the pork carcass fatty acid composition, evaluation of the dietary IV can be used to predict fatty acid composition of pork fat and consequently help manage soft pork fat concerns in practical pork production (Madsen, et al., 1992; Cast, 2010)

Fatty acid profile

Fatty acid profile describes the fatty acid composition of lipids and is usually determined by a gas chromatograph, which separates the methyl esters of the fatty acids (Method 996.06; AOAC, 2010). Fatty acid profile is an important determination in the

evaluation of lipid quality because this assay provides information regarding the types and proportions of the major fatty acids present in lipids. Fatty acid composition of fat not only influences the digestion and absorption of the lipids by pigs, but also affects the types of lipids deposited into pork fat. In addition, the carbon chain length and the degree of unsaturation of lipids determine the hardness (titer) and IV of pork fat. For example, vegetable oils contain high degrees of unsaturated fatty acids and IV, while animal fats are more saturated and have lower IV. Thus, measurements such as iodine number and titer are useful in predicting the general degree of unsaturation, but the actual fatty acid profile of the lipid is more useful.

MEASUREMENTS OF LIPID PEROXIDATION

Lipids are highly susceptible to oxidation, especially lipids rich in unsaturated fatty acids (Mayes 1996). Many toxic primary and secondary lipid peroxidation products are produced during the lipid peroxidation process. Many lipids used in animal feeds are subjected to various heating processes which accelerate lipid peroxidation (Canakci, 2007). Therefore, the diverse lipids used in animal feeds not only differ in fatty acid content, but also contain various concentrations of toxic oxidation products, which may affect their energy value. Therefore, understanding the degree of lipid peroxidation is important when evaluating lipid quality. Methods specific for evaluating lipid peroxidative stability can be divided into indicative and predictive tests. Indicative tests describe the presence of lipid peroxidation products in lipids and include: conjugated dienes, peroxide value (**PV**), anisidine value (**AnV**), thiobarbituric acid reactive substances (**TBARS**), hexanal value, 2, 4-decadienal (**DDE**), and 4-hydroxynonenal

(**HNE**). Predictive tests, including active oxygen method (**AOM**), oxygen stability index (**OSI**), and oxygen bomb method (**OBM**), measure the stability or susceptibility of lipids to oxidation. In predictive tests, the lipid is subjected to specific accelerated peroxidation conditions and a peroxidation endpoint is defined to determine the degree of peroxidation damage. One advantage of the predictive tests is that they have the ability to assess the relative effectiveness of different antioxidants.

Indicative tests

Conjugated dienes

Conjugated dienes are initial lipid peroxidation products and produced during the formation of hydroperoxides from unsaturated fatty acids. Conjugated dienes have been used to evaluate lipid peroxidation beginning in the 1960's (Antolovich et al., 2002). Conjugated dienes exhibit an intense absorption at 234 nm, and an increase in ultraviolet light (**UV**) absorption theoretically is proportional to the formation of primary peroxidation products in lipids. Good correlations between conjugated dienes and PV have been found (Shahidi et al, 1994; Wanasundara et al., 1995). The advantages of using UV detection of conjugated dienes to predict lipid peroxidation are that it is simple, fast, and only small amounts of sample are required. However, this method can be less sensitive compared to direct PV analysis (Gordon, 2001; Antolovich et al., 2002). Furthermore, the presence of other compounds such as carotenoids, which also have absorptivity in the same range of spectrum, contributes to inaccurate results (Shahidi and Wanasundara, 2002). Therefore, caution must be used when interpreting lipid peroxidation results using the conjugated dienes assay.

Peroxide value

Peroxide value measures of the concentration of primary lipid peroxidation products including peroxides and hydroperoxides formed during the initial phase of lipid peroxidation. Because peroxides and hydroperoxides are well documented for their potential toxicity (Kaneko et al., 1988), PV may provide useful information for predicting animal growth performance when lipids of various degrees of peroxidation are fed. Although PV is one of the most widely used indicators to evaluate the degree of peroxidation in lipids, it may be misleading when characterizing highly peroxidized lipids. A high PV indicates high concentrations of peroxides and hydroperoxides present in a lipid and can provide reliable information, but moderate or low PV may be due to the decomposition of hydroperoxides previously formed during lipid peroxidation since hydroperoxides are unstable when exposed to high temperatures (Frankel, 1998). Thus, p-anisidine value (**AnV**) and TBARS methods have been developed to quantify secondary peroxidation products and are more accurate measures of the degree of lipid peroxidation.

Anisidine value (AnV)

Anisidine value measures the amount of high molecular weight saturated and unsaturated aldehydes in lipids. The basic principle of AnV is that p-anisidine reacts with aldehydes in acetic acid to generate a yellowish color which can be detected and quantified at 350 nm. Muik et al. (2005) reported that the color intensity depends on the amount of aldehydes as well as on their structure (e.g. the diunsaturated aldehydes showing a more intensive color than the monounsaturated and saturated aldehydes).

However, like the PV assay, only one measurement of AnV may not provide reliable information of the degree of peroxidation for lipids exposed to an extreme oxidative challenge because AnV exhibits a bell shape curve response as a function of oxidation time (DeRouchey et al., 2004).

Thiobarbituric acid reactive substances (TBARS)

Thiobarbituric acid reactive substances analysis is another common method used to quantify the secondary lipid peroxidation products due to its simple and fast characterization. Compared to AnV, which measures high molecular weight of aldehydes, the TBARS assay detects malondialdehyde (MDA) concentration based on the fact that each MDA molecule can react with 2 molecules of thiobarbituric acid (TBA) to form a pink pigment MDA-TBA complex, which can be quantified by a spectrophotometer (Gutteridge, 1981). The cytotoxic and mutagenic effect of malondialdehyde has been extensively studied and it has been shown to be present in many heated oils. However, other carbonyl-containing secondary lipid peroxidation products also can react with TBA and produce a positive TBARS result (Kosugi et al. 1989; Esterbauer et al., 1991). In spite of this possibility, the TBARS assay may provide useful information for evaluating the quality of dietary lipid because malondialdehyde is highly reactive and potentially mutagenic (Marnett 1999).

Hexanal value

Hexanal is one of the major secondary lipid peroxidation products generated during the termination phase of peroxidation of linoleic acid (C18:2 n-6) and other n-6 fatty acids. Hexanal is well recognized as a good indicator of lipid peroxidation (Shahidi

et al., 1967; Ha et a., 2011), because the hexanal content is directly proportional to off-flavors caused by lipid peroxidation, and can be easily detected due to its low odor threshold (Shahidi and Pegg, 1994; van Ruth et al., 2000; Kalua et a., 2007). However, because hexanal is volatile at high temperatures, a low hexanal value does not necessarily indicate a low level of peroxidation in lipids because hexanal may have been lost depending on when the assay is performed in the preoxidation process.

2,4-decadienal (DDE)

The compound DDE is a byproduct derived from peroxidized linoleic acid during storage or heating (National Toxicology Program, 1993; Wu et al., 2003). Compared to the PV, Anv, and TBARS analyses, assay of DDE is more complicated and expensive requiring gas chromatography and mass spectrophotometry (Matthew et al., 1971; Selke and Frankel, 1987; Lasekan and Abbas, 2010). Low levels of DDE generate a deep-fried flavor, but excessive amounts of this specific dienaldehyde induce many undesirable effects, including peroxidative stress and proinflammatory reactions in human lung cells (Chang et al., 2005), cellular toxicity in liver and kidney tissues (Hageman et al., 1991), cellular proliferation in gastrointestinal epithelial cells (National Toxicology Program, 1993), and carcinogenic effects in the gastrointestinal tract (Hageman et al., 1991). Thus, with respect to the considerably negative impact of DDE on animal health, quantification of DDE may provide valuable information regarding the quality of dietary lipids. However, to date, no research had been conducted to investigate the effect of DDE on growth performance and health status in pigs.

4-Hydroxynonenal (HNE)

The peroxidation compound, 4-hydroxynonenal, is one of the α, β-unsaturated lipophilic aldehydes formed from lipid peroxidation of n-6 polyunsaturated fatty acids, such as arachidonic or linoleic. 4-Hydroxynonenal is well known for its cytotoxic and mutagenic effects (Witz, 1989; Esterbauer et al., 1991). Similar to the assay of DDE, measurement of HNE is quite complicated and expensive (Zanardi et al., 2002; Fitzmaurice et al., 2006). The reactive groups (an aldehyde, a double-bond at carbon 2, and a hydroxy group at carbon 4) of HNE make it a good marker of lipid peroxidation (Zarkovic, 2003). Furthermore, previous studies demonstrated that the consumption of fat sources containing 4-HNE are likely to increase the oxidative load in the mammalian metabolic system because 4-HNE readily conjugates to glutathione, thus depleting this important antioxidant (Seppanen and Csallany, 2002; Uchida, 2003). Treating cells directly with 4-HNE increases the activation of stress pathways (Biasi et al., 2006; Yun et al., 2009) and alters immune signaling pathways. However, to date, no information regarding the effect of HNE on growth performance and health status in pigs has been reported.

Predictive tests

Active oxygen method (AOM)

Active oxygen method is a predictive method where purified air is bubbled through a lipid sample held at 97.8°C, and the PV of the lipid is determined at regular intervals. Active oxygen method is defined as the time required to reach a PV of 100 mEq/kg lipid (recorded as hours), or the PV of the lipid is measured at a predetermined amount of time, 20 hours (recorded as meq/kg lipid). Because the PV are plotted over

time and because of the bell shape curve of PV is a function of oxidizing time, AOM can reflect the degree of lipid oxidation much more accurately than a single PV assay. The more stable the fat, the longer it will take to reach that level or the lower the PV at the predetermined amount of time. The AOM method is included in compendia published by AOCS (Method Cd 12-57), however, the AOM method is labor-intensive and time-consuming (Jebe et al., 1993). Furthermore, because the AOM results are determined by measuring unstable hydroperoxides, another disadvantage of the AOM test is its low reproducibility (Jebe et al., 1993).

Oxygen stability index (OSI)

Oxygen stability index is another predictive measure of lipid oxidation potential and is similar to the AOM method in theory. In the OSI test, air passes through the lipid solution under a specific temperature, whereupon volatile acids that are decomposed from artificial lipid peroxidation are driven out by the air and subsequently dissolved in the water which increases its conductivity. Conductivity of the water is measured constantly and the OSI value is defined as the hours required for the rate of conductivity to reach a predetermined value. The advantages of OSI tests over the AOM method include: multiple samples can be analyzed easily and simultaneously, and the results from OSI tests are highly reproducible because the volatile acids are relative stable tertiary oxidation products compared to hydroperoxides (Jebe et al., 1993; Mendez, 1996). Like AOM, the OSI test provides information regarding the changing trends in the concentration of volatile lipid peroxidation products by constantly monitoring the conductivity. Thus, OSI can provide a better evaluation of the degree of lipid

peroxidation than a single indicative assay. However, because the OSI test depends on monitoring conductivity by quantification of the volatile fatty acids, the OSI test may have similar disadvantages as those using pAV, TBARS, HNE, and hexanal due to the potential loss of volatile fatty acids derived from lipid peroxidation before the OSI test is conducted.

Oxygen bomb method (OBM)

Oxygen bomb method is used frequently to evaluate the oxidative stability of food products or extract lipid samples. Compared to AOM and OSI, OBM method is not only restricted to pure oils or fats and it is frequently used to assess the oxidative stability of food products and feed products (Gearhart et al. 1957, Inglis and Willington 1976, Shermer and Giesen 1997). The oxygen bomb machine includes a stainless steel container (bomb) connected to a pressure recorder. In the oxygen bomb test, a weighed sample is inserted into the bomb which is filled with certain amount of oxygen. The change of oxygen pressure is constantly recorded. And the process of lipid peroxidation requires incorporating different amount of oxygen in to the analyzed samples, which is proportional to the degree of lipid peroxidation, and can be measure by monitoring the reduction of the oxygen pressure. Thus, the degree of peroxidation products in lipids can be calculated by recording the decreases of the oxygen pressure in the bomb. For lipids or feed or food products with a fast and high amount of oxygen uptake, they are more prone to peroxidative degradation (Gearhart et al. 1957; Blankens et al. 1973). The susceptibility to peroxidation of products in the oxygen bomb apparatus is based mainly on their total fat content.

RESEARCH QUESTIONS TO BE ANSWERED

Negative effects from feeding peroxidized lipids to animals have been reported in many previous studies. Kimura et al. (1984) noted that feeding oxidized soybean oil not only impaired growth performance, but also caused diarrhea in rats. Alexander et al. (1987) and Behniwal et al. (1993) also reported that rats fed diets containing oxidized corn or peanut oil had impaired growth rates. Similarly in broiler studies, ingestion of oxidized poultry fat led to impaired growth compared with birds fed fresh poultry fat (Cabel et al., 1988; Dibner et al., 1996a). Furthermore, DeRouchey et al. (2004) reported that growth rate of pigs decreased as the rancidity of choice white grease consumed increased, while Fernandez-Duenas (2009) reported that feeding oxidized corn oil reduced pig growth performance. However, despite consistent negative response from feeding peroxidized lipids from several sources to multiple species, very little is known about the mechanism responsible for the poor growth performance of pigs fed with thermally-oxidized lipids.

In addition, feed formulators need a better understanding of the physiological effects and the energy value of oxidized lipids from various sources to enable nutritionists to assess value and optimize feeding applications of peroxidized lipids in swine diets. Furthermore, this knowledge also has important implications for understanding the potential effects of using DDGS, a common feed ingredient that contains a relatively high content of unsaturated lipid which may be highly oxidized in swine feeds.

Evaluation of methods to determine peroxidation of lipids

Measurements of lipid peroxidation provide useful information to evaluate the degree of peroxidation. However, assessment regarding the degree of lipid peroxidation may not be valid due to the drawbacks of the method used for characterizing peroxidation and the stage of the peroxidation process when the analysis occurred. Lipid peroxidation is a complex process and is generally considered to consist of 3 phases: (1) an initiation phase which involves the formation of free lipid radicals with hydroperoxides as primary reaction products, (2) a propagation phase where hydroperoxides formed are decomposed into secondary peroxidation products, and (3) a termination phase involving the formation of tertiary peroxidation products (Gutteridge, 1995; Yong and McEneny, 2001). As such, the increased and decreased amount of various lipid peroxidation products over time during each of these phases increases the difficulty in accurately measuring and assessing the extent of lipid peroxidation. Therefore, it is essential to understand the advantages and disadvantages of different methodologies used to evaluate lipid peroxidation.

Effects of feeding thermally-oxidized lipid on lipid metabolism of animals

Many studies have been performed to evaluate the effect of feeding peroxidized lipids on lipid metabolism in rats (Cohn, 2002). In those experiments, authors have found consistently that feeding peroxidized lipids to rats results in a change in their ability to regulate lipid metabolism by activating the peroxisome proliferator-activated receptor-α (PPAR-α), and enhancing the expression of PPAR-α target genes that participate in fatty acid oxidation (Chao et al., 2001, 2004, 2005; Sülzle et al., 2004). The exact mechanism for the activation of PPAR-α pathway by thermally-oxidized lipids is still unknown. One

major reason for this effect of peroxidized lipids on lipid metabolism may be attributed to the presence of hydroxyl- and hydroperoxy fatty acids, including hydroxyl octadecadienoic and hydroperoxy octadecadienoic acid, both of which are potent activators of PPAR-α (Mishra et al., 2004; König and Eder, 2006). The activation of the PPAR-α pathway is responsible for increased transcription of PPAR-α target genes. The increased transcription and expression of PPAR-α target genes results in the increased activities of fatty acid transport across the cell membrane, intracellular lipid trafficking, mitochondrial and peroxisomal fatty acid uptake, and both mitochondrial and peroxisomal fatty acid β-oxidation, gluconeogenesis, and ketogenesis (Konig and Eder, 2006; Rakhshandehroo et al., 2010). Thus, the activation of the PPAR-α parthway contributes to the reduction in triacylglyceride levels in the liver, plasma, and adipose tissues (Mandard et al., 2004). In addition, PPAR-α activation may also stimulate the expression ofthe proteolytic activation of sterol regulatory element-binding proteins (**SREBP**), which are major transcription factors that control fatty acid synthesis and cholesterol hemostasis (Patel et al., 2001; Knight et a., 2005; Köng et al., 2006). Therefore, in addition to enhancing β-oxidation resulting in decreased liver and serum triglycerides, activation of PPAR-α caused by peroxidized lipids also contributes to reduced liver and serum cholesterol levels (Huang et al., 1988; Chao et al., 2001, 2004, 2005; Sülzle et al. 2004). Two major uses of absorbed triglycerides include incorporation into adipose tissue to function as stored energy, or providing energy for maintaining body functions. Thus, decreased growth performance in pigs fed thermally-oxidized lipids may be related to the activation of PPARα pathway, which may lead to decreased lipid

availability for adipose tissue accretion and protein synthesis. However, no experiments have been conducted to characterize the metabolic effects of feeding diets containing peroxidized lipids to pigs.

Effects of feeding thermally-oxidized lipids on apparent lipid digestibility and energy content

One of the most important functions of adding lipids to animal diets is to provide increased caloric density to improve feed efficiency (Pettigrew and Moser, 1991). Therefore, understanding factors that limit energy value of lipids is essential for optimizing application of lipids and reducing the cost for pork production.

Lipids have been generally recognized as highly digestible energy sources (Babatunde et al., 1968; Cera et al., 1988a,b; 1989a; 1990; Li et al., 1990; Jones et al., 1992; Jorgensen et al., 2000). The apparent digestibility of various lipids in nursery pigs has been shown to increase with age (Hamilton and McDonald, 1969; Frobish et al., 1970). The digestibility of the lower digestible animal fat sources (lard and tallow) increased to a greater extent with pig age compared to digestibility of vegetable oils (Cera et al., 1988a,b; 1989a, 1990). Furthermore, many studies have been conducted to characterize the quality of lipids as energy ingredients (Cera et al., 1988, 1989; Li et al., 1990; Jones et al., 1992). However, these research efforts have focused mainly on the effects of the unsaturated to saturated fatty acid ratio (Powles et al., 1993, 1994, 1995), fatty acid chain length (Hamilton and McDonald, 1969; Cera et al., 1989; Straarup et al., 2006), and FFA content of the fat sources (Sklan, 1979; Tso et al., 1981; DeRouchey et al., 2004). Few studies have evaluated the contribution of lipid peroxidation products on the energy value

of lipids. Large quantities of lipids produced from food processing facilities or restaurants are rendered and have been used as economical sources of energy in animal feeds (Canakci, 2007). Thermally-oxidized lipids may contain various concentrations of toxic lipid peroxidation products (Lin et al., 1989; Adam et al., 2008) because lipids containing high concentrations of polyunsaturated fatty acids are thermally sensitive to lipid peroxidation and are unstable when exposed to high temperatures for a considerable length of time (Frankel et al., 1984; Linfield et al., 1985). Thus, there is a critical need to understand the impact of thermally-oxidized lipids on lipid digestibility as well as DE and ME content.

Effects of feeding thermally-oxidized lipids on oxidative status

Oxidative stress is the imbalance between the production or manifestation of free radicals and a biological system's ability to readily detoxify the free radicals or to repair the resulting damage. Free radicals are substances with single unpaired electrons, which can result in damage to all components of the cell, including proteins, lipids, and DNA. In contrast, antioxidants are compounds that can neutralize free radicals and thus, prevent healthy cells and repair damaged cells from their negative effects (Rahman, 2007). Free radicals are normal products of animal reduction-oxidation metabolism and the production and removal of free radicals are in constant equilibrium in healthy biological systems (Droge, 2002). However, external sources of free radicals, including dietary intake of excess peroxidized lipids, contribute to oxidative stress.

Animal growth (Lin et al., 1989) as well as meat quality (Lin et al., 1989; Lauridsen et al., 1999b) may be impaired depending on the degree of oxidative stress.

Oxidative stress caused by ingestion of peroxidized lipids has been reported in rats (Yoshida and Kajimoto, 1989; Liu and Huang, 1996; Ammouche et al., 2002), broilers (Engberg et al., 1996), and rabbits (Hennig et al., 1995). Furthermore, Luci et al. (2007) and Ringseis et al. (2007) found that feeding oxidized sunflower oil to pigs resulted in oxidative stress whereby the amount of *in vivo* antioxidant was decreased compared to pigs fed unoxidized lipids. In addition, excessive consumption of dietary PUFA contributed to oxidative stress (Kleinveld et al., 1993; Hennig et al., 1995; Lauridsen, et al., 1999a), because unsaturated fatty acids are particularly susceptible to autoxidation, forming primary and secondary peroxidation products, and consequently deplete antioxidants (Sherwin, 1978). However, no study has been conducted to systematically investigate the effect of lipid source and peroxidation level on the metabolic oxidative status of young pigs.

Effect of feeding thermally oxidized lipids on gut barrier function

The gastrointestinal tract serves to digest and absorb nutrients from the intestinal lumen and acts as the first protective barrier between the intestinal lumen and the body (Sanderson, 1998). Changes in gastrointestinal tract structure can be associated with changes in its nutrient absorption and pathogen resistance. The protective function of the gastrointestinal tract is known primarily as gut barrier functions and is highly related to the integrity of the gastrointestinal tract (Rowlands et al., 1999; Fasano and Shea-Donohue. 2005). Poor gut barrier function may reduce the resistance of the animal to infectious substances such as endotoxins or pathogenic bacteria, and may cause activation of the immune system.

Oxidized lipids, especially the secondary lipid peroxidation products such as MDA and HNE, can have a negative direct effect on enterocytes causing membrane perturbations, and consequently contribute to increased membrane permeability (Dibner et al., 1996a). Results from a previous study by Dibner et al. (1996a) showed that feeding oxidized poultry fat to broilers resulted in structural injury to the intestine as indicated by a decreased half-life of enterocytes. Assimakopoulos et al. (2004) found that intestinal peroxidative stress was a key factor resulting in intestinal physical injury as indicated by decreased villous density and total mucosal thickness. In addition, feeding thermally-oxidized sunflower oils to growing pigs increased markers of oxidative stress at the small intestinal level (Ringseis et al. 2007). Thus, it is possible that consumption of thermally-oxidized lipids results in intestinal injury and gut barrier dysfunction. However, no information has been reported regarding the effects of feeding peroxidized lipids on intestinal architecture or gut barrier function in pigs.

Effects of feeding thermally oxidized lipids on immunity

The immune system is a complicated system of biological structures and processes within an organism that protects against disease. The primary function of the immune system is to detect a wide variety of infectious and toxic substances ranging from viruses to parasitic worms, and distinguish them from the organism's own healthy tissues. Activation of stress pathways or over expression of inflammatory mediators not only causes redistribution of nutrients away from growth processes in support of the immune system (Liu et al., 2010), but may also inhibit IGF-1 mRNA expression (Thissen and Verniers, 1997), both of which will contribute to poor growth performance. On the

contrary, immunodeficiency, often described as a poor immune response, also contributes to poor growth performance as result of an increase in the incidence of viral, bacterial, and parasitic infections.

The heating/peroxidation of unsaturated fat sources leads to the production of reactive lipid oxidation products including MDA, HNE, DDE and others (Seppanen and Csallany, 2002). Of these compounds, 4-hydroxynonenal (4-HNE; Uchida, 2003) is best characterized for its adverse physiological effects. In addition to depleting *in vivo* antioxidants by conjugating glutathione (Uchida, 2003), consumption of specific hydroxylated aldehydes, or treating cells with 4-HNE has been shown to influence immune responses by increasing the activation of stress pathways (Biasi et al., 2006; Yun et al., 2009). Altering the immune pathway stimulates the expression of inflammatory mediators in macrophages (Kumagai et al., 2004), decreased the ability of IgA to bind bacterial antigens (Kimura et al., 2006), and blocks macrophage signaling pathways (Kim et al., 2009). Therefore, it is likely that consumption of a peroxidized unsaturated fat source directly alters the mucosal immune system through the direct action of compounds such as 4-HNE that are produced by lipid peroxidation.

CHAPTER 2

EVALUATION OF METHODS TO DETERMINE PEROXIDATION OF LIPIDS

Measurements of peroxidation can provide useful information regarding the degree of lipid peroxidation, but limitations of each test should not be overlooked. The objective of this experiment was to evaluate peroxidation in 4 lipids, each with 3 degrees of peroxidation. Lipid sources were: corn oil (CN), canola oil (CA), poultry fat, and tallow. Peroxidation levels were: original lipids (OL), slow-oxidized lipids (SO), and rapid-oxidized lipids (RO). To produce peroxidized lipids, OL were either heated at 95°C for 72 h to produce SO, or heated at 185°C for 7 h for producing RO. Five indicative measurements [peroxide value (PV), p-anisidine value (AnV), thiobarbituric acid reactive substance concentration (TBARS), hexanal concentration, 4-hydroxy nonenal concentration (HNE), and 2,4-decadienal (DDE)] and 2 predictive tests [active oxygen method stability (AOM) and oxidative stability index (OSI)] were performed to quantify the degree of oxidation of the subsequent 12 lipids of varying degrees of peroxidation. Analysis showed that a high PV accurately indicated the high degree of lipid peroxidation, but a moderate or low PV may be misleading due to the unstable characteristics of hydroperoxides as indicated by the unchanged PV of rapidly oxidized CN and CA compared to their original state (OL). However, additional tests which measure secondary peroxidation products such as AnV, TBARS, hexanal, HNE, and DDE may provide a better indication of lipid peroxidation than PV for lipids subjected to a high degree of peroxidation. Similar to PV analysis, these tests may also not provide

irrefutable information regarding the extent of peroxidation due to the volatile characteristics of secondary peroxidation products and the ever changing stage of lipid peroxidation. For the predictive tests, AOM accurately reflected the increased lipid peroxidation caused by SO and RO as indicated by the increased AOM value in CN and CA, but not in poultry fat and tallow, which indicates a potential disadvantage of the AOM test. Oxidative stability index successfully showed the increased lipid peroxidation caused by SO and RO in all lipids, but it too may have disadvantages similar to AnV, TBARS, hexanal, DDE, and HNE because OSI directly depends on quantification of the volatile secondary peroxidation products. To accurately analyze the peroxidation damage in lipids, measurements should be determined at appropriate time intervals by more than one test and include different types of peroxidation products simultaneously.

Keywords: hydroperoxides, lipid oxidation, secondary oxidation products

INTRODUCTION

Energy is one of the most expensive components of swine diets. Lipids are commonly added to swine diets as concentrated energy sources to improve feed efficiency (Pettigrew and Moser, 1991), but they also reduce feed dust, supply fat soluble vitamins and essential fatty acids, and improve diet palatability. Lipids used in animal production not only vary in fatty acid composition due to their origin, but may also contain various concentrations of primary and secondary lipid peroxidation products depending upon their fatty acid composition, storage length and conditions, and effects of processing (Canakci, 2007).

Lipid peroxidation, especially when subjected to heat during processing or cooking, may deplete endogenous antioxidants (Seppanen and Csallany, 2002) and generate an assortment of peroxidation products (Lin et al., 1989; Adam et al., 2008) which increase intestinal oxidative stress (Suomela et al., 2005; Ringseis et al., 2007), damage small intestinal structure (Dibner et al., 1996a,b), and impair immune function (Takahashi and Akiba, 1999). In addition, DeRouchey et al. (2004) reported that feeding thermally-oxidized choice white grease to pigs may also reduce growth performance. Therefore, the need to accurately evaluate lipid peroxidation is essential for optimizing the value of lipids as an energy source in swine diets.

Although many analytical methods are used commonly to characterize lipid peroxidation, limitations of each method should not be overlooked due to the complexity of lipid peroxidation reactions which produce a chemically diverse group of oxidation compounds. In the current experiment, 4 types of lipids varying in their fatty acid

composition were oxidized thermally by 2 different heating methods to assess commonly used methodology to evaluate lipid peroxidation for use in animal feeds.

MATERIALS AND METHODS

Lipid Preparation

In the current experiment, 4 lipids, each with 3 degrees of peroxidation were evaluated. Lipid sources were: corn oil (**CN**; ADM, Decatur, IL), canola oil (**CA**; ConAgra Foods, Omaha, NE), poultry fat (**PF**; American Protein, Inc., Hanceville, AL), and tallow (**TL**; Darling International, Wahoo, NE). Peroxidation levels were: original lipids (**OL**), slow-oxidation (**SO**), and rapid-oxidation (**RO**). To generate peroxidized lipids, OL were either heated at 95°C for 72 h to produce SO, or heated at 185°C for 7 h to produce RO. Both heating processes were accompanied with a constant flow of compressed air of 12,000 cm^3/min at a temperature of 22 to 24°C. All of the OL, SO, and RO lipid sources were stored at -20°C and no antioxidant was added prior to laboratory analysis.

Analysis of Chemical Properties of Lipids

Crude fat (Method 920.39 A; AOAC, 2010), free fatty acids (Method 940.28, AOCS, 2009), moisture (Method Ca 2c-25; AOCS, 2009), insolubles (Method Ca 3a-46; AOCS, 2009), unsaponifiables (Method Ca 6a-40; AOCS, 2009), and fatty acid profile (Method 996.06; AOAC, 2010) of experimental lipids were analyzed at the University of Missouri Agricultural Experiment Station Chemical Laboratories (Columbia, MO). The vitamin E concentration of experimental lipids was analyzed at a commercial lab (Minnesota Valley Testing Laboratories, New Ulm, MN) using a modified method of

971.30 (AOAC, 1971) by HPLC with a fluorescence detector. The term "vitamin E" is the generic descriptor for all tocol and tocotrienol derivatives that exhibit qualitatively the biological activity of α-tocopherol (Ball, 2006).

Peroxide value (PV, method Cd 8-53; AOCS, 1993), thiobarbituric acid reactive substance concentration (**TBARS**, Buege and Aust, 1978), and 4-hydroxynonenal (**HNE,** Zanardi et al., 2002; Fitzmaurice et al., 2006) were analyzed at the University of Minnesota. The active oxygen method (**AOM**) is a predictive method where purified air is bubbled through a lipid sample at 97.8°C, and the PV of the lipid is determined at regular intervals to determine the time required to reach a PV of 100 mEq/kg lipid (recorded as h), or the PV of the lipid is determined at a predetermined time endpoint, such as the 20 h time period used in this study (recorded as mEq/kg lipid). In principle, the oxidative stability index (**OSI**) method is similar to the AOM method whereupon air passes through a lipid under a specific temperature, at which point volatile acids decomposed from lipid peroxidation are driven out by the air and subsequently dissolved in water thereby increasing its conductivity. The conductivity of the water is constantly measured, and the OSI value is defined as the hours required for the rate of conductivity to reach a predetermined level. Unlike most other tests described herein, for the OSI test, a high value means that less peroxidation has occurred (i.e. more hours to reach a pre-determined level of peroxidation). Active oxygen method (PV at 20 h of Method Cd 12-57; AOCS, 2009), oxidative stability index (Method Cd 12b-92; AOCS, 1997), p-anisidine value (**AnV**, method Cd 18-90, AOCS, 2009), and hexanal (GC/MS methodology, proprietary methodology) were analyzed by a commercial laboratory

(Eurofins Scientific Inc., Des Moines, IA) while 2, 4-decadienal (**DDE**) was analyzed by gas chromatography using a flame ionization detector (detector temperature, 260°C; injection temperature, 250°C) employing a ramp temperature program from 40°C to 320°C with a run time of 12 minutes, a Zebron ZB column (Phenomenex, Torrance, CA), and 1.0 µl injection at a another commercial laboratory (Kemin, Des Moines, IA).

Statistical Analysis

Relationships between measures of lipid peroxidation and various variables were evaluated by simple linear correlation (Pearson correlation coefficients) analysis using the CORR procedure of SAS (SAS Inst. Inc., Cary, NC). The mean of triplicate samples from each lipid peroxidation measures was used as the experimental unit. Treatment effects were considered significant if $P < 0.05$, whereas values between $0.05 \leq P \leq 0.10$ were considered statistical trends. .

RESULTS AND DISCUSSION

Compositional Changes of Lipids

Lipid peroxidation is a complex process and is generally considered to consist of 3 phases: (1) an initiation phase which involves the formation of free lipid radicals and hydroperoxides as primary reaction products, (2) a propagation phase where hydroperoxides formed are decomposed into secondary peroxidation products, and (3) a termination phase involving the formation of tertiary peroxidation products (Gutteridge, 1995; Yong and McEneny, 2001). As such, the increased and decreased amount of various lipid peroxidation products over time during each of these phases increases the

difficulty of accurately measuring and assessing the extent of lipid peroxidation. Therefore, it is essential to understand the disadvantages of different methodologies used to evaluate lipid peroxidation.

The current experiment utilized 4 types of dietary lipids that varied greatly in fatty acid composition depending on their origin (Table 2.1). Compared to PF and TL, CN and CA had higher concentrations of unsaturated fatty acids (89% and 84% vs. 64% and 37%, respectively), higher ratios of unsaturated to saturated fatty acids (14.2 and 6.2 vs. 2.1 and 0.7, respectively), and accordingly higher iodine values (**IV**; 103 and 121 vs. 75 and 34, respectively). As expected, the most abundant unsaturated fatty acid in CA was oleic acid (66%), while linoleic acid was the most abundant in CN (54%). Of the animal fat sources, PF had a greater concentration of unsaturated fatty acids (64%) compared with TL (37%), and subsequently had a higher unsaturated to saturated fatty acid ratio (2.1:1) and a greater IV (75) compared to TL (0.7:1 and 34, respectively). In general, the fatty acid composition of the four original lipids was in agreement with data reported in previous publications (NRC, 1998; AOCS, 2006).

Each OL was heated in two ways. The SO process of applying a relatively low temperature (95°C) for a relatively long period of time (72 h) reflects the peroxidation that might occur during the rendering of animal fats (Meeker and Hamilton, 2006). The RO process was used to simulate the heating process that normally occurs in the restaurant industry where lipids may be heated in fryers for up to 18 h daily at a temperature of approximately 180°C (Frankel et al., 1984). Unsaturated fatty acids, especially polyunsaturated fatty acids (**PUFA**), are highly susceptible to oxidation

(Frankel et al., 1984; Linfield et al., 1985) and their oxidation can ultimately lead to the generation of free radicals, peroxides and hydroperoxides, and undesirable substances with intrusive odor and toxic properties (Lin et al., 1989; Adam et al., 2008). In addition to the fatty acid composition of lipids from different origins, the thermal oxidation conditions applied (e.g., temperature and duration of heating, addition of oxygen and catalysts and water activity) play an important role in determining the variable concentration of lipid peroxidation products (Chang et al., 1978). Generally, autoxidation of lipids at a low temperature without catalysis mainly results in the formation of primary lipid peroxidation products such peroxides and hydroperoxides, while large quantities of secondary oxidation products such as aldehydes, carbonyls, and ketones will be produced when lipids are heated at relative high temperatures because hydroperoxides are not heat-stable, and will further break down into numerous secondary oxidation products when exposed to prolonged heating (Esterbauer et al., 1991).

After the heating processes (Table 2.1), small changes were noted in crude fat, FFA, and the sum of moisture, impurities, and unsaponifiables, among OL, SO, and RO in all sources of lipids. In general, the concentration of PUFA (namely linoleic and linolenic acid) was reduced for all lipids, which was likely due to the PUFA being highly susceptible to lipid peroxidation (Sherwin, 1978; Seppanen and Csallany, 2002) forming hydroperoxides, and further decomposing into other oxidation products compared to saturated fatty acids. Except for these two minor changes, the fatty acid composition within each lipid source was unaltered.

Traces of total tocopherols were only measurable in the original CA (0.40 IU/g)

and CN (0.29 IU/g), whereas the total tocopherols in original PF or TL was lower than the detection limit (< 0.10 IU/g) of the assay. Slow oxidation and RO decreased the total tocopherol level of original CA and CN to a level lower than the detection limit (< 0.10 IU/g). The depletion of antioxidants in lipids has been observed in other studies (Lea and Ward, 1959; Seppanen and Csallany, 2002), where the loss of vitamin E is partly due to its antioxidant effect, because antioxidants can be quickly consumed by lipid free radicals derived from fatty acid peroxidation at high temperatures (Ullrey, 1981), and partly because of the heat sensitive characteristics of antioxidants (Park et al., 2004). These results suggest that although the presence of the total tocopherols helped minimize lipid peroxidation, their protective potential was greatly diminished by both SO and RO heating processes used in the current experiment.

Indicator Measures of Lipid Peroxidation

Peroxide value measures lipid peroxides and hydroperoxides which are primarily formed in the initial phase of lipid peroxidation. Because peroxides and hydroperoxides are well documented for their potential toxicity (Holman and Greenberg, 1958; Kaneko et al., 1988), PV may provide useful information for predicting animal growth performance. Peroxide value analysis in the current experiment showed that SO of lipids led to a relatively high PV compared to OL or RO lipids. The lack of a high PV in oils that had been RO suggests that the production of peroxides and hydroperoxides may have been further degraded to secondary or tertiary oxidation products. This is supported by the results from AnV and TBARS tests (measures of carbonyl-containing secondary lipid oxidation products formed from the decomposition of hydroperoxides) which remained

high in rapidly oxidized CN and CA because these lipids are more prone to oxidation, and only slightly higher in PF and TL, which are lipids less prone to oxidation. These results indicate that a single PV analysis may cause a misleading assessment of lipid oxidation because the hydroperoxides formed were unstable at a high temperature. As expected, the increased PV in CN or CA caused by SO was much higher than that in TL, with PF being intermediate. These responses are consistent with their concentrations of unsaturated fatty acids because unsaturated fatty acids are more susceptible to lipid oxidation than saturated fatty acids.

Measures such as AnV, TBARS, HNE, and hexanal have also been utilized to determine the production of secondary oxidation products derived from the decomposition of initially produced hydroperoxides. p-Anisidine value measures the amount of high molecular weight saturated and unsaturated aldehydes. In the current experiment, the AnV of all OL were low (CN, < 1.0; CA, 1.4; PL, < 1.0; TL, 4.3), but oxidation by SO and RO resulted in a higher AnV compared to the OL. The higher degree of lipid oxidation caused by RO, as indicated by the increased AnV, were in contrast to their unchanged PV, which suggests that measurement of secondary oxidation products such as AnV, may provide a better assessment of lipid oxidation than PV for the lipids that have experienced a relatively high degree of peroxidation. However, measurement of only AnV may not provide reliable information of lipid peroxidation because AnV of lipids exposed to extreme oxidative conditions have been shown to exhibit a bell shaped curve (DeRouchey et al., 2004), and thus their respective values are time dependent.

The TBARS analysis is another method used to quantify secondary lipid oxidation products. Compared to AnV, the TBARS assay was developed to detect malondialdehyde, although other carbonyl compounds can also contribute to TBARS values (Gutteridge, 1981). However, the TBARS assay may provide useful information regarding the quality of dietary lipids because malondialdehyde is highly reactive and potentially mutagenic (Marnett, 1999) which can be a factor in causing intestinal oxidative stress (Suomela et al., 2005; Ringseis et al., 2007), and ultimately lead to poor growth performance in animals fed thermally oxidized lipids (DeRouchey et al., 2004). As expected, all of the OL had a low TBARS value, and the increase in TBARS caused by SO in CN, CA, and PF was greater than in TL due to these lipids having a higher concentration of unsaturated fatty acids compared to TL. Although original PF had the potential to produce a higher TBARS value due to its high amount of unsaturated fatty acids (7% of palmitic acid, 38% of oleic acid, and 18% of linoleic acid), the TBARS of rapid oxidized PF was only slightly higher than that of the original PF, which may be due to the loss of the secondary oxidation products because of their volatile characteristics (Seppanen and Csallany, 2002). These results suggest that a single low TBARS value may also be misleading because of the loss of volatile secondary oxidation products.

Hexanal is one of the major secondary lipid oxidation products produced from the termination phase during the oxidation of linoleic and other ω-6 fatty acids. Hexanal has become a well-known indicator of lipid peroxidation (Shahidi et al., 1987; Ha et a., 2011), because the production of hexanal is directly related to oxidative off-flavors (Shahidi and Pegg, 1994; van Ruth et al., 2000; Kalua et a., 2007). The hexanal content of the initial

CN (< 1.0 mg/kg), CA (1 mg/kg), PF (3 mg/kg), and TL (4 mg/kg) were all increased by the SO and RO heating process (Table 2.1). It was expected that heated CN would result in a higher hexanal value compared to CA, PF and TL, because CN contains the highest concentration of linoleic acids among all the lipids evaluated, and because ω-6 fatty acids are prone to peroxidation. However, because hexanal is volatile at high temperatures, a single low hexanal value may not necessarily indicate a low level of peroxidation in a lipid sample because, like other secondary lipid oxidation products, hexanal can be lost during subsequent processing and storage.

The compound, DDE, is a by-product derived from oxidized linoleic acid formed during storage or heating (National Toxicology Program, 1993; Wu et al., 2003). Low levels of DDE result in a deep-fried flavor, but excessive amounts of this specific dienaldehyde induce many undesirable effects, including oxidative stress and proinflammatory reactions in human lung cells (Chang et al., 2005), cellular toxicity in liver and kidney tissues (Hageman et al., 1991), cellular proliferation in gastrointestinal epithelial cells (National Toxicology Program, 1993), and carcinogenic effects in the gastrointestinal tract (Hageman et al., 1991). In the current study, low levels of DDE were detected among all OL (Table 2.1) and were increased by SO and RO. Because of the potential negative influence of DDE and its widespread presence in heated lipids, monitoring the concentration of these compounds provides additional information regarding the degree of lipid peroxidation.

The compound HNE, is an α, β-unsaturated lipophilic aldehyde formed from the lipid peroxidation of polyunsaturated ω - 6 fatty acids, such as arachidonic or linoleic

acid, and is well known for its cytotoxic and mutagenic effects (Witz, 1989; Esterbauer et al., 1991). The reactive groups (an aldehyde, a double-bond at carbon-2, and a hydroxy group at carbon-4) of HNE have generated a great deal of attention for its potential to serve as a marker of lipid peroxidation (Zarkovic, 2003). In the current study, no HNE was detected in any of the OL (Table 2.1), while SO and RO caused HNE generation mainly in CN (194 and 594 μmol/kg, respectively) and CA (105 and 221 μmol/kg, respectively). Although HNE was produced in TL (13 and 6 μmol/kg for SO and RO, respectively), it was only produced by SO in PF (2 μmol/kg). Because HNE is a secondary peroxidation product derived from peroxidation of n-6 fatty acids, and CN contained the highest concentration of linoleic acid among all 4 lipid sources, it was expected that the concentration of HNE would be much greater after SO and RO in CN than in the other heated lipid sources. Although the original PF contained about 18% of linoleic acid (18:2, n-6), which was much higher than that in the original TL (3%), the HNE concentration in SO and RO of PF (2 and 0 μmol/kg, respectively) was slightly less than that in SO and RO of TL (13 and 6 μmol/kg, respectively). One explanation for the lower HNE in heated PF might due to the HNE being already volatized before the analysis (Seppanen and Csallany, 2002), which suggests that HNE analysis has a similar disadvantage as with PV and TBARS to evaluate oxidative deterioration in dietary lipid sources. Another disadvantage of using the HNE analysis as an indicator of lipid oxidation might relate to its high cost and complexity of analysis.

Our results are in agreement with those reported by Brandsch et al. (2004) where SO (heating a mixture of sunflower oil and linseed oil at 50°C over 16 d) led to a greater

production of total primary lipid peroxidation products, such as peroxides and hydroperoxides reflected by relatively high PV, and total secondary lipid peroxidation products, such as aldehydes, carbonyls, ketones, reflected by a relatively high TBARS. Rapid oxidation in the current study contributed to a higher PV value than that of OL only in CA, but not in other 3 lipid sources, and the PV of RO of each source of lipid were much lower than that of SO of the corresponding lipid. This lower PV in RO versus SO can be explained by the fact that hydroperoxides are thermally unstable under high temperature heating (Frankel, 1998), and the hydroperoxides generated had possibly been decomposed into secondary peroxidation products during the RO process. In addition, the increased TBARS concentration in RO compared to those in OL of CN and CA also indicate that the decomposition of hydroperoxides into secondary peroxidation products occurred. It was expected that heating vegetable oils would lead to a greater production of primary and secondary lipid peroxidation products compared to heating animal fats due to the heat sensitive characteristics of PUFA and because PUFA are more concentrated in vegetable oils than in animal fats. The results from these chemical analyses of experimental lipids indicate that lipids from different origins vary in fatty acid composition, and the two different heating processes altered the peroxidation products of these dietary lipids through the formation of both primary and secondary peroxidation products.

Predictive Measures of Lipid Peroxidation

In addition to the indicator measurements of lipid peroxidation previously discussed, predictive tests also are used to measure the stability or susceptibility of lipids

to oxidation. In these tests, a lipid is subjected to a specific accelerated condition and an endpoint is defined to determine the degree of peroxidation damage. In the current experiment, after 20-h of accelerated conditions, the AOM of OL, SO, and RO of CN or CA were greatly increased (as reflective by a greater PV) and the AOM of SO and RO were much higher than that of OL in CN or CA. These results are in agreement with the results obtained utilizing TBARS, AnV, and hexanal analysis. Within PF, only SO but not RO, had a higher AOM compared to the original PF, while within TL only RO but not SO, had an increased AOM compared to the original TL. The unchanged AOM of RO poultry fat and SO tallow may have been due to the decomposition of primary hydroperoxides formed during the heating process, which has been suggested as a reason for the relatively low reproducibility of the AOM test (Jebe et al., 1993).

Another limitation of AOM method is that it is labor-intensive and time-consuming (Jebe et al., 1993). Therefore, a faster and more automated oxygen stability index method has been developed. Advantages of OSI tests over the AOM method include multiple samples can be analyzed easily and simultaneously because a computer software program controls the instrument configuration and data collection, and the results from OSI tests are highly reproducible because the volatile acids are relative stable tertiary oxidation products compared to hydroperoxides (Jebe et al., 1993; Mendez, 1996). Like AOM, the OSI test provides useful information regarding the changes in the concentration of volatile lipid peroxidation products over time by constantly monitoring the conductivity. Therefore, OSI can provide a better evaluation of the degree of lipid peroxidation than a single indicator assay. As expected, the OSI of original CN (8.4 h) and CA (9.2 h) were

comparable and were lower than the OSI of original PF (24.6 h) and TL (12.1 h) due to their unsaturated fatty acid composition differences. These results are in agreement with the AnV and hexanal analysis, indicating that SO and RO caused lipid peroxidation as indicated by the low OSI values for CN, CA, PF, and TL (less than 1 h for all lipids). However, because the OSI test depends on monitoring conductivity by quantification of the volatile fatty acids, the OSI test may have similar disadvantage as AnV, TBARS, HNE, and hexanal due to the volatile fatty acids derived from lipid peroxidation being lost before the OSI test.

Correlation Among Measures of Peroxidation

Lipid peroxidation is a complex process and is affected by several factors including degree of saturation, temperature, oxygen, heavy metals, undissociated salts, water, and other nonlipidic compounds (AOCS, 2005). Lipid hydroperoxides initially formed during the lipid peroxidation process not only have a potential impact on lipid quality, and therefore on animal health and performance, but the formation of secondary and tertiary oxidation products (aldehydes, ketones, alcohols, hydrocarbons, volatile organic acids, and epoxy compounds) often have additional effects on lipid quality and animal productivity. Peroxidation reactions occur concurrently with a wide range of oxidative compounds produced and modified during the peroxidation process (Liu, 1997). To date, however, limited data regarding the relationship between these tests have been published and, under practical conditions, there may be advantages in time and cost savings in predicting lipid peroxidation by understanding these relationships.

Correlations among lipid peroxidation measurements are summarized in Table 2.2. Even though some correlations were found to be significant among various composition and peroxidation measures, caution must be taken when interpreting these data because significant correlations do not infer a cause and effect relationship due to the potential confounding of lipid source and the peroxidation method used. For example, moisture, insolubles, and MIU were positively correlated to OSI (r = 0.81, 0.78, and 0.70, respectively; $P < 0.01$). In animal fats, however, the greater OSI was most likely because animal fats have a lower level of unsaturation and not because they had higher level of moisture and insoluble as shown in table 2.1. In addition, the negative correlation noted between moisture and U:S (r = -0.51, $P < 0.10$) was likely due to the fact that the CN and CA utilized for this project were more refined compared to the PF and TL, which were obtained largely unprocessed from their respective rendering locations. Another example is that even though palmitic acid was negatively correlated to TBARS (r = -0.59, $P < 0.01$), lipids that had higher palmitic acid in the current study (PF and LT) also had a lower U:S compared to CN and CA. As expected, there was a positive correlation of U:S, oleic acid, and linolenic acid with TBARS (r = 0.62, 0.70, and 0.60, respectively; $P < 0.05$), but no significant association was noted between linoleic acid and TBARS. And there was a negative correlation between vitamin E and FFA (r = 0.47, $P < 0.05$), which was mainly due to the presence of vitamin E preventing the production of FFA from lipid oxidation. However, no correlation was found between any peroxidation measurements and vitamin E. Linoleic acid (e.g., CN) was correlated positively to DDE and HNE (r = 0.56 and 0.54, respectively; $P < 0.10$), but not to other measures of peroxidation.

Table 2.2 also lists correlations among peroxidation measures, where PV was positively associated with TBARS, hexanal, and DDE (r = 0.75, 0.76, and 0.61, respectively; $P < 0.05$); AnV was correlated positively to HNE (r = 0.67, $P < 0.05$) and AOM (r = 0.53, $P < 0.10$), but associated negatively to OSI (r = -0.57, $P < 0.05$); TBARS was positively correlated to AOM (r = 0.51, $P < 0.10$); hexanal was positively associated to DDN (r = 0.94, $P < 0.05$) and AOM (r = 0.57, $P < 0.10$); DDN was positively correlated to HNE (r = 0.49, $P < 0.10$) and AOM (r = 0.65, $P < 0.05$); HNE was positively associated to AOM (r = 0.66, $P < 0.05$); and AOM was negatively correlated to OSI (r = -0.58, $P < 0.05$). It was not surprising that correlations among several of the oxidation measures were not always significant given that peroxidation reactions occur concurrently during the peroxidation process with primary, secondary and tertiary oxidation products being produced and degraded at different rates depending upon the stage of oxidation (Gutteridge, 1995; Liu, 1997; Yong and McEneny, 2001). The negative correlation between AOM and OSI are in agreement with the findings by Woestenburg and Zaalberg (1986) and Laubli and Bruttel (1986).

Measurements of lipid peroxidation provide important information in the assessment of lipid quality because of the potential impact on animal health and performance from secondary and tertiary peroxidation products. Unfortunately, the assessment of the degree of lipid peroxidation is challenging because of the drawbacks of each method used. Our results suggest that there is no single method that seems to adequately describe or predict lipid peroxidation due to the complexity of lipid composition and the phases involved in lipid peroxidation. To accurately analyze the amount of lipid damage caused by peroxidation, it may be advantageous to determine the degree of lipid peroxidation at several time intervals using more than one test. However, a high value of peroxide value, p-anisidine value, thiobarbituric reactive substances, hexanal, 2, 4-dicadienal, 4-hydroxylnoneal, or active oxygen method as well as a low value of oxidative stability index indicate a high degree of lipid peroxidation. If a lipid undergoes a mild degree of lipid peroxidation and most of the hydroperoxides formed had not been decomposed, it is economical and feasible to use peroxide value as a primary measure of peroxidation because there is less concern about decomposition of hydroperoxides and peroxide value can accurately reflect the degree of lipid peroxidation. However, if a lipid is subjected to a high degree of lipid peroxidation, and most of the hydroperoxides formed have already been decomposed to yield secondary or tertiary lipid peroxidation products, a single measure of secondary oxidation products, such as p-anisidine value or thiobarbituric acid substances may be more acceptable and economical because the production of secondary lipid peroxidation products can be measured more accurately than a single peroxide value analysis. Furthermore, for better evaluation of

lipid peroxidation of lipids that have been subjected to extreme peroxidation, future research should focus on development of measures used to assess lipid peroxidation based on quantification of the triacylglycerol dimers or triacylglycerol polymers. The amount of dimers and polymers formed during lipid peroxidation keeps increasing as the heating time is increased (Sanchez-Muniz et al., 1993; Takeoka et al., 1997).

Table 2.1. Characteristics of the experimental lipids[1]

	Corn oil			Canola oil			Poultry fat			Tallow		
	OL	SO	RO	OL	SO	RO	OL	SO	RO	OL	SO	RO
Crude fat, %	99.34	99.36	99.26	99.16	99.50	99.26	95.52	96.42	98.23	98.04	98.68	99.02
Free fatty acids, %	0.28	0.48	0.65	0.36	0.57	0.58	3.62	3.65	3.17	1.99	3.10	2.28
Total MIU[2]	1.00	1.02	1.22	1.01	0.89	0.96	2.24	1.01	1.23	0.78	0.60	0.64
Moisture, %	0.06	0.00	0.06	0.08	0.00	0.00	0.19	0.02	0.07	0.15	0.10	0.07
Insolubles, %	0.02	0.04	0.08	0.02	0.02	0.02	1.08	0.08	0.22	0.22	0.16	0.23
Unsaponifiables, %	0.92	0.98	1.06	0.91	0.87	0.94	0.97	0.93	0.94	0.41	0.34	0.34
Fatty acids, %												
Myristic (14:0)	0.06	0.06	0.07	0.08	0.09	0.08	0.63	0.63	0.65	3.03	3.21	3.29
Palmitic (16:0)	10.76	11.90	12.11	3.95	4.39	4.43	24.69	24.49	24.68	24.50	24.68	25.94
Palmitoleic (16:1)	0.10	0.10	0.12	0.22	0.23	0.23	7.11	7.39	7.19	2.55	2.71	2.55
Stearic (18:0)	1.71	1.91	1.93	1.78	1.93	1.95	5.93	5.62	5.80	21.59	20.00	21.97
Oleic (18:1)	27.70	29.84	29.80	64.57	65.47	66.82	38.07	39.16	39.20	32.03	33.48	30.62
Linoleic (18:2)	57.18	52.73	52.32	17.90	16.51	15.93	18.50	17.59	17.10	2.80	1.83	1.84
Linolenic (18:3)	0.79	0.62	0.63	7.09	5.73	5.01	0.77	0.67	0.69	0.22	0.12	0.11
U:S[3]	6.85	6.01	5.87	15.45	13.72	13.62	2.06	2.11	2.06	0.77	0.80	0.69
Iodine value[4]	125	119	118	105	100	98	73	73	72	35	35	32
Vitamin E, IU/g	0.40	< 0.10	< 0.10	0.29	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10	< 0.10
Oxidation products												
PV[5], mEq/kg	1	151	2	1	239	12	1	57	2	1	29	3
p-Anisidine[6]	< 1	61.4	142.9	1	37.0	154.8	3	88	22	4	120	19
TBARS[7], µmol/kg	16	225	119	45	968	622	79	151	58	58	61	41
Hexanal, mg/kg	< 1	390	83	1	180	59	3	88	22	4	120	19
2, 4-decadienal, ppm	72	3728	1345	7	1091	511	30	442	169	47	261	125
HNE[8], µmol/kg	0	194	594	0	105	221	0	2	0	0	13	6
AOM[9], mEq/kg	103	575	528	112	419	533	4	298	5	< 2	6	446
OSI[10], h	8.4	< 1.0	< 1.0	9.2	< 1.0	< 1.0	24.6	< 1.0	< 1.0	12.1	< 1.0	< 1.0

[1]OL: Lipids were stored as received without antioxidants or heating; SO, lipids heated for 72 h at 95°C with constant compressed air flow rate at 12 L/min; RO, lipids heated for 7 h at 185°C with constant compressed air flow rate at 12 L/min.

[2]Total of moisture, insolubles, and unsaponifiables.

[3]Unsaturated to saturated fatty acid ratio.

[4]Iodine value was calculated by the following equation: iodine value = (C16:1) × 0.95 + (C18:1) × 0.86 + (C18:2) × 1.732 + (C18:3) × 2.616 (Method Cd 1-25; AOCS, 1998).

[5]PV = peroxide value

[6]There is no unit for p-anisidine value.

[7]TBARS = thiobarbituric acid reactive substances

[8]HNE = 4-hydroxynonenal.

[9]AOM = active oxygen method measured as the peroxide value at 20 h of oxidation.

[10]OSI =Oxygen stability index.

Table 2.2. Correlation matrix among lipid composition and various oxidation measures[1]

	CF	FFA	MIU	Mo	In	Usap	Myr	Pal	Pmo	Ste	Ole	Lin	Linol	US	IV	VE	PV	AnV	TBARS	Hex	DDE	HNE	AOM	OSI
CF	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
FFA	-0.81 0.01	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MIU	-0.66 0.02	NS	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mo	-0.57 0.05	0.50 0.10	NS	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
In	-0.77 0.01	0.60 0.04	0.80 0.01	0.77 0.01	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Usap	NS	NS	0.58 0.05	NS	NS	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Myr	NS	NS	NS	NS	NS	-0.97 0.01	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pal	-0.64 0.03	0.89 0.01	NS	0.57 0.05	0.51 0.09	-0.52 0.08	0.69 0.01	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pmo	-0.86 0.01	0.93 0.01	NS	NS	0.60 0.04	NS	NS	0.77 0.01	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ste	NS	NS	NS	NS	NS	-0.96 0.01	0.99 0.01	0.71 0.01	NS	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ole	NS	NS	NS	NS	NS	NS	NS	-0.66 0.02	NS	NS	1.0	-	-	-	-	-	-	-	-	-	-	-	-	-
Lin	NS	-0.56 0.06	NS	NS	NS	0.68 0.02	-0.68 0.02	NS	NS	-0.68 0.01	NS	1.0	-	-	-	-	-	-	-	-	-	-	-	-
Linol	NS	-0.54 0.07	NS	NS	NS	NS	NS	-0.80 0.01	NS	-0.49 0.10	0.95 0.01	NS	1.0	-	-	-	-	-	-	-	-	-	-	-
US	0.52 0.09	-0.76 0.01	NS	-0.51 0.09	NS	NS	-0.65 0.02	-0.96 0.01	-0.63 0.03	-0.67 0.02	0.83 0.01	NS	0.94 0.01	1.0	-	-	-	-	-	-	-	-	-	-
IV	NS	-0.72 0.01	NS	-0.51 0.09	NS	0.85 0.04	-0.92 0.01	-0.79 0.01	NS	-0.93 0.01	NS	0.85 0.01	NS	0.66 0.02	1.0		-	-	-	-	-	-	-	-
VE	NS	-0.47 0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.0								
PV	NS	NS	NS	-0.57 0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.0	-	-	-	-	-	-	-
AnV	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	1.0	-	-	-	-	-	-
TBARS	NS	NS	NS	-0.58 0.05	NS	NS	NS	-0.59 0.04	NS	NS	0.70 0.01	NS	0.60 0.04	0.62 0.03	NS	NS	0.75 0.01	NS	1.0	-	-	-	-	-
Hex	NS	NS	NS	-0.57 0.06	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.50 0.10	NS	0.76 0.01	NS	NS	1.0	-	-	-	-
DDE	NS	NS	NS	-0.53 0.08	NS	NS	NS	NS	NS	NS	NS	0.56 0.06	NS	NS	NS	NS	0.61 0.04	NS	NS	0.94 0.01	1.0	-	-	-
HNE	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.54 0.07	NS	NS	NS	NS	NS	0.67 0.02	NS	NS	0.49 0.10	1.0	-	-
AOM	NS	-0.51 0.09	NS	-0.75 0.01	NS	NS	NS	NS	-0.50 0.10	NS	NS	NS	NS	NS	NS	NS	NS	0.53 0.08	0.51 0.09	0.57 0.06	0.65 0.02	0.66 0.02	1.0	-
OSI	-0.60 0.04	NS	0.70 0.01	0.81 0.01	0.78 0.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	-0.57 0.05	NS	NS	NS	NS	-0.58 0.05	1.0

[1] Abbreviations: CF = crude fat, FFA = free fatty acids, MIU = moisture, insolubles, and unsaponifiables, Mo = moisture, In = insolubles, Unsap = unsaponifiables, Myr = myristic acid, Pal = palmitic acid, Pmol = palmitoleic acid, Ste = stearic acid, Ole = oleic acid, Lin = linoleic acid, Linol = linolenic acid, US = unsaturated:saturated ratio, IV = iodine value, VE = vitamin E, PV = peroxide value, AnV = p-ansidine value, TBARS = thiobarbituric acid reactive substances, Hex = hexanal, DDE = 2, 4-decadinal, HNE = 4-hydroxy nonenal, AOM = active oxygen method, and OSI = oxidative stability index. Top value represents correlation (r value) and bottom value represents significance (*P* value). If no value is given, it was not found to be different at $P \leq 0.10$ and listed as NS = non-significant.

CHAPTER 3

INFLUENCE OF THERMALLY-OXIDIZED VEGETABLE OILS AND ANIMAL FATS ON GROWTH PERFORMANCE, LIVER GENE EXPRESSION, AND LIVER AND SERUM CHOLESTEROL AND TRIGLYCERIDES IN YOUNG PIGS

To evaluate the effect of feeding thermally-oxidized vegetable oils and animal fats on growth performance, liver gene expression, and liver and serum fatty acid and cholesterol concentration in young pigs, 102 barrows (6.67 ± 0.03 kg BW) were divided into 3 groups and randomly assigned to dietary treatments in a 4 × 3 factorial arrangement. The main factors were lipid source [n = 4: corn oil (CN), canola oil (CA), poultry fat (PF), and tallow (TL)] and lipid peroxidation level [n =3: original lipids (OL), slow oxidation (SO) through heating at 95°C for 72 h, or rapid oxidation (RO) through heating at 185°C for 7 h]. Pigs were provided *ad libitum* access to diets in group pens for 28 d, followed by controlled feed intake in metabolism crates for 10 d. On d 39, all pigs were euthanized for liver samples to determine liver weight, lipid profile, and gene express patterns. Lipid oxidation analysis indicated that compared to the OL, SO and RO had a markedly increased concentrations of primary and secondary peroxidation products, and the increased lipid peroxidation products in CN and CA were higher than those in PF and TL. After a 28-d *ad libitum* feeding period, pigs fed RO tended to have reduced ADFI ($P = 0.09$), and ADG ($P < 0.05$) compared to pigs fed OL, and pigs fed CA had reduced G:F ($P < 0.05$) compared to pigs fed all other lipids. Pigs fed RO lipids tended to have increased liver weight ($P = 0.09$) compared to pigs fed OL. Liver triglyceride

concentration (LTG) in pigs fed OL was greater ($P < 0.05$) than in pigs fed RO, and tended to be greater ($P < 0.07$) than in pigs fed SO. The reduced LTG were consistent with increased ($P < 0.05$) mRNA expression of PPARα factor target genes (acyl-CoA oxidase, carnitine palmitoyltransferase-1, and mitochondrial 3-hydroxy-3-methylglutary-CoA synthase) in pigs fed SO and RO lipids compared with pigs fed OL. Pigs fed CN or CA tended to have increased LTG ($P = 0.09$) compared to pigs fed TL. Liver cholesterol concentration in pigs fed CN was less ($P < 0.05$) than pigs fed PF, and tended to be less ($P = 0.06$) than pigs fed TL, whereas pigs fed CA had a reduced ($P < 0.05$) liver cholesterol compared to pigs fed PF or TL. In conclusion, feeding thermally-oxidized lipids negatively affected growth performance and liver triglyceride concentrations of young pigs.

Keywords: cholesterol, growth performance, liver, pigs, thermally oxidized lipids, triglycerides

INTRODUCTION

Lipids are commonly added into swine diets as concentrated energy sources to improve feed efficiency (Pettigrew and Moser, 1991). In addition, supplementing lipids into swine diets provides advantages of reducing dust, supplying fat soluble vitamins and essential fatty acids, and improving diet palatability. Large quantities of lipids produced from food processing facilities or restaurants are rendered and can be used as economical sources of energy in animal feeds (Canakci, 2007). However, these lipids normally are heated for a considerable length of time at a high temperature (Frankel et al., 1984) and may be thermally sensitive and unstable and thus, sensitive to lipid peroxidation depending on their degree of unsaturation (Sherwin, 1978; Linfield et al., 1985). Heating lipids may also generate toxic lipid peroxidation products (Lin et al., 1989; Frankel, 1998; Adam et al., 2008) and may deplete endogenous antioxidants (Seppanen and Csallany, 2002). Kimura et al. (1984) reported that feeding oxidized soybean oil not only impaired growth performance, but induced diarrhea in rats. Alexander et al. (1987) and Behniwal et al. (1993) also reported that rats fed diets containing oxidized corn or peanut oil had impaired growth rate. Similarly in broilers, ingestion of oxidized poultry fat reduced growth compared with birds fed unoxidized poultry fat (Cabel et al., 1988; Dibner et al., 1996a). In pigs, DeRouchey et al. (2004) reported that gain decreased as the rancidity of choice white grease consumed increased, while Fernandez-Duenas (2009) reported that feeding oxidized corn oil reduced growth performance. Neither of these studies, however, evaluated any metabolic effects of consuming oxidized lipids in pigs. This study was conducted to investigate the effects of feeding thermally-oxidized vegetable oils and

animal fats on growth performance, liver gene expression, and liver and serum fatty acid composition in young pigs.

MATERIALS AND METHODS

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

Animals, Experimental Design, and Diets

Weaned barrows (n = 102; initial BW of 6.67 ± 0.03 kg) were divided into 3 groups of 34 and housed at the Southern Research and Outreach Center (Waseca, MN) for this study. Pigs were terminal offspring of Yorkshire × Landrace sows (TOPIGS USA, Des Moines, IA) sired by Duroc boars (Compart Boar Store, Nicollet, MN). In each group, pigs were assigned randomly to 1 of 12 dietary treatments, resulting in 2 or 3 pigs/replicate pen and 1 replicate pen/treatment for each group.

To generate oxidized lipids, original lipids (**OL**) were either heated at 95°C for 72 h to produce slow-oxidation (**SO**) lipids or heated at 185°C for 7 h to produce rapid-oxidation (**RO**) lipids. Both heating processes were accompanied with constant flow of compressed air of 12,000 cm^3/min at a temperature between 22 to 24°C. Before feed mixing, the OL, SO, and RO lipids were stored at -20°C, and no antioxidant was added before or during diet preparation. Treatments consisted of 12 corn-soybean meal based diets supplemented with 10% lipids and were arranged in a 4 × 3 factorial design. The two main factors were lipid source [corn oil (**CN**; ADM, Decatur, IL), canola oil (**CA**; ConAgra Foods, Omaha, NE), poultry fat (**PF**; American Protein, Inc., Hanceville, AL),

and tallow (**TL**; Darling International, Wahoo, NE)] and oxidation level (OL, SO, and RO).

Experimental diets were formulated based on a 2-phase program. To compensate for the expected reduction in feed intake caused by increased caloric density of the lipid supplemented diets, a constant nutrient to ME ratio was used based on the ME content of CA, which had the highest ME concentration of all the lipid sources evaluated (8,410 kcal ME/kg, NRC, 1998). Canola oil diets were formulated first by adjusting the standardized ileal digestible Lys, Met, Thr, Trp, total Ca, and available P to ME ratio recommended by NRC (1998). Other lipid supplemented diets were then formulated by replacing 10% CA with the other lipids. Phase-1 diets (Table 3.1) were formulated based on NRC (1998) recommendations for pigs weighing 7 to 19 kg and were fed to pigs from d 1 to 24 post-weaning. Due to slower than expected growth of pigs during Phase-1, Phase-2 diets (Table 3.1) were formulated based on the NRC (1998) recommendation for pigs weighing 13 to 20 kg and were fed to pigs from d 25 to 39 of the experiment. Diets for each phase and for each of the 3 groups were mixed 4-d before they were fed initially and stored at 4°C throughout the feeding period of each phase.

During the first 28 d of each group, 2 or 3 pigs from the same dietary treatment were housed in one pen in an environmentally controlled room (27 to 28°C) and were provided *ad libitum* access to feed and water. Body weight and feed consumption of pigs in each pen were determined on d 1, 9, 17, 25, and 29 to calculate ADG, ADFI, and G:F. From d 29 to d 39, pigs were housed individually in metabolism crates in an environmentally controlled room (25 to 27°C) and fed an amount of diet equivalent to 4% of their BW daily (2% at 0700 h and 2% at 1900 h). Pigs were allowed *ad libitum* access

to water. After the morning feeding at 0700 h on d 37, all pigs were fasted for 24 h and a blood sample was collected at 0700 h of d 38 to obtain fasted serum. Approximately 8 mL of blood was obtained by jugular venipuncture using a 10-mL serum tube (Becton Dickinson, Franklin Lakes, NJ). Blood samples were centrifuged at 2,500 × g (Heraeus Biofuge 22R Centrifuge, Hanau, Germany) for 15 min at 4°C and serum was harvested. Serum samples were frozen immediately and stored at -20°C for subsequent triglyceride (**TG**) and cholesterol (**CH**) analysis. At 0700 h of d 39, all pigs were euthanized with 1 mL pentobarbital sodium solution (390 mg/ml, Fatal-Plus Powder, Vortech Pharmaceuticals, Dearborn, MI) per 4.53 kg of BW by intracardiac injection. Livers were excised and weighed to calculate liver weight as a percentage of BW, frozen immediately on dry ice, and stored at -80°C until subsequent liver lipid profile and gene analysis was conducted. The chemical and oxidative status of the experimental lipids is described in detail in Chapter 2.

Serum and Liver Cholesterol and Triglycerides

Total lipids from the liver were extracted using the modified method of Folch et al. (1957). Briefly, about 0.25 g of frozen liver tissue was weighed and transferred into a 2-mL flat-bottom centrifuge tube containing 0.5 mL methanol. After homogenization, 0.5 mL of chloroform and 0.4 mL of water were added to the liver homogenate and mixed by vortexing. The lipid fraction in chloroform was separated from the aqueous fraction and liver debris by centrifuging for 10 min at 14,000 × g, and was then transferred to a new glass tube. After drying under N_2, the lipid fraction was reconstituted in butanol for further analysis of TG and CH. Both TG and CH in serum and n-butanol solution of liver lipid extraction were determined enzymatically using commercial kits (T7531 for TG,

C7509 for CH, Pointe Scientific, Canton, MI). These measurements were performed using a spectrometer (SpectraMAX 250, Molecular Devices, Sunnyvale, CA) following the manufacturer's instructions.

Gene Expression Analysis

Total RNA from liver tissue was isolated using trizol reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. The concentration and quality of RNA were measured using a spectrophotometer at 260 and 280 nm (NanoDrop 2000C, Thermo Fisher Scientific, Wilmington, DE). The reverse transcription of 1 μg of total RNA to cDNA was conducted using SuperScript II Reverse Transcriptase (Invitrogen Life Technologies, Carlsbad, CA). The expression levels of targeted genes were measured by real-time reverse transcriptase PCR using SYBR Green PCR Master Mix in a StepOne Plus system (Applied Biosystems, Carlsbad, CA). For determination of mRNA concentration, a threshold cycle and amplification efficiency was obtained from each amplification curve using the StepOne system (Applied Biosystems, Carlsbad, CA). Quantification of the relative mRNA concentration was calculated using the comparative threshold cycle method (Livak and Schmittgen, 2001). The GAPDH was used as the housekeeping gene as described by others (Kerr et al., 2005; Paczkowski et al., 2011) because the abundance of this gene transcript remained stable in gene expression (not statistically different and data are not provided).The sequences of customized primers used in the RT-PCR reactions (Integrated DNA Technologies, Coralville, IA) are listed in Table 3.2.

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS (Version 9.2; SAS Inst. Inc., Cary, NC). Two-way ANOVA was conducted to evaluate the main effects of lipid source (CN, CA, PF, and TL), lipid peroxidation level (OL, SO, and RO), and any 2-way interactions in a 4 × 3 factorial arrangement of treatments. The corresponding statistical model included the fixed effects of lipid source, peroxidation level, and lipid source × peroxidation level interactions. Group was included as a random effect. Pen was used as the experimental unit in analysis of growth performance responses, while individual pig was used as the experimental unit for all other responses. Initial BW on d 1 was also used as a covariate in analysis of growth performance data. All results are reported as least squares means. Mean comparisons were achieved by the PDIFF option of SAS with the Tukey-Kramer adjustment. In addition, relationships between measures of lipid peroxidation and various variables were evaluated by simple linear correlation (Pearson correlation coefficients) analysis. Treatment effects were considered significant if $P < 0.05$, whereas values between $0.05 \leq P \leq 0.10$ were considered statistical trends.

RESULTS

Characterization of Experimental Lipids

The various characteristics of the experiment lipids have been described in detail previously (Chapter 2) and are shown in Table 2.1. Briefly, the concentration of crude fat, moisture, insolubles and unsaponifiables were similar among the 12 experimental lipids. As expected, CN and CA had higher concentrations of unsaturated fatty acids than TL, with PF being intermediate. Averaged among lipid sources, both SO and RO decreased the linoleic acid and linolenic acid concentrations compared to the OL, but changes in

concentration of other major fatty acids were not observed. As expected, lipid peroxidation tests indicated that all of the OL were relatively unoxidized, but SO and RO led to a marked increase in the production of primary and secondary peroxidation products, and the production of these peroxidation products caused by SO and RO in CN and CA was much higher than that in PF and TL (Chapter 2).

Growth Performance

During the first 28 d, no lipid source or lipid source × peroxidation interactions were observed for ADFI and ADG (Table 3.3). In contrast, peroxidation level affected both ADFI ($P = 0.09$) and ADG ($P = 0.04$). Compared to pigs fed OL, pigs fed RO lipids tended to have reduced ADFI ($P = 0.09$) and reduced ADG ($P = 0.03$). No differences in ADG or ADFI were found between pigs fed SO and OL lipids, or between pigs fed SO and RO lipids. There was no lipid source × peroxidation level interaction noted for G:F, but lipid source affected G:F ($P = 0.02$), where pigs fed CA supplemented diets had a lower G:F compared to pigs fed the other three lipid supplemented diets ($P < 0.05$).

Liver Weight

No lipid source or lipid source × peroxidation level interaction was noted for liver weight (Figure 3.1). Liver weight tended to be greater for pigs fed RO lipids compared to pigs fed OL ($P < 0.09$), but no other differences due to lipid peroxidation were observed.

Serum and Liver Cholesterol and Lipid Content

No lipid source, peroxidation level, or lipid source × peroxidation level interaction effects were noted for TG or CH in serum collected from pigs after a 24 h fast (Figure 3.2). For liver TG, no lipid source × peroxidation level interaction was noted, but peroxidation level and lipid source affected ($P < 0.05$) liver TG concentrations. Liver TG

concentration in pigs fed OL was less than in pigs fed RO lipids ($P < 0.05$), and tended to be less than pigs fed SO lipids ($P = 0.07$). No differences in TG concentrations in liver were noted between pigs fed SO and RO lipid supplemented diets. Pig fed either CN or CA supplemented diets tended to have increased liver TG concentrations compared to pigs fed TL supplemented diets ($P = 0.09$). However, no differences in liver TG concentrations were found between pigs fed PF and TL diets, or between pigs fed CN and CA diets.

No oxidation level or interaction effects were observed for liver CH concentration (Figure 3.2). However, a lipid source effect was observed for liver CH concentration ($P < 0.01$). Pigs fed CN had a lower ($P < 0.05$) liver CH concentration compared to pigs fed PF, and tended to have reduced ($P = 0.06$) liver CH concentration compared to pigs fed TL. Pig fed CA had a lower ($P < 0.05$) liver CH concentration compared to pigs fed PF or TL. No differences in liver CH concentration were found between pigs fed CN and CA or between pigs fed PF and TL.

Relative mRNA Levels of Genes in the Liver

Because there were significant changes in hepatic TG concentrations as well as liver weight between pigs fed OL and thermally-oxidized lipids, the mRNA level of genes encoding enzymes or transcription factors involved in fatty acid anabolism and catabolism in the liver were analyzed (Table 3.4). Genes analyzed included: peroxisome proliferator activated receptor α (**PPARα**, a target gene encoding the enzyme catalyzing the first step of fatty acid β-oxidation in peroxisomes), acyl-CoA oxidase (**ACO**, a classical PPARα factor), carnitine palmitoyltransferase 1 (**CPT-1**, a classical PPARα target gene encoding the enzyme essential for transportation of fatty acids into the

mitochondria for β-oxidation), mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase (**mHMG-CoA-S**, a PPARα target gene encoding the enzyme involved in ketone genesis), stearoyl-CoA desaturase (**SCD**, a key enzyme in fatty acid metabolism which catalyzes the formation of oleic acid from steric acid), sterol regulatory element-binding protein-1 (**SREBP-1**, a target gene encoding the enzymes that serve as the rate-limiting enzymes catalyzing the synthesis of monounsaturated fatty acids and is associated with regulating the genes required for *de novo* lipogenesis), and sterol regulatory element-binding protein-2 (**SREBP-2**, a transcription factor that binds to the sterol regulatory element of the DNA sequence and is responsible for regulating the genes required for cholesterol metabolism).

No effect of lipid source or lipid source × peroxidation level interaction was found in mRNA analysis of PPARα, ACO, CPT-1, and mHMG-CoA-S (Table 3.4). However, a peroxidation level effect in liver mRNA level of these genes was observed ($0.05 < P < 0.10$). Pigs fed either SO or RO supplemented diets had greater mRNA levels of ACO, CPT-1, and mHMG-CoA-S than those fed OL supplemented diets ($P < 0.05$). Oxidation level tended to increase the mRNA expression level of PPARα ($P = 0.08$), but no differences mRNA abundance of PPARα among OL, SO, and RO were observed. Lastly, no lipid source, peroxidation level, or lipid source × peroxidation level interaction effects were found in the mRNA expression level of SCD, SREBP-1, and SREBP-2.

Correlation Analysis

Since lipids with various degrees of peroxidation, measured using different peroxidation tests, result in differences in growth performance, liver weight, liver TG concentrations, and expression of certain liver genes (PPARα, ACO, CPT, and HMG-

CoA-S) of pigs, a correlation analysis between various measures of lipid peroxidation and growth performance, liver weight, liver TG concentration, and expression of liver genes was conducted to determine the importance of various lipid peroxide measures in predicting different biological responses (Table 3.5). Due to the limited number of animals used in the current experiment, correlations where the *P*-value was 0.2 or less were considered. For growth performance, only a negative correlation ($P = 0.09$; r = -0.29) between thiobarbituric acid reactive substances (**TBARS**) and ADG was observed. Except for PV and OSI tests, negative correlations were found between p-anisidine (**pAV**) and liver weight and ($P = 0.06$; r = 0.19), between TBARS and liver weight ($P = 0.11$; r = 0.16), between hexanal (**HEX**) and liver weight ($P = 0.17$; r = 0.14), between 2, 4-decadinal (**DDE**) and liver weight ($P = 0.13$; r = 0.15), between 4-hydroxynonenal (**HNE**) and liver weight ($P = 0.02$; r = 0.24), and between active oxygen method (**AOM**) and liver weight ($P = 0.03$; r = 0.22). All the measures of lipid oxidation were correlated with the gene expression of PPARα, ACO, CPT, and HMG-CoA-S as shown in Table 3.5 No correlation was found between TG and any measures of lipid oxidation.

DISCUSSION

Lipid Composition and Oxidation

In the current study, the chemical and oxidative status of the experimental lipids varied greatly due to lipid source and peroxidation status as described in detail by Liu et al. (2012).

Pig Performance

All the pigs were allowed a 28-d nursery adaptation where pigs were provided *ad libitum* access to feed in a pen, followed by a 10-d controlled feeding program in

metabolism crates. During the first 28-d nursery adaptation period, one pig from the first group fed original CA and one pig from the second group fed slow oxidized PF died. Overall, lipid supplemented diets had 8 or 9 observations per treatment. After the 28-d *ad libitum* feeding, the growth performance results observed in the current study are consistent with those reported in other studies (Alexander et al., 1987; Behniwal et al. 1993; Dibner et al., 1996a) where feeding diets supplemented with heated CN, peanut oil, or PF to rats or poultry reduced ADFI and ADG. In the current experiment, pigs fed the RO lipids had a lower ADG than those fed OL, but the growth rate of pigs fed SO and OL were not different, potentially due to our use of only 3 replications per treatment for the growth performance portion of this study. Reduced growth rate in pigs fed thermally-oxidized lipids may be caused by several factors. First, reduced growth rate could be a result of rancidity of thermally-oxidized lipids, which can reduce diet palatability, and thereby decrease feed intake leading to a poor growth rate (DeRouchey et al., 2004). Secondly, impaired growth may be a consequence of the toxic effects of the lipid peroxidation products. Secondary lipid peroxidation products, such as α, β-unsaturated hydroxy aldehydes, are of particular interest because some of them are highly toxic and readily absorbed (Kanazawa et al., 1985; Grootveld et al., 1998; Kim et al., 1999). Reactive secondary lipid peroxidation products are capable of modifying proteins *in vivo* by damaging the intestinal brush border membrane (Kanazawa et al., 1985; Witz, 1989; Comporti, 1993) which may result in poor nutrient absorption and feed conversion, leading to growth depression. Weight loss and high mortality in experimental animals consuming thermally-oxidized oils has been reported by others (Crampton et al., 1953; Giani et al., 1985; Chow, 1989). In addition, results from the current experiment showed

that pigs fed CA had the poorest gain efficiency compared with those fed the other 3 lipid sources, which may be due to CA having the highest total secondary oxidation products as indicated by the highest TBARS concentration in the present study. Correlation analysis of ADG and measures of lipid oxidation indicate that TBARS value of the lipid was inversely correlated ($P = 0.09$; r = -0.29) with the ADG in pigs. This finding might be helpful to predict the differences in ADG when using lipids with high TBARS values during diet formulation.

Liver Weight

Liver weight, expressed as a percentage of final BW, is important to evaluate general toxicity because the liver is an important organ for nutrient metabolism and is a sensitive indicator of toxicity (Amacher et al., 2006; Juberg et al., 2006). In the current experiment, after feeding diets containing 10% oxidized lipids for 38 d, the toxic effect of secondary lipid peroxidation products was observed in pigs fed diets containing RO lipids, which was likely due to RO lipids having the highest concentrations of HNE and TBARS. Since lipid peroxidation products, especially α, β-unsaturated hydroxyaldehydes such as HNE, are readily absorbed (Kanazawa et al., 1985; Grootveld et al., 1998), and are highly reactive, it is likely that this led to hepatic oxidative stress. The development of oxidative stress by consumption of thermally-oxidized fats has been reported in previous studies in pigs and other animals including guinea pigs, rats, and broilers (Zhang et al., 2011, Eder et al., 2004; Keller et al., 2004). In addition, many previous studies indicated that animals fed oxidized lipids experience a greater rate of hepatic lipid metabolism and stress responses (Liu and Huang, 1995, 1996; Liu et al., 2000). Thus, the increase in liver weight may be a result of increased secretion of stress hormones, greater metabolic

activity, or was reflective of increased expression of mRNA levels for lipid metabolizing enzymes in hepatocytes. Correlation analysis of liver organ weight and measures of lipid peroxidation showed that positive correlations existed between liver weight and pAV ($P = 0.06$; $r = 0.19$), between liver weight and TBARS ($P = 0.11$; $r = 0.16$), between liver weight and HEX ($P = 0.17$; $r = 0.14$), between liver weight and DDE ($P = 0.13$; $r = 0.15$), between liver weight and HNE ($P = 0.02$; $r = 0.24$), and between liver weight and AOM ($P = 0.03$; $r = 0.22$). Although there were significant positive correlations between liver weight and HNE and AOM, they explained a low percentage of the variation in liver weight. Among all of these measures of lipid oxidation, HNE concentration provided the most accurate prediction of liver weight, which can be explained by the well-known cytotoxic and mutagenic effects of HNE (Witz, 1989; Esterbauer et al., 1991).

Serum and Liver Cholesterol and Lipid Content

Pigs fed both SO or RO lipids had reduced liver TG concentrations compared to pigs fed OL, regardless of the lipid source. Decreased concentration of TG in the liver caused by consumption of oxidized soybean oil or a mixture of lard and sunflower oil has been reported in rats (Huang et al., 1988; Eder, 1999; Chao et al., 2004). Because there were significant changes in hepatic TG concentrations in the current study, as well as increased liver weight between pigs fed OL and thermally-oxidized lipids, mRNA levels of genes encoding enzymes or transcription factors involved in fatty acid anabolism and catabolism were analyzed in the liver. As expected, regardless of lipid source fed, both SO and RO of lipids caused activation of PPARα in the liver, as indicated by the increased expressions of typical PPARα targeted genes, including ACO, CPT-1, and mHMG-CoA-S. Activation of PPARα caused by feeding oxidized lipids had been

reported from studies in both rats and pigs (Chao et al., 2001; Chao et al., 2005; Luci et al., 2007). The mechanism of activation of PPARα is possibly related to the presence of hydroxyl- and hydroperoxy fatty acids in the thermally oxidized fat, which can function as potent activators of PPARα (Delerive et al., 2000; Luci et al., 2007). The activation of PPARα can stimulate the expression of the PPARα target genes, which in turn contributes to reduced liver TG concentrations due to the increased activities of fatty acid transportation across the cell membrane, intracellular lipid trafficking, mitochondrial and peroxisomal fatty acid uptake, and both mitochondrial and peroxisomal fatty acid β-oxidation and ketogenesis. The decreased concentrations of liver TG in pigs fed SO and RO support the assumption that oxidized lipids activated PPARα pathway as well. The correlation analysis between gene expression and measures of lipid oxidation suggest that all of the tests of lipid peroxidation including PV, pAV, TBARS, HEX, DDE, HNE, AOM, and OSI were helpful in predicting gene expression of PPARα, ACO, CPT, and HMG-CoA-S. Compared to other measures of lipid peroxidation, pAV, AOM, and OSI may contribute more to the gene expression of PPARα, ACO, CPT, and HMG-CoA-S because they had a higher correlation with the relative gene expression. In addition to fatty acid β-oxidation and fatty acid ketogenesis, fatty acid *de novo* synthesis is controlled by the hepatic balance of fatty acids. The effect of feeding oxidized fat on gene expression of lipogenic enzymes is controversial. Luci et al. (2007) reported an up-regulation effect of oxidized sunflower oil on SREBP-1 and its target genes ACC and SCD in pigs, while other researchers reported a down regulation of lipogenic enzymes in rats fed oxidized soybean oil (Eder and Kirchgessner, 1998) or a mixture of sunflower oil and lard (Eder et al., 2003). Results from our study showed that the hepatic mRNA levels

of SREBP-1 (a transcription factor controlling fatty acid synthesis) and its target gene SCD (a key enzyme that controls *de novo* fatty acid synthesis) were not influenced by either SO or RO. The exact mechanisms are still unknown, and the various effects of oxidized lipids on fatty acid synthesis might be a result of the different degrees of peroxidation products in the oxidized lipids. Considering that all pigs were fed with adequate dietary TG, and the mRNA levels of lipogenic genes were similar among OL, SO, and RO, we speculate that the decreased hepatic TG concentrations in pig fed SO and RO lipid containing diets was mainly due to the activation of the PPARα caused by feeding thermally-oxidized lipids. Two major pathways of TG metabolism include incorporating into adipose tissue to function as stored energy, or providing energy for maintaining essential life functions including lean tissue synthesis. Thus, the decreased growth performance observed in animals fed thermally-oxidized lipids may be related to the activation of PPARα pathway by the thermally-oxidized lipids, and subsequently may lead to decreased lipid availability for adipose tissue accretion and protein synthesis.

The effect of feeding highly oxidized vegetable oils, fish oil, or mixtures of vegetable oils and animal fats on TG metabolism has been extensively studied in rats (Hochgraf et al., 1997; Eder, 1999; Chao et al., 2005). However, studies conducted to determine the differential effect of thermally-oxidized oils and animal fats on the lipid profiles in pigs are limited. In the current experiment, regardless of the peroxidation level, pigs fed either CN or CA had increased liver TG concentrations compared to those fed TL, but liver TG concentrations were not different between pigs fed PF and TL. Furthermore, there were no differences in hepatic mRNA levels of genes involved in PPARα pathway and fatty acid *de novo* synthesis (SREBP-1 and SCD) among pigs fed

different lipid sources. We suspect that the different fatty acid profiles of the lipids evaluated in this study resulted in differences in fatty acid digestibility, which would account for the differences in liver TG concentrations.

In the current study, oxidation level of lipids did not affect serum or liver CH concentrations regardless of lipid source. Similarly, Luci et al. (2007) also reported that liver and serum concentration of CH of pigs fed fresh or oxidized lipids were not different, although they did report that oxidized lipids could stimulate CH synthesis by up-regulation of SREBP-2, a transcription factor which controls CH synthesis by activating the transcription of genes for CH synthesis. However, the effect of oxidized lipids on genes controlling CH synthesis, such as SREBP-2 is not clear. In a study by Konig et al. (2007), they reported an opposite effect where oxidized fat suppressed gene expression of SREBP-2 and its target genes, leading to reduced CH synthesis in rats. In the current experiment, there were no differences in hepatic gene expression of SREBP-2 among pigs fed different degrees of oxidized lipids.

Pigs fed different sources of lipid tended to have different hepatic CH concentrations. Generally, pigs fed vegetable oils had relatively lower liver CH concentrations compared to pigs fed PF or TL. Pigs fed CN diets had lower liver CH concentration compared to pigs fed PF, and tended to have lower liver CH than pigs fed TL. Pigs fed CA had a lower liver CH concentration compared to pigs fed either PF or TL. There were no differences in hepatic mRNA levels of SREBP-2 found among pigs fed different sources of lipids. Previous studies in rats (Smith et al., 1993; Takeuchi et al., 1995) and pigs (Eder and Stangl, 2000) demonstrated that feeding lipids with high concentrations of mono- or poly-unsaturated fatty acids increased concentrations of

plasma triiodothyronine relative to fat sources that contain predominantly saturated fatty acids, such as lard and TL. Furthermore, the inverse correlation between the circulating concentrations of CH and thyroid hormone are well known (Engelken and Eaton, 1981; Aviram et al., 1982; Eder and Stangl, 2000). Thus, the reduced liver CH concentrations in pigs fed CN and CA diets compared with pigs fed PF and TL might be a consequence of increased thyroid hormone, due to the greater total amount of unsaturated fatty acids in CN and CA compared with those in PF and TL (86% and 89% vs. 64% and 37%, respectively). Another reason for the greater hepatic CH concentrations in animal fat diets may be related to the greater CH concentration in PF and TL than in CN and CA, which subsequently may have led to greater absorption and uptake of CH in the liver.

In conclusion, pigs fed rapidly oxidized lipids had reduced growth performance and increased liver weight compared to those fed the original lipids. Measures of lipid peroxidation may provide helpful information for predicting various biological responses in pigs. In addition, both SO and RO, regardless of lipid source, decreased liver triglycerides presumably by the activation of the PPARα pathway. Finally, regardless of lipid peroxidation level, lipids derived from different sources will contribute to different hepatic triglyceride and cholesterol concentrations, which indicate that feeding lipids that are markedly different in fatty acid profiles will lead to different liver triglyceride and cholesterol concentrations.

Table 3.1. Diet and nutrient composition of Phase-1 and Phase-2 diets (as-fed basis)

	Phases[1]	
	Phase-1	Phase-2
Ingredients, %		
Corn	43.30	48.65
Lipid source	10.00	10.00
Soybean meal (46%)	25.34	38.18
Fish meal, menhaden	9.60	--
Whey powder	10.00	--
Limestone	0.49	0.97
Dicalcium phosphate	0.06	1.38
NaCl	0.18	0.17
L-Lys HCl	--	0.10
DL-Met	0.03	0.05
Premix[2]	0.50	0.50
Mecadox[3]	0.50	--
Total	100.00	100.00
Calculated nutrients[4], %		
CP	22.50	21.66
Total P	0.66	0.64
Available P	0.42	0.35
Ca	0.87	0.79
SID[4] Lys	1.26	1.16
SID Met	0.72	0.66
SID Thr	0.79	0.73
SID Trp	0.23	0.23

[1] Phase 1 diets were formulated based on NRC (1998) recommendations for pigs weighing 7 to 19 kg and were fed to pigs from d 1 to 24 post-weaning. Due to slower than expected growth of pigs during phase 1, phase 2 diets were formulated based on the NRC (1998) recommendation for pigs weighing 13 to 20 kg and were fed to pigs from d 25 to 39 of the experiment.

[2]Vitamin and mineral premix provided the following per kilogram of diet: vitamin A, 7,716 IU; vitamin D_3, 1,929 IU; vitamin E, 39 IU; vitamin B12, 0.04 mg; riboflavin, 12 mg; niacin, 58 mg; pantothenic acid, 31 mg, Cu (oxide), 35 mg; Fe (sulfate), 350 mg; I (CaI), 4 mg; Mn (oxide) 120 mg; Zn (oxide), 300 mg; Se (Na_2SeO_3), 0.3 mg.

[3]Mecadox is the trade name for carbadox (Phibro Animal Health Corporation, Ridgefield Park, NJ).

[4]Calculated analysis was based on the NRC (1998) recommended values for all ingredients. The calculated ME of canola oil, corn oil, poultry fat, and tallow supplemented diets of Phase-1 were 3,814, 3,813, 3,791, and 3,741 kcal/kg, respectively. The calculated ME of canola oil, corn oil, poultry fat, and tallow supplemented diets of Phase-2 were 3,702, 3,701, 3,679, and 3,629 kcal/kg, receptively.

[4]SID AA represented standardized ileal digestible AA.

Table 3.2. Characteristics of the primers used for real-time PCR analysis

Gene[1]	Forward primer (from 5' to 3')	Reverse primer (from 5' to 3')	Product length	Temperature, °C	NCBI GenBank
ACO	GCTTACACACATCCTGGACGGCA	ACCTCGTAACGCTGGCTTCGA	132	59	AF185048
CPT-1	TCGCTGCGGAATGGGTTCGT	AGGGCCTTTTGTCCCGTGGT	143	59	AF288789
GAPDH	AGCCACAAGGTTCGAGGACTGGT	TTCCTCCCCCTCAACCCGCAAT	135	60	AF017079
mHMG-CoA-S	ACCCACTGGTGGATGGGAAGCT	TCGCTCGATGCCAGCTTGCTT	116	60	U90884
PPARα	GCCTGTGAAGGTTGCAAGGGCT	GGCCGAGAGGCACTTGTGGAAA	141	59	DQ437887
SCD	CGCCATCGTGCTCAATGCCA	AGTTGTGGAAGCCCTCACCCACA	137	58	NM_213781
SREBP-1	GCCTTGCACTTTCTGACCCGCT	TGCATGGCAACAGGCACCGA	86	60	NM_214157
SREBP-2	AGTGCTCAAGTCAGCCCTCGGT	AAAGTGAGCACGCACAGCCG	112	60	DQ020476

[1]ACO, acyl-CoA oxidase; CPT-1, carnitine palmitoyltransferase 1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; mHMG-CoA-S, mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase; PPARα, peroxisome proliferator activated receptor α; SCD, stearoyl-CoA desaturase; SREBP-1 and -2, sterol regulatory element-binding protein-1 and -2.

Table 3.3. Growth performance of pigs fed vegetable oils and animal fats of differing oxidation status[1]

	Corn oil			Canola oil			Poultry fat			Tallow					*P* value[2]	
	OL	SO	RO	OL	SO	RO	OL	SO	RO	OL	SO	RO	SEM	S	O	S × O
d 1-29																
ADFI, g	463	411	391	430	385	339	394	402	343	376	413	336	52	0.51	0.09[a]	0.94
ADG, g	320	293	246	276	206	209	278	282	215	262	274	226	43	0.21	0.04[b]	0.86
G/F	0.69	0.71	0.63	0.63	0.53	0.61	0.70	0.68	0.62	0.70	0.66	0.67	0.04	0.02[c]	0.17	0.34

[1]Data are least square mean of 3 observations per treatment. OL, lipids were stored as received without antioxidants or heating; SO, lipids heated for 72 h at 95°C with constant compressed air flow rate at 12 L/min; RO, lipids heated for 7 h at 185°C with constant compressed air flow rate at 12 L/min.

[2]S, lipid source; O, oxidation level; S × O, lipid source x oxidation level interaction.

[a]Pigs fed RO lipids tended to have reduced ADFI ($P = 0.09$) compared to pigs fed OL lipids.

[b]Pigs fed RO lipid had a reduced ADG ($P = 0.03$) compared to pigs fed OL lipids.

[c]Pigs fed CA supplemented diets had a reduced G/F compared to pigs fed other source of lipids ($P = 0.05$)

Table 3.4. Expression levels of mRNAs in livers of pigs fed vegetable oils and animal fats[1]

	Corn oil			Canola oil			Poultry fat			Tallow				*P* value[3]		
Gene[2]	OL	SO	RO	OL	SO	RO	OL	SO	RO	OL	SO	RO	SEM	S	O	S × O
PPARα	1.00	1.27	1.26	1.19	1.51	1.52	1.25	1.55	1.50	1.03	1.23	1.24	0.54	0.19	0.08	0.98
ACO	1.00	1.43	1.45	1.10	1.47	1.52	1.01	1.34	1.32	1.00	1.21	1.19	0.60	0.63	0.04	0.98
CPT-1	1.00	1.65	1.53	1.00	1.75	1.66	1.02	1.46	1.41	1.02	1.29	1.39	0.73	0.70	0.01	0.98
mHMG-CoA-S	1.00	2.04	2.18	1.03	2.22	2.13	1.03	2.03	1.92	1.03	1.85	1.76	0.67	0.66	< 0.01	0.98
SREBP-1	1.00	1.14	0.97	0.98	1.17	0.98	0.95	1.02	1.03	0.99	1.06	1.17	0.36	0.92	0.43	0.91
SREBP-2	1.00	1.25	1.24	1.01	1.08	1.07	1.11	1.27	1.30	1.03	1.15	1.13	0.54	0.72	0.46	0.99
SCD	1.00	1.00	1.02	1.10	1.03	1.10	1.11	1.26	1.27	0.97	1.23	1.23	0.40	0.37	0.55	0.94

[1]Data are least square mean of 8 observations per treatment; OL, lipids were stored as received without antioxidants or heating; SO, lipids heated for 72 h at 95°C with constant compressed air flow rate at 12 L/min; RO, lipids heated for 7 h at 185°C with constant compressed air flow rate at 12 L/min.

[2]ACO, acyl-CoA oxidase; CPT-1, carnitine palmitoyltransferase 1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; mHMG-CoA-S, mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase; PPARα, peroxisome proliferator activated receptor α; SCD, stearoyl-CoA desaturase; SREBP-1 and -2, sterol regulatory element-binding protein-1 and -2.

[3]S, lipid source; O, oxidation level; S × O, lipid source x oxidation level interaction.

Table 3.5. The correlation coefficients between tests of lipid oxidation (TLO) and various evaluations[1]

	TLO[2]							
	PV	AnV	TBARS	HEX	DDE	HNE	AOM	OSI
Growth performance								
ADFI	NS	NS	NS	NS	NS	NS	NS	NS
ADG	NS	NS	-0.29	NS	NS	NS	NS	NS
			0.09					
Organ weight								
Liver	NS	0.19	0.16	0.14	0.15	0.24	0.22	NS
		0.06	0.11	0.17	0.13	0.02	0.03	
Gene expression[3]								
PPARα	0.14	0.20	0.16	0.17	0.15	0.14	0.21	-0.24
	0.17	0.05	0.13	0.11	0.15	0.18	0.04	0.02
ACO	0.16	0.22	0.20	0.18	0.18	0.19	0.24	-0.23
	0.12	0.03	0.05	0.08	0.08	0.06	0.02	0.02
CPT-1	0.23	0.22	0.25	0.23	0.21	0.18	0.28	-0.27
	0.03	0.03	0.01	0.02	0.04	0.08	< 0.01	0.01
mHMG-CoA-S	0.29	0.42	0.32	0.33	0.29	0.31	0.41	-0.48
	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Liver lipid concentration[4]								
TG	NS	NS	NS	NS	NS	NS	NS	NS

[1]Top value represents correlation (r value) and bottom value represents significance (*P* value). If no value is given, it was not found to be different at $P \leq 0.10$ and listed as NS = non-significant.
[2]PV = peroxide value, AnV = p-ansidine value, TBARS = thiobarbituric acid reactive substances, HEX = hexanal, DDE = 2, 4-decadinal, HNE = 4-hydroxy nonenal, AOM = active oxygen method, and OSI = oxidative stability index.
[3]ACO = acyl-CoA oxidase, CPT-1 = carnitine palmitoyltransferase 1, mHMG-CoA-S = mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase; PPARα = peroxisome proliferator activated receptor α.
[4]TG = triglyceride

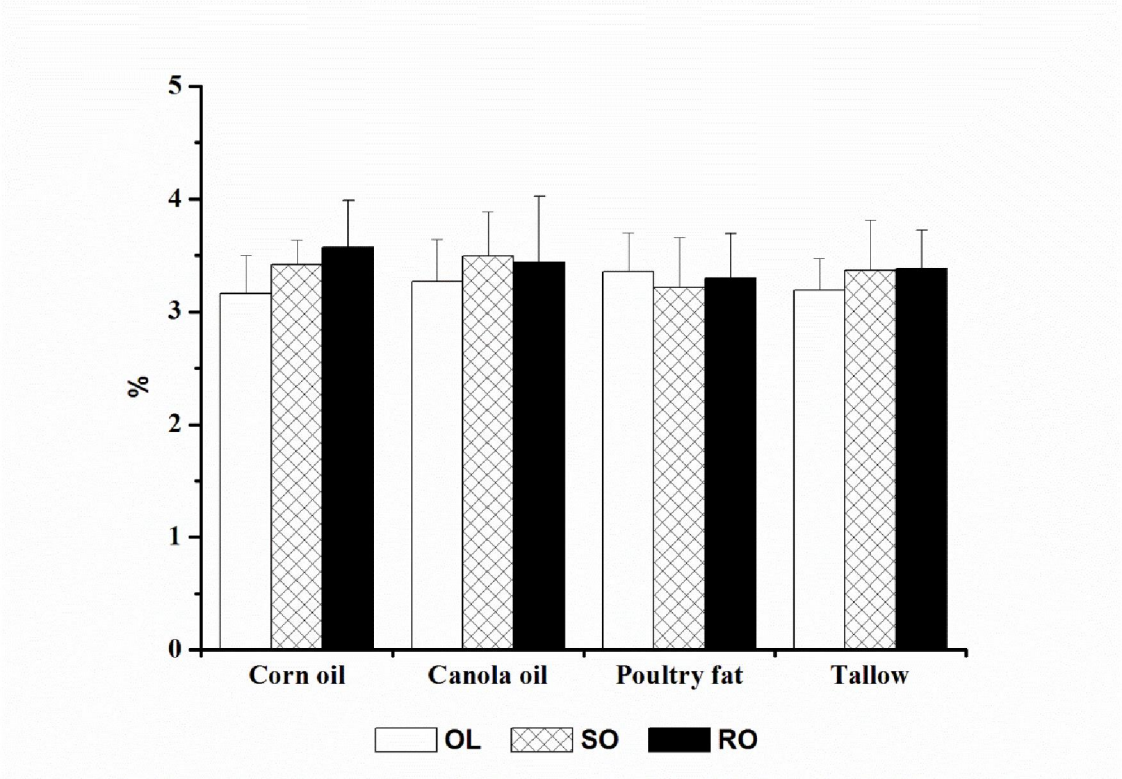

Figure 3.1. Effect of thermally-oxidized vegetable oils and animal fats on liver weight of young pigs. Original lipids (OL) were stored at -4°C without heating or antioxidants, slow oxidation (SO) lipids were heated for 72 h at 95°C with constant compressed air flow rate at 12 L/min, and rapid oxidation (RO) lipids were heated for 7 h at 185°C with constant compressed air flow rate at 12 L/min. Each bar represents the mean ± SE of 8 pigs. *P* value for lipid source effect = 0.58. *P* value for oxidation level effect = 0.09 (pigs fed RO tended to increase the liver organ weight compared to those fed the OL).

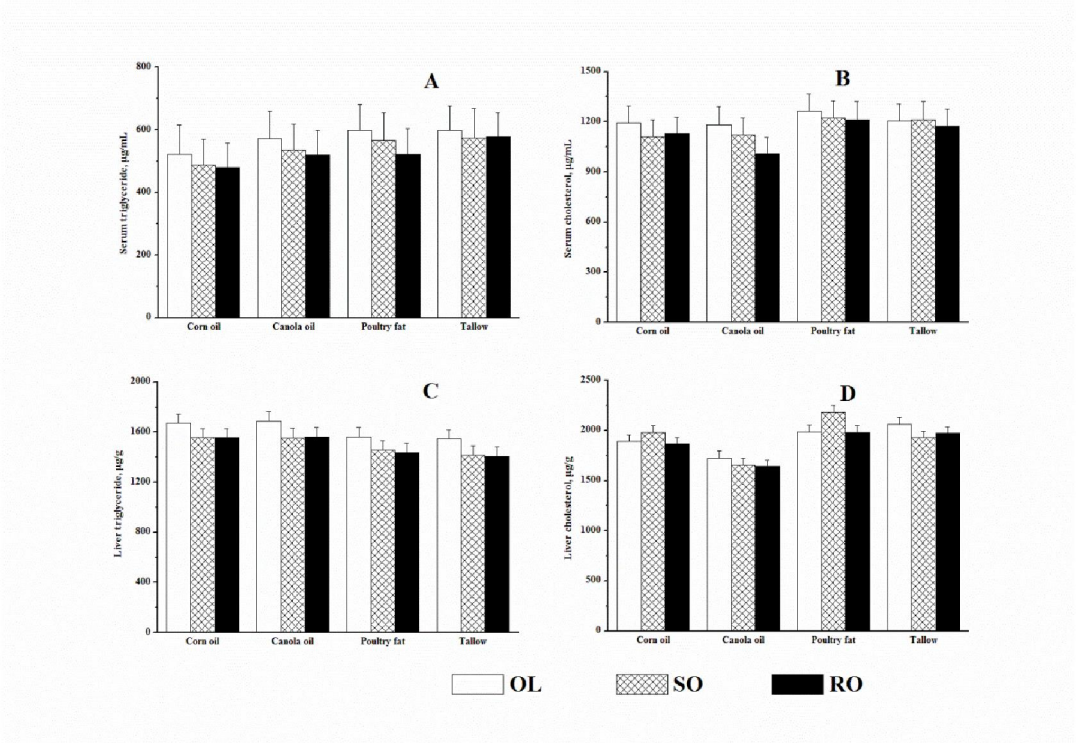

Figure 3.2. Effects of thermally-oxidized vegetable oils and animal fats on serum triglycerides (A), serum cholesterol (B), liver triglyceride (C), and liver cholesterol (D) concentrations of young pigs. Original lipids (OL) were stored at -4°C without heating or antioxidants, slow oxidation (SO) lipids were heated for 72 h at 95°C with constant compressed air flow rate at 12 L/min, and rapid oxidation (RO) lipids were heated for 7 h at 185°C with constant compressed air flow rate at 12 L/min. Each bar represents the mean ± SE of 8 pigs. No lipid source, oxidation level, or lipid × oxidation interaction effects were found for serum triglyceride and cholesterol. For liver triglyceride concentrations (C), *P* value for oxidation level effect = 0.03 [Rapid oxidation decreased ($P < 0.05$) and SO lipids tended to decrease ($P = 0.07$) the liver triglyceride concentration compared to OL]. *P* value for lipid source effect = 0.03 [pigs fed either corn or canola oil tended to have increased liver triglyceride concentration compared to those fed tallow (*P*

= 0.09)]. For liver cholesterol concentration (D), *P* value of lipid source effect < 0.01 [pigs fed corn oil had a lower (*P* < 0.05) liver cholesterol concentration than those fed poultry fat, and tended to have a lower concentration (*P* = 0.06) of liver cholesterol than pigs fed tallow. Pigs fed canola oil had less (*P* < 0.05) liver cholesterol than those fed poultry fat or tallow].

CHAPTER 4

INFLUENCE OF THERMALLY-OXIDIZED VEGETABLE OILS AND ANIMAL FATS ON ENERGY AND NUTRIENT DIGESTIBILITY IN YOUNG PIGS

A total of 108 barrows (6.67 ± 0.03 kg BW) were assigned to 12 dietary treatments in a 4 × 3 factorial design, plus a corn-soybean meal control diet to evaluate the effect of lipid source and peroxidation level on DE, ME, and apparent total tract digestibility (ATTD) of DM, GE, ether extract (EE), nitrogen (N), and carbon (C) in young pigs. Main effects were lipid source [corn oil (CN), canola oil (CA), poultry fat (PF), and tallow (TL)] and peroxidation level [original lipids (OL), slow oxidation (SO) of lipids heated for 72 h at 95°C, or rapid oxidation (RO) of lipids heated for 7 h at 185°C]. Pigs were provided *ad libitum* access to diets for 28-d, followed by an 8-d period of controlled feed intake equivalent to 4% BW daily. Diets were formulated based on the ME content of CA with the standardized ileal digestible Lys, Met, Thr, Trp, total Ca, and available P:ME balanced relative to NRC (1998) recommendations. Lipid peroxidation analysis indicated that compared to the OL, SO and RO had a markedly increased concentrations of primary and secondary peroxidation products, and the increase in these peroxidation products in CN and CA were higher than those in PF and TL. Addition of lipids to diets increased ($P < 0.05$) ATTD of EE and tended to improve ($P = 0.06$) ATTD of GE compared to pigs fed the control diet. Feeding CN or CA increased ($P < 0.05$) ATTD of DM, GE, EE, N, and C compared to feeding TL, while feeding PF improved ($P < 0.05$) ATTD of GE and EE, and tended to increase ($P = 0.06$) ATTD of C compared to TL. Pigs fed CN had increased ($P = 0.05$) percentage N retention than pigs fed TL. No

peroxidation level effect or interaction between lipid source and peroxidation level on DE and ME was observed. Lipid source tended ($P = 0.08$) to affect DE, but not ME values of experimental lipids ($P > 0.12$). Digestible energy values for CA (8,846, 8,682, and 8,668 kcal/kg) and CN (8,867, 8,648, and 8,725 kcal/kg) were about 450 kcal/kg higher than that of TL (8,316, 8,168, and 8,296 kcal/kg), with PF being intermediate (8,519, 8,274, and 8,511 kcal/kg) for OL, SO, and RO, respectively. In conclusion, lipid source affected ATTD of dietary DM, GE, EE, N, and C, and N retention rate; and tended to influence the DE value of the lipid, but did not significantly affect their ME value. Rapid and slow heating of lipids evaluated in this study increased lipid peroxidation products, but had minor effects on nutrient and energy digestibility as well as DE and ME values of the various lipids.

Keywords: energy, lipid source, nitrogen retention, oxidation level, young pigs

INTRODUCTION

Energy is one of the most expensive components of swine diets. Lipids are commonly added to swine diets as concentrated energy sources to improve feed efficiency (Pettigrew and Moser, 1991). Better knowledge of the energy value of lipids will help to increase the ability of nutritionists to successfully utilize lipids in swine diets.

Several studies have characterized the quality of lipids as energy ingredients (Cera et al., 1988, 1989; Li et al., 1990; Jones et al., 1992). However, those research efforts have focused mainly on the effects of unsaturated to saturated fatty acid ratio (Powles et al., 1993, 1994, 1995), fatty acid chain length (Hamilton and McDonald, 1969, Cera et al., 1989, Straarup et al., 2006), and FFA content (Sklan, 1979; Tso et al., 1981; DeRouchey et al., 2004). Few studies have evaluated the effect of lipid peroxidation products on energy value of lipids.

Most lipids are subjected to heating and potential oxidative processes before being supplemented in swine diets (Canakci, 2007), and some lipids are highly susceptible to peroxidation depending on their degree of unsaturation (Frankel et al., 1984, Seppanen and Csallany, 2002). Therefore, lipids used in animal feeds may not only differ considerably in fatty acid composition, but they may also contain various concentrations of peroxidation products which may affect their DE and ME content. Recently, DeRouchey et al. (2004) showed that increasing the rancidity of choice white grease did not affect fatty acid digestibility, but they did not determine the DE or ME content of the lipids. The objective of the current experiment was to determine the effects of lipid source and peroxidation level on DE and ME content, and on apparent total tract

digestibility (**ATTD**) of DM, GE, ether extract (**EE**), nitrogen (**N**), and carbon (**C**) in lipid-supplemented diets fed to young pigs.

MATERIALS AND METHODS

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

Animals, Experimental Design, and Diets

Weaned barrows (n = 108; initial BW of 6.67 ± 0.03 kg) were divided into 3 groups of 36 and housed at the Southern Research and Outreach Center (Waseca, MN) for this study. Pigs were terminal offspring of Yorkshire × Landrace sows (TOPIGS USA, Des Moines, IA) sired by Duroc boars (Compart Boar Store, Nicollet, MN). In each group, pigs were randomly assigned to 1 of 13 dietary treatments, resulting in 2 or 3 pigs per replicate pen and 1 replicate pen per treatment for each group.

In each group, pigs were assigned randomly to 1 of 13 dietary treatments, including one control diet and 12 corn-soybean meal based diets supplemented with 10% lipids. Lipid supplemented diets were arranged in a 4 × 3 factorial design. The main factors were lipid source [corn oil (**CN**; ADM, Decatur, IL), canola oil (**CA**; ConAgra Foods, Omaha, NE), poultry fat (**PF**; American Protein, Inc., Hanceville, AL), and tallow (**TL**; Darling International, Wahoo, NE)] and oxidation level [original lipids (**OL**), slow-oxidation **(SO)** lipids, and rapid-oxidation (**RO**) lipids]. To generate oxidized lipids, OL were either heated at 95°C for 72 h to produce SO or heated at 185°C for 7 h for producing RO. Both heating processes were accomplished using constant flow of compressed air of 12,000 cm^3/min at a temperature between 22 to 24°C. Before mixing feed, the OL, SO, and RO

lipid sources were stored at -20°C and no antioxidant was added before or during diet preparation.

Experimental diets were formulated based on a 2-phase feeding program. To compensate for the expected reduction in feed intake caused by increased caloric density of the lipid supplemented diets, a constant nutrient to ME ratio was used based on the ME content of CA, which had the highest ME concentration of all the lipid sources evaluated (8,410 kcal ME/kg; NRC, 1998). Canola oil diets were formulated first by adjusting the standardized ileal digestible Lys, Met, Thr, Trp, total Ca, and available P to ME ratio relative to recommendations in NRC (1998). Other lipid supplemented diets were then formulated by replacing 10% CA with the other lipids. Phase-1 diets (Table 3.1) were formulated based on NRC (1998) recommendations for pigs weighing 7 to 19 kg and were fed to pigs from d 1 to 24 post-weaning. Due to slower than expected growth of pigs during Phase-1, Phase-2 diets (Table 3.1) were formulated based on the NRC (1998) recommendation for pigs weighing 13 to 20 kg and were fed to pigs from d 25 to 39 of the experiment. Diets for each phase and for each of the 3 groups were mixed 4-d before they were fed initially and stored at 4°C throughout the feeding period of each phase.

During the first 28 d for each group, 2 or 3 pigs from the same dietary treatment were housed in one pen in an environmentally controlled room (27 to 28°C) and were provided *ad libitum* access to feed and water. After the 28-d diet adaptation phase, pigs were weighed (BW = 13.98 ± 2.37 kg) and moved to individual metabolism crates on d 29. Pigs were fed an amount of diet equivalent to 4% of their BW twice daily (2% at 0700 h and 2% at 1900 h) for an additional 5 d (i.e. d 29 to d 34) followed by a 3-d total urine and fecal collection period. All pigs had constant feed intake and fecal output

starting during the adaptation period through the end of the collection period. Thus, we chose to use total collection rather than marker to marker methodology for this experiment. Feces and urine were collected for 72 h beginning on the evening at 1900 of d 34 and ending on the evening at 1900 of d 37. During the collection period, fecal samples were collected daily at 0700 and 1900 h and stored at -18˚C. At the end of the collection period, fecal samples from each pig were pooled, weighed, and dried in a forced-draft oven at 55˚C. After drying, fecal samples were ground through a 1-mm screen and a homogeneous subsample was obtained for subsequent analysis. Total urine output was collected in plastic containers located under the metabolism cages at the same time as fecal collection. To limit microbial growth and reduce ammonia loss, 30 mL of 6 *N* HCl was added to the urine collection containers during the 3-d collection period. Urine volume was recorded twice daily and a subsample consisting of 20% of the urine excreted from each pig was collected and stored in a freezer at approximately -18˚C. At the end of the collection period, urine samples were pooled by mixing all the thawed urine sample from each pig and a subsample was obtained for subsequent analysis. Any unconsumed feed was removed, dried and weighed, and subtracted from the amount added to determine net feed consumption. The chemical and oxidative status of the experimental lipids is described in Chapter 2.

Chemical Analysis

Gross energy of lipids, diets, feces, and urine samples were determined using an isoperibol bomb calorimeter (Model Number 1281; Parr Instrument Co., Moline, IL) with benzoic acid used as a standard. Each sample was analyzed in duplicate. For urine GE, 3 mL of filtered urine subsample was added to 0.5 g of dried cellulose and subsequently

dried at 50°C for 72 h. The urinary energy was calculated by subtracting the energy measured in cellulose from the energy in the samples containing both urine and cellulose. From these data, the DE and ME content of all the diets were calculated by subtracting the GE excreted in feces and urine from GE intake over the 3-d collection period. The concentrations of DE or ME of lipids were calculated by subtracting the DE or ME contributed by the control diet from the DE or ME of the lipid containing diets then dividing by the dietary inclusion rate of the lipid.

Ether extract of the experimental diets and feces was analyzed in duplicate using an accelerated solvent extraction system (ASE 350, Thermo Scientific, Waltham, MA). Briefly, the sample was dispersed in sand and loaded into an extraction cell. The cell was filled with petroleum ether and then heated and pressurized. The solvent containing the extract was pumped out, using additional solvent, into a pre-weighed glass vial. The extraction process was repeated two more times for each sample, with the solvent being collected into the same vial each time. When all cells had been extracted, the solvent was evaporated using a N_2 evaporation system (Multivap Model 118, Organomation Associates Inc, Berlin, MN). The glass vial was then re-weighed and the percentage of EE was calculated.

Carbon, N, and sulfur (S) were analyzed by thermocombustion (VarioMAX CNS, Elementar Analysensysteme GmbH, Hanau, Germany) which uses catalytic tube combustion to volatilize the sample. The analyzer cleaned up the targeted gases by removing the unwanted substances, and the target gases are converted to N_2, CO_2, and SO_2, which are separated from each other using adsorption columns, and after heating, are measured using a thermal conductivity detector.

Apparent total tract digestibility of DM, GE, EE, N, C, and S in each diet was calculated using the following equation: ATTD = [(Nt − Nf)/Nt] × 100%, where Nt = the total consumption of DM (g), energy (kcal), or nutrient over the 3-d fecal total collection period and Nf = the total fecal excretion of DM (g), energy (kcal), or nutrient during the 3-d fecal total collection period.

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS 9.2 (SAS Inst. Inc., Cary, NC). Two-way ANOVA was conducted to evaluate the main effects of lipid source (CN, CA, PF, and TL), lipid peroxidation level (OL, SO, and RO), and any 2-way interactions in a 4 x 3 factorial arrangement. The corresponding statistical model included the fixed effects of lipid source, peroxidation level, and lipid source × peroxidation level interactions. One-way ANOVA was conducted to evaluate the differential effect between the control diets and lipid containing diets on all response criteria. Individual pig was used as the experimental unit for all other responses. Group was included as a random effect. All results are reported as least squares means. Mean comparisons were achieved by the PDIFF option of SAS with the Tukey-Kramer adjustment. The significance level chosen was $\alpha = 0.05$. Treatment effects were considered significant if $P < 0.05$, whereas values between $0.05 \leq P \leq 0.10$ were considered statistical trends.

RESULTS

Characterization of Experimental Lipids

The various composition and peroxidation characteristics of the experiment lipids have been described in detail (Chapter 2) and are shown in table 2.1. Briefly, the concentration of crude fat, moisture, insolubles and unsaponifiables were similar among

the 12 experimental lipids. As expected, CN and CA had higher concentrations of unsaturated fatty acids than that of TL, with PF being intermediate. Averaged within lipid source, both SO and RO decreased the linoleic acid and linolenic acid concentrations compared to OL, but changes in concentrations of other major fatty acids were not observed. As expected, lipid peroxidation measures indicated that all of the OL were relatively unoxidized, but SO and RO led to the marked increases in primary and secondary peroxidation products, and these peroxidation products were much higher for SO and RO of CN and CA than for PF and TL (Chapter 2).

Lipid Digestible and Metabolizable Energy Content

There was no effect of peroxidation level or lipid source × peroxidation level interaction noted for lipid DE (Table 4.1). Lipid source tended to affect ($P = 0.08$) the DE content on an as-fed basis, where the DE content of CN and CA (8,747 and 8,732 kcal/kg, respectively) were numerically higher than tallow (8,260 kcal/kg), with poultry fat being intermediate (8,435 kcal/kg).

No lipid source, peroxidation level, or lipid source × peroxidation level interaction effects were observed for ME content of the different lipids (Table 4.1). The ME content of different lipids had similar trends relative to their DE content, with the CN and CA (8,453 and 8,456 kcal/kg, respectively) having the highest ME, PF being intermediate (8,167 kcal/kg), and TL having the lowest ME (7,978 kcal/kg).

ATTD of DM, GE, EE, N, C, and S

Lipid Diets versus Control: Pigs fed diets supplemented with lipids had a higher ($P < 0.01$) ATTD of EE and tended to have a higher ($P = 0.06$) ATTD of GE compared to pigs fed the control diet. No differences in ATTD of DM, N, C, and S, or in percentage

nitrogen retention between pigs fed the control diet and pigs fed the diets containing lipids were observed (Table 4.2).

Among Lipids: There was no peroxidation level or lipid source × peroxidation level interaction noted for ATTD of DM, GE, EE, N, C, and S among diets containing various lipid sources (Table 4.2). Lipid source affected ATTD of DM, GE, EE, N, and C ($P < 0.01$), but did not affect ATTD of S. Pigs fed diets containing either CN or CA had increased ATTD of GE (Main effect mean of CN or CA vs. main effect mean of TL was 88.78 or 88.57 vs. 86.50), EE (Main effect mean of CN or CA vs. main effect mean of TL was 83.73 or 83.15 vs. 79.52), N (Main effect mean of CN or CA vs. main effect mean of TL was 89.15 or 88.78 vs. 85.95), and C (Main effect mean of CN or CA vs. main effect mean of TL was 89.29 or 89.11 vs. 87.26) compared to pigs fed diets containing TL ($P < 0.05$). Pigs fed diets containing PF also had a higher ATTD of GE and EE ($P < 0.05$), and tended to have a higher ATTD of C ($P = 0.06$) compared to pigs fed diets supplemented with TL.

Nitrogen Retention

There was no peroxidation level or lipid source × peroxidation level interaction observed for percentage N retention ($P > 0.05$). The only difference in N retention among lipid sources was for pigs fed diets containing CN having greater N retention ($P < 0.05$) than pigs fed diets containing TL.

DISCUSSION

General: Lipids are commonly added to swine diets to serve as concentrated energy sources and consequently, to improve feed efficiency (Pettigrew and Moser, 1991). Large quantities of lipids produced from the rendering industry as well as food processing

facilities and restaurants are subjected to heating processes and are used exclusively in animal feeds (Canakci, 2007). However, because the lipids are normally heated for a considerable length of time at a high temperature (Frankel et al., 1984), these lipids are highly susceptible to peroxidation. Therefore, lipids used in animal diets not only differ in their fatty acid profile, but also contain various concentrations of toxic peroxidation products, which may contribute to differences in energy concentrations as well as have effects on digestibility of other nutrients. In the current study, 4 different sources of lipids (CN, CA, PF, and TL) were evaluated and differed greatly in fatty acid composition as well as in lipid peroxidation status (OL, SO, and RO) as described by Liu et al. (2012a).

Lipids were included in the diet at 10% to maximize differences between pigs fed the control and lipid containing diets, to maximize differences in fatty acid composition and peroxidation levels among lipid sources, and to minimize errors associated with determining energy values of lipids when using the difference method for DE and ME determinations. Previous studies have demonstrated that the apparent digestibility of various lipids in nursery pigs increases with age, stabilizing around 4 wk of age (Hamilton and McDonald, 1969; Frobish et al., 1970; Cera et al., 1988). As a result, we utilized a 28 d adaptation of diets allowing for an accurate estimation of the maximum energy potential of the various lipids evaluated.

DE and ME: For comparative purposes, the DE and ME of the basal diet used in Phase-2 was 3,293 and 3,173 kcal/kg (as-fed basis), respectively, which are similar to values calculated based on NRC (1998) ingredient values. Close agreement of our experimental values with NRC (1998) values suggests good collection and analytical methods used in the current experiment. All of the experimental lipids had similar GE

values of 9,384 ± 43 kcal/kg and were close to average GE value of 9,410 ± 121 kcal/kg of 8 lipids including 3 animal fats, 2 soybean oils, 1 palm oil, 1 palm mix oil, and 1 vegetable oil by-product reported by Jorgensen and Fernandez (2000). Similar GE values were expected considering that results from most published experiments show that lipids contain a high concentration of EE (above 96%) and low amounts (usually less than 3%) of moisture, impurities, and unsaponifiables. The nearly equal GE values of lipids used in the current experiment suggest that neither the fatty acid composition nor the different concentrations of lipid peroxidation products are related to the GE value of lipids.

The DE or ME content of each source of lipids determined in the current experiment are similar to those for CN (8,755 and 8,405 kcal/kg, respectively), CA (8,760 and 8,410 kcal/kg, respectively), PF (8,520 and 8,180 kcal/kg, respectively), and TL (8,000 and 7,680 kcal/kg, respectively) as reported in the NRC (1998). This is encouraging given that the DE content of various lipids reported in the NRC (1998) were estimated based on an equation accounting for the concentration of FFA and the unsaturated:saturated fatty acid ratio, and ME was predicted as 96% of DE (Powles et al., 1995).

Nutrient and GE Digestibility: The different DE or ME content of various lipids in the current experiment were consistent relative to their corresponding EE digestibility. Regardless of peroxidation level, CN and CA had the highest ATTD of EE, with PF being intermediate, and the TL having the lowest ATTD of EE. Lipid digestibility can be affected by several factors. Because unsaturated fatty acids are easier to form micelles for absorption compared to saturated fatty acids, the concentration of various fatty acids and the ratio of unsaturated to saturated fatty acids are important factors in lipid digestibility

(Freeman et al., 1968; Stahly, 1984; Powles et al. 1995). In addition, chain length of fatty acids also plays an important role in lipid digestibility, because fatty acids with a chain length of less than 14 carbons have a higher digestibility than those with a longer chain length (Cera et al., 1988; Straarup et al., 2006). Free fatty acid concentrations may also affect lipid digestibility (NRC, 1998). Free fatty acids are less water soluble than monoglycerides or diglycerides and lipids with a higher FFA concentration have a lower incorporation rate into micelles leading to reduced absorption efficiency (Sklan, 1979; Tso et al., 1981). However, results from a recent study suggest that FFA concentrations of at least 53% do not adversely affect utilization of choice white grease in nursery pigs (DeRouchey et al., 2004). In the current experiment, the various DE and ME values among different lipid sources can be explained by their different concentrations of unsaturated fatty acids, given that only 10% of each lipid was added to the diet and the range in FFA was only from 0.28 to 3.65% among lipid sources.

In addition to the influence of lipid source on apparent EE digestibility, ATTD of DM, GE, N, and C were also affected. Lipids are an important source of GE and C in animal diets. As a result, the higher ATTD of GE and C in pigs fed diets containing CN (GE digestibility was 88.66, 88.42, and 88.76% for OL, SO, and RO corn oil, respectively; C digestibility was 89.41, 89.07, and 89.39% for OL, SO, and RO corn oil, respectively), CA (GE digestibility was 88.76, 88.21, and 87.35% for OL, SO, and RO canola oil, respectively; C digestibility was 89.86, 89.28, and 88.20% for OL, SO, and RO canola oil, respectively), and PF (GE digestibility was 87.35, 88.00, and 87.51 % for OL, SO, and RO poultry fat, respectively; C digestibility was 89.01, 88.34, and 88.33% for OL, SO, and RO poultry fat, respectively) compared to pigs fed diets containing TL

(GE digestibility was 86.83, 86.66, and 86.21% for OL, SO, and RO tallow, respectively; C digestibility was 87.56,87.43, and 86.79% for OL, SO, and RO tallow, respectively). These differences can be attributed to a higher ATTD of EE in diets supplemented with CN, CA, and PF compared to pigs fed diets containing TL. In the current experiment, ATTD of N in pigs fed diets containing CN or CA was also higher compared to pigs fed diets containing TL. One of the important functions of dietary lipids is to serve as an essential structural component of biological membranes, and as such, dietary lipids may affect composition of the enterocyte cell membrane. Consequently the physiological integrity of the membranes may change when dietary lipid source changes (Jorgensen and Fernandez, 2000). This concept is supported by results reported by Lindley et al. (1995) who showed that feeding rats diets containing polyunsaturated fatty acids had improved absorptive functions. Thus, the higher apparent N digestibility in pigs fed diets containing CN or CA compared to pigs fed diets containing TL in the current experiment might have resulted from the higher concentration of polyunsaturated fatty acids in the CN or CA compared to that in TL, which contributed to increased intestinal absorptive function. Another reason for the higher apparent N digestibility in pigs fed diets CN or CA compared to pigs fed diets containing TL might have resulted from the differential impact of lipid source on microflora in the large intestine. Bacterial protein synthesis in the large intestine plays an important role in altering apparent N digestibility (Li and Sauer, 1994). However, the detailed mechanism of the effects of lipid source on microflora in the large intestine is unknown. In the current experiment, the higher ATTD of N can be largely explained by the higher N retention rate in pigs fed diets containing CN compared to pigs fed diets containing TL.

A key objective of the current experiment was to evaluate the effects of peroxidation level in lipids on their DE and ME content. However, no effect of peroxidation level (OL versus SO versus RO) on the DE or ME content was observed. The lack of an effect of peroxidation on DE or ME content among OL, SO, and RO lipids were agreement with their corresponding ATTD of EE, which was also not affected by peroxidation level. Similar to our results, DeRouchey et al. (2004) showed that pigs fed diets supplemented with choice white grease with different degrees of peroxidation had similar ATTD of EE. Overall, these results indicate that thermal oxidation processes that increase lipid peroxidation product concentration have little to no effect on lipid digestibility, and consequently do not influence their DE or ME values. In contrast, it may be possible that digestibility coefficients and(or) DE and ME determinations may not be sensitive enough to detect the effects lipid peroxidation on pig performance and gene expression (Chapter 3) or intestinal barrier function and immunity (Chapter 5).

The ratio of unsaturated to saturated fatty acids is recognized as one of the important indicators of the lipid digestibility (Powles et al. 1993, 1994, 1995). In the current experiment, lipid peroxidation methods used resulted in significant changes in various peroxidative measures of the lipids utilized in this study, but had little effect on the composition of major fatty acids or the subsequent unsaturated to saturated fatty acid ratio. This observation suggests that measures of lipid peroxidation may not be as sensitive as the unsaturated to saturated fatty acid ratio in predicting lipid digestibility and subsequent DE and ME values.

Pigs fed diets supplemented with lipids had a higher ATTD of EE and tended to have a higher ATTD of GE compared to pigs fed control diet. This was expected because

the majority of dietary lipids in the control diet were bound lipids (lipids existing within cell membranes) while most of the dietary lipids in the lipid-supplemented diets were unbound lipids. These results agree with those reported by others (Adams and Jensen, 1984; Li et al., 1990; Kil et al., 2010) where pigs fed diets containing supplemental lipids had a higher ATTD of EE compared to pigs fed diets containing only bound lipids. In addition, increased dietary fat helps to delay gastric emptying (Hunt and Knox, 1968) which may result in a slower rate of passage of the diet in the small intestine resulting in higher carbohydrate, AA, and EE digestibility in lipid-supplemented diets (Li and Sauer, 1994). Therefore, the improved ATTD of GE in pigs fed the lipid supplemented diets in the current experiment could be a consequence of an overall enhancement in nutrient digestibility.

In conclusion, the increase in lipid peroxidation products produced by heating of lipids did not affect the ATTD of various nutritional components and had no effect on subsequent DE or ME of the lipids evaluated. In addition, results from this study support the notion that nutrient and energy digestibility, and consequently DE and ME values, are mainly dependent on their fatty acid composition rather than their level of peroxidation.

Table 4.1. Effect of thermally-oxidized vegetable oils and animals fats on lipid energy values for growing pigs[1]

	Control	Corn oil			Canola oil			Poultry fat			Tallow				*P* value[3]		
	diet	OL	SO	RO	OL	SO	RO	OL	SO	RO	OL	SO	RO	SEM	S	O	S×O
Obs[2]	6	9	9	9	8	8	8	8	8	8	9	8	8	--	--	--	--
Energy content, kcal/kg as-fed basis																	
GE	3,813	9,435	9,434	9,328	9,454	9,362	9,401	9,386	9,348	9,356	9,412	9,337	9,352	--	--	--	--
DE	3,293	8,846	8,682	8,668	8,867	8,648	8,725	8,519	8,274	8,511	8,316	8,168	8,296	268	0.08	0.60	0.99
ME	3,173	8,522	8,417	8,429	8,551	8,371	8,436	8,324	7,960	8,217	8,033	7,891	8,009	287	0.12	0.63	0.98

[1]Data are least squares mean (for control n = 6, for lipids diets n = 8 or 9); OL: lipids were stored as received without antioxidants or heating; SO: lipids heated for 72 h at 95°C with constant compressed air flow rate at 12 L/min; RO: lipids heated for 7 h at 185°C with constant compressed air flow rate at 12 L/min. Data for the Control represents the control diet while data for all lipid sources represents the energy of the lipid itself.

[2]Number of observations per treatment.

[3]S, lipid source; O, oxidation level; S×O, lipid source by oxidation level interaction.

Table 4.2. Apparent total tract digestibility (ATTD) of DM, GE, ether extract (EE), nitrogen (N), carbon (C), and sulfur (S), and N retention (NR) of diets (as-fed basis)[1]

		Corn oil			Canola oil			Poultry fat			Tallow				*P* value[3]			
	Control	OL	SO	RO	OL	SO	RO	OL	SO	RO	OL	SO	RO	SEM	S	O	S×O	C vs L
Obs[2]	6	9	9	9	8	8	8	8	8	8	9	8	8	--	--	--	--	--
ATTD, %																		
DM	86.78	88.66	88.42	88.76	88.76	88.21	87.35	87.35	88.00	87.51	86.83	86.66	86.21	0.74	< 0.01	0.42	0.91	0.29
GE	86.35	88.92	88.59	88.84	89.35	88.67	87.69	88.44	87.56	87.81	86.83	86.53	86.15	0.78	< 0.01	0.23	0.91	0.06
EE	21.50	83.34	85.02	82.82	84.94	83.55	80.96	83.59	82.27	82.49	80.04	78.33	80.19	1.50	< 0.01	0.23	0.24	< 0.01
N	86.81	88.73	89.34	89.39	88.63	89.24	88.47	87.76	86.59	87.86	85.57	86.46	85.83	1.30	< 0.01	0.90	0.81	0.83
C	87.34	89.41	89.07	89.39	89.86	89.28	88.20	89.01	88.34	88.33	87.56	87.43	86.79	2.30	< 0.01	0.22	0.88	0.13
S	73.16	72.24	73.61	71.20	72.50	69.11	71.71	71.28	71.63	71.49	72.34	72.18	71.4	2.30	0.90	0.91	0.93	0.58
NR, %[4]	62.08	64.14	65.66	64.95	64.81	62.76	63.07	64.29	60.05	63.17	56.32	60.44	58.20	4.00	0.05	0.99	0.89	0.95

[1]Data are least squares mean (for control n = 6, for lipids diets n = 8 or 9); OL: lipids were stored as received without antioxidants or heating; SO: lipids heated for 72 h at 95°C with constant compressed air flow rate at 12 L/min; RO: lipids heated for 7 h at 185°C with constant compressed air flow rate at 12 L/min.

[2]Number of observations per treatment.

[3]S, lipid source; O, oxidation level; S × O, lipid source by oxidation level interaction; C vs L, control versus lipids.

[4]Nitrogen retention as a percentage of N intake.

CHAPTER 5

INFLUENCE OF THERMALLY-OXIDIZED VEGETABLE OILS AND ANIMAL FATS ON INTESTINAL BARRIER FUNCTION AND IMMUNE VARIABLES IN YOUNG PIGS

To evaluate the effect of feeding thermally-oxidized lipids on metabolic oxidative status, gut barrier function, and immune response of young pigs, 108 barrows (6.67 ± 0.03 kg BW) were assigned to 12 dietary treatments in a 4 × 3 factorial design in addition to a corn-soybean meal control diet. Main effects were lipid source [n = 4: corn oil (CN), canola oil (CA), poultry fat (PF), and tallow (TL)] and oxidation level [n = 3: original lipids (OL), slow oxidation (SO) of lipids heated for 72 h at 95°C, or rapid oxidation (RO) of lipids heated for 7 h at 185°C]. Pigs were provided *ad libitum* access to diets for 28 d, followed by controlled feed intake for 10 d. After a 24-h fast on d 38, serum was collected and analyzed for α-tocopherol (α-T), thiobarbituric acid reactive substances (TBARS), endotoxin, haptoglobin, IgA, and IgG. On the same day following serum collection, lactulose and mannitol were fed and subsequently measured in the urine to evaluate gut permeability. There was a source × peroxidation interaction for serum α-T concentration where pigs fed SO or RO had decreased ($P < 0.05$) serum α-T concentration compared to pigs fed OL in CA and CN diets, but not in pigs fed PF and TL diets. There was no source × peroxidation interaction for serum TBARS, but among all lipid sources, pigs fed SO or RO lipids had increased ($P < 0.05$) serum TBARS compared with pigs fed OL. In addition, pigs fed CN or CA had higher ($P < 0.05$) serum TBARS compared to pigs fed PF or TL diets. There was no lipid source × peroxidation level

interaction**,** nor lipid source or peroxidation level effects observed for serum endotoxin, haptoglobin, IgA, or IgG. Pigs fed lipid supplemented diets tended to have increased serum endotoxin ($P = 0.06$), IgA ($P = 0.10$), and IgG ($P = 0.09$) compared to pigs fed the control diet. There was no lipid source × peroxidation level interaction, nor lipid source or peroxidation level effects noted for urinary TBARS and lactulose to mannitol ratio. Compared to pigs fed the control diet, pigs fed diets containing lipids had a lower a lactulose to mannitol ratio ($P < 0.01$). In conclusion, feeding weaning pigs diets containing 10% thermally-oxidized lipids for 38 d, especially vegetable oils containing high concentration of polyunsaturated fatty acids, appeared to impair oxidative status, but had little influence on gut barrier function or serum immunity parameters.

Keywords: gut barrier function, immunity, oxidative stress, thermally-oxidized lipids, young pigs

INTRODUCTION

Lipids produced from food processing facilities or restaurants have been thermally processed and are used commonly as economical sources of energy in animal feeds (Canakci, 2007). However, these lipids may be heated for a considerable length of time (Frankel et al., 1984), and may therefore contain various amounts of peroxidation products (Lin et al., 1989; Adam et al., 2008). Kimura et al. (1984) reported that feeding oxidized soybean oil impaired growth performance and caused diarrhea in rats. Alexander et al. (1987) and Behniwal et al. (1993) also reported that rats fed diets containing oxidized corn or peanut oil impaired growth rate. Similarly, broilers fed oxidized poultry fat exhibited impaired growth rates (Cabel et al., 1988; Dibner et al., 1996a), while DeRouchey et al. (2004) observed reduced growth rates in pigs consuming rancid choice white grease. The biological mechanisms to explain these observations are largely unknown.

Two lipid peroxidation products, malondialdehyde (**MDA**) and 4-hydroxynonenal (**HNE**) have been shown to increase metabolic peroxidation in animals (Seppanen and Csallany, 2002; Uchida, 2003). Feeding oxidized sunflower oil increased markers of oxidative stress in the small intestine of pigs (Ringseis et al., 2007) and feeding oxidized poultry fat to broilers decreased intestinal villus length (Dibner et al., 1996a). Feeding peroxidized lipids or treating cells with specific lipid peroxidation products decreases *ex vivo* primary antibody production to a bacterial pathogen (Takahashi and Akiba, 1999) and activates stress pathways (Biasi et al., 2006; Yun et al., 2009). However, little information has been reported regarding the effect of feeding peroxidized lipids on intestinal health or immune function in pigs. The following study was conducted to

investigate the effect of feeding diets containing thermally-oxidized lipids on metabolic oxidation status, gut barrier function, and immune response in young pigs.

MATERIALS AND METHODS

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

Animals, Experimental Design, and Diets

Weaned barrows (n = 108; initial BW of 6.67 ± 0.03 kg) were divided into three groups of 34 and housed at the Southern Research and Outreach Center (Waseca, MN) for this study. Pigs were terminal offspring of Yorkshire × Landrace sows (TOPIGS USA, Des Moines, IA) sired by Duroc boars (Compart Boar Store, Nicollet, MN). In each group, pigs were randomly assigned to 1 of 13 dietary treatments, resulting in 2 or 3 pigs/replicate pen and 1 replicate pen/treatment for each group.

To generate peroxidized lipids, original lipids (**OL**) were either heated at 95°C for 72 h to produce slow-oxidation (**SO**) lipids or heated at 185°C for 7 h to produce rapid-oxidation (**RO**) lipids. Both heating processes were accomplished with a constant flow of compressed air at 12,000 cm^3/min and a temperature between 22 to 24°C. Before feed mixing, the OL, SO, and RO lipids were stored at -20°C, and no antioxidant was added prior to or during diet preparation. Treatments consisted of 12 corn-soybean meal based diets supplemented with 10% lipids and were arranged in a 4 × 3 factorial design. The two main factors were lipid source [corn oil (**CN**; ADM, Decatur, IL), canola oil (**CA**; ConAgra Foods, Omaha, NE), poultry fat (**PF**; American Protein, Inc., Hanceville, AL),

and tallow (**TL**; Darling International, Wahoo, NE)] and oxidation level (OL, SO, and RO).

Experimental diets were formulated based on a 2-phase feeding program. To compensate for the expected reduction in feed intake caused by increased caloric density of the lipid supplemented diets, a constant nutrient to ME ratio was used based on the ME content of CA, which had the highest ME concentration of all the lipid sources evaluated (8,410 kcal ME/kg; NRC, 1998). Canola oil diets were formulated first by adjusting the standardized ileal digestible Lys, Met, Thr, Trp, total Ca, and available P to ME ratio recommended by NRC (1998). Other lipid supplemented diets were then formulated by replacing 10% CA with the other lipids. Phase-1 diets (Table 3.1) were formulated based on NRC (1998) recommendations for pigs weighing 7 to 19 kg and were fed to pigs from d 1 to 24 post-weaning. Due to slower than expected growth of pigs during Phase-1, Phase-2 diets (Table 3.1) were formulated based on the NRC (1998) recommendation for pigs weighing 13 to 20 kg and were fed to pigs from d 25 to 39 of the experiment. Diets for each phase and for each of the 3 groups were mixed 4-d before they were initially fed and stored at 4°C throughout the feeding period of each phase.

During the first 28 d of each group, 2 or 3 pigs from the same dietary treatment were housed in one pen in an environmentally controlled room (27 to 28°C) and were provided *ad libitum* access to feed and water. From d 29 to d 39, pigs were housed individually in metabolism crates in an environmentally controlled room (25 to 27°C) and fed an amount of diet equivalent to 4% of their BW daily (2% at 0700 h and 2% at 1900 h). Pigs were allowed *ad libitum* access to water. After the morning feeding at 0700 h on d 37, all pigs were fasted for 24 h. A blood and urine sample was collected at 0700 h of d

38 to obtain fasted serum and urine. Approximately 8 mL of blood was obtained by jugular venipuncture using a 10-mL serum tube (Becton Dickinson, Franklin Lakes, NJ). Blood samples were centrifuged at 2,500 × g (Heraeus Biofuge 22R Centrifuge, Hanau, Germany) for 15 min at 4°C and serum was harvested. Serum samples were frozen immediately and stored at -20°C for subsequent serum α-tocopherol (**α-T**), thiobarbituric reactive substance analysis (**TBARS**), endotoxin, haptoglobin, IgG, and IgA analysis. Chlorhexidine (Bimeda, Inc., Oakbrook Terrace, IL) was placed into each plastic urine collection container to prevent microbial contamination, and plastic containers were then placed under each funnel of the metabolism cages to collect approximately 200 mL of urine from each pig beginning at 0700 h for about 4 h on d-38. After collection, all urine samples were stored in a freezer at -20˚C for subsequent analysis. At 1200 h of d 38, after a 29-h fast, all pigs were fed 100 g of their assigned experimental diets with an additional 10 g of lactulose (Sigma, St. Louis, MO) and 2 g of mannitol (Sigma, St. Louis, MO) as markers of intestinal permeability. Plastic containers with 2 ml chlorhexidine (Bimeda, INC., Oakbrook Terrace, IL) were again placed under the funnel of each metabolism cage for a period of 6 hours following this feeding for urine collection. After urine was collected, it was stored at -20˚C for subsequent lactulose and mannitol analysis. At 0700 h of d 39, all pigs were euthanized with 1 mL pentobarbital sodium solution (Fatal-Plus Powder, Vortech Pharmaceuticals, Dearborn, MI) per 4.53 kg of BW by intracardiac injection. Spleen and kidney were excised and weighed to calculate their organ weight as a percentage of BW. The chemical and oxidative status of the experimental lipids is described in detail in Chapter 2.

Serum and Urine sample analysis

Serum α-T concentration was analyzed (Method 996.06; AOAC) at a commercial laboratory (Michigan State University DCPAH, Lansing, MI). Serum and urine TBARS concentrations were analyzed using Animal Models of Diabetic Complications Consortium Protocols by Feldman (2004). Serum endotoxin concentration was measured by a commercial kit (PyroGene recombinant factor C endotoxin detection system, Lonza, Walkersville, MD). The concentration of serum haptoglobin, a major acute phase protein in pigs, was measured by a colorimetric assay (Phase haptoglobin assay, Tridelta Development Limited, Kildare, UK). Serum IgA and IgG were determined using commercial ELISA kits (E100-102 for IgA and E100-104 for IgG, Bethyl Laboratories Inc., Montgomery, TX) following the product instructions. Lactulose and mannitol concentrations in urine were determined by HPLC and the ratio of lactulose and mannitol was used as an *in vivo* indicator of small intestinal permeability according to the method described by Kansagra et al. (2003).

Statistical Analysis

All data were analyzed using the MIXED procedure of SAS (Version 9.2; SAS Inst. Inc., Cary, NC). A two-way ANOVA was conducted to evaluate the main effects of lipid source (CN, CA, PF, and TL), lipid peroxidation level (OL, SO, and RO), and any 2-way interactions in a 4 × 3 factorial arrangement. The corresponding statistical model included the fixed effects of lipid source, peroxidation level, and lipid source × peroxidation level interactions. Group was included as a random effect. Individual pig was used as the experimental unit for all responses. All results are reported as least squares means. Mean comparisons were achieved by the PDIFF option of SAS with the Tukey-Kramer

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

RESULTS

Characteristics of Experimental Lipids

The characteristics of the experimental lipids used in this study have been described in detail Chapter 2 and are shown in Table 2.1. Briefly, the concentration of crude fat, moisture, insolubles and unsaponifiables were similar among the 12 experimental lipids. As expected, CN and CA had higher concentrations of unsaturated fatty acids than TL, with PF being intermediate. Averaged among lipid sources, both SO and RO decreased the linoleic acid and linolenic acid concentrations compared to OL, but changes in concentrations of other major fatty acids were not observed. Lipid peroxidation measurements indicated that all of the OL were relatively unoxidized, but SO and RO led to a marked increase in the production of primary and secondary oxidation products, with the concentrations of these peroxidation products being much higher in both SO and RO thermal treatments for CN and CA than for PF and TL.

Metabolic Oxidative Status

Serum α-T: A lipid source × peroxidation level interaction ($P < 0.01$) was observed for serum α-T concentration (Table 5.1), which was mainly caused by pigs fed CN and CA that were SO or RO resulting in decreased ($P < 0.05$) serum α-T concentration, but not in pigs fed PF or TL that were SO or RO. In addition, pigs fed the control diet had a higher serum α-T concentration compared to pigs fed lipid diets ($P < 0.05$).

Serum TBARS: No lipid source × peroxidation level interaction was observed for serum TBARS (Table 5.1). Peroxidation level significantly affected serum TBARS ($P <$

0.05), where pigs fed SO or RO lipids had higher serum TBARS than pigs fed diets containing OL ($P < 0.05$). Lipid source also influenced serum TBARS ($P < 0.05$) in that pigs fed either CN or CA supplemented diets had a higher serum TBARS than pigs fed PF or TL ($P < 0.05$).

Urinary TBARS: There was no lipid source × peroxidation level interaction, nor were there lipid source or peroxidation level effects on urinary TBARS (Table 5.1).

Intestinal Barrier Function

Urinary Lactulose to Mannitol Ratio: The ratio of lactulose to mannitol was higher ($P < 0.05$) in pigs fed the control diet compared to pigs fed the lipid supplemented diets (Table 5.1). Lipid source tended ($P = 0.09$) to affect the ratio of lactulose to mannitol . The ratios of lactulose to mannitol for CN (0.07, 0.05, and 0.05) and CA (0.06, 0.04, and 0.07) were lower than that of PF (0.26, 0.05, and 0.13), with TL being intermediate (0.19, 0.09, and 0.05) for OL, SO, and RO, respectively. However, there were no statistically significant differences for lactulose to mannitol ratio between any two sources of lipids. There was no peroxidation level effect or lipid source × peroxidation level interaction for lactulose to mannitol ratio.

Serum Endotoxin analysis: Pigs fed diets supplemented with lipids tended to have a higher ($P = 0.06$) endotoxin concentrations than pigs fed the control diet (Table 3). No lipid source × peroxidation level interaction, or lipid source and peroxidation level effects were noted in the 24-h fasted serum endotoxin analysis.

Immune Responses

Serum Haptoglobin: No lipid source × peroxidation level interaction, or lipid source or peroxidation level effects were found in serum haptoglobin concentration

(Table 5.1). In addition, no difference in serum haptoglobin was found between pigs fed lipid supplemented diets and pigs fed the control diet.

Serum IgA and IgG: No lipid source × peroxidation level interaction, or lipid source or peroxidation level effects were found for serum IgA or IgG analysis (Table 5.1). Pigs fed the lipid supplemented diets tended to have an increased serum IgA and Ig G compared to pigs fed the control diet ($P = 0.10$ and 0.09, respectively).

Organ Weight

Spleen: No differences spleen weight were observed between pigs fed the control and the lipids diets, and no oxidation level or lipid source × oxidation level interaction was observed. A lipid source effect was observed where pigs fed TL diets had increased spleen weight compared to pigs fed CN diets ($P = 0.02$), and also tended to have increased spleen weight compared to pigs fed PF ($P = 0.07$).

Correlation Analysis

Lipids with various degrees of peroxidation, measured using different peroxidation assays, affected serum α-T and serum TBARS concentration of pigs. As a result, correlation analysis between measures of lipid peroxidation and serum α-T and TBARS concentration was conducted to determine the association of various lipid peroxide measures in predicting these biological responses (Table 4). Due to the limited number of animals used in the current experiment, correlations where the *P*-value was 0.2 or less were considered. For serum α-T concentration, negative correlations between serum α-T concentration and peroxide value (**PV**, $P < 0.05$). p-anisidine value (**AnV**, $P < 0.01$), thiobarbituric acid reactive substances (**TBARS**, $P < 0.01$), hexanal (Hex, $P < 0.01$), 2, 4-decadienal (**DDE**, $P < 0.05$), 4-hydroxynonenal (**HNE**, $P < 0.05$) active oxygen method

(**AOM**, $P < 0.01$) were observed. Oxidative stability index (**OSI**) value also had a positive correlation with serum α-T concentration ($P = 0.15$). All measures of lipid peroxidation (PV, AnV, TBARS, Hex, DDE, HNE, AOM, and OSI) were correlated with the serum TBARS concentration as shown in Table 5.2.

DISCUSSION

The chemical composition and peroxidative status of the experimental lipids used in this study varied greatly as described by Chapter 2. All lipids were included in the diet at 10%, which although higher than might be utilized in commercial diets, was done to help delineate differences among treatments if they existed. Because the original CN (400 IU/kg lipid) and CA (290 IU/kg lipid) contained a relative high amount of total tocopherols compared to other 10 experimental lipids (< 100 IU/kg lipid), the calculated total tocopherols concentration were higher in the diets containing original CN (75.1 IU/kg diet) or original CA (64.1 IU/kg diet) than that in the control (39 IU/kg diet) or in other lipid supplemented diets (35.1 IU/kg). However, the calculated daily consumption of total tocopherols based on the ADFI and the calculated dietary concentration of total tocopherols in both the control diet (22.3 IU/d) or diets containing supplemental lipids (> 18.6 IU/d) were still higher than NRC (1998) recommended level for the young pigs weighing less than 20 kg (11 IU/d).

Alpha-tocopherol is the most active isomer of the vitamin E family, and is the principle lipid-soluble antioxidant in tissues and blood (Rigotti, 2007). After absorption, α-T is transported in serum by lipoproteins where it initially functions to protect unsaturated fatty acids from free radical damage (Chung et al., 1992). In the current experiment, although all pigs had higher daily consumption of dietary α-T than NRC

(1998) recommendations, pigs fed lipids that had been subjected to slow- or rapid-oxidation exhibited lower serum α-T than pigs fed OL within the CN or CA treatment. Oxidative stress in animals fed peroxidized lipids has been well documented and can be explained by the enhanced turnover or catabolism rate of antioxidants caused by the oxidative stress (Benedetti et al., 1987; Liu and Huang, 1996; Eder, 1999). No decrease in serum α-T concentration was noted in slow or rapid oxidized PF and TL. This finding is consistent with the relatively low concentrations of peroxidation products found in PF and TL compared to the concentrations found in CN and CA, and is most likely due to differences in fatty acid composition among lipid sources. In addition, correlations were found between serum α-T and all measures of lipid peroxidation (PV, AnV, TBARS, Hex, DDE, HNE, AOM, and OSI) in the current experiment, which suggests that measuring the degree of lipid peroxidation may provide a helpful information regarding prediction the oxidative status of pigs. Furthermore, as we expected, pigs consuming lipid diets had reduced serum α-T compared to pigs fed the control diet which can explained by the high amounts of peroxidation products present in SO and RO lipids. As a result, the dietary antioxidant requirement of pigs may be increased due to consumption of thermally-oxidized lipids, especially for the lipids which contain a high concentration of PUFA.

One of the most frequently used biomarkers in prediction of the overall metabolic oxidative status in animals is the concentration of serum MDA. Malondialdehyde is one of the typical by-products of lipid peroxidation and is well documented for its cellular toxicity by interacting with lysine, albumin, DNA, and other cellular components (Del-Rio et al., 2005). Therefore, the serum TBARS assay, which is primarily based on quantification of serum MDA, can be used to evaluate the metabolic peroxidation status

of animals (Nielsen et al., 1997). In the current experiment, pigs fed diets containing SO or RO lipids had a higher serum TBARS than pig fed diets containing OL. In rats, feeding oxidized sunflower oil increases serum TBARS (Garrido-Polonio et al., 2004). Likewise, Juskiewicz et al. (2000) found that rats fed oxidized fat with high PV (> 160 meq/kg) led to a higher serum MDA concentration as well. An increase in blood TBARS had also been observed in broilers after consumption of oxidized sunflower oil (Sheehy et al., 1993, 1994), and in broilers consuming a mixture of oxidized rapeseed and soybean oil (Engberg et al., 1996). The increased level of lipid peroxidation products in SO and RO lipids, as indicated by their higher PV, TBARS, and HNE values is also supportive of an increase in serum TBARS reported by Sheehy et al. (1993, 1994) and Engberg et al. (1996). In the current experiment, we also observed a correlation between all the measures of lipid oxidation (PV, AnV, TBARS, Hex, DDE, HNE, AOM, and OSI) and serum TBARS concentration, which may be useful in predicting the oxidative status of pigs when pigs feeding various degrees of peroxidized lipids. Pigs fed CN or CA had higher TBARS compared to pigs fed PF or TL, which is consistent with their differences in lipid peroxidation products. The higher potential of metabolic oxidative stress caused by CN and CA is also consistent with the early findings in humans (Kleinveld et al., 1993), rabbits (Hennig et al., 1995), and rats (Csallany et al., 2000) where excessive consumption of dietary PUFA increased the risk of lipid peroxidation. The increased level of oxidative stress in pigs fed CN and CA may be due to the consumption of a high amount of unsaturated fatty acids which are particularly susceptible to autoxidation forming of fatty acid radicals (Sherwin, 1978).

Measurement of urinary secondary peroxidation products, such as MDA, can be biased as a marker of oxidative stress *in vivo* by ingestion of dietary lipid (Draper et al., 2000). Because of this, 24-h fasted urine was collected to avoid the influence induced by the different dietary intake of peroxidized lipids. However, no lipid source or peroxidation level effects were found for urinary TBARS. One explanation might be due to the fact that thiobarbituric acid may react with the variety of compounds other than aldehydes in the urine, resulting in a lack of either sensitivity or specificity in urine TBARS analysis (Draper et al., 2000; Grotto et al., 2009; Campos et al., 2011). Serum α-T and TBARS results in the current study indicate that feeding weaned pigs diets containing 10% of thermally-oxidized lipids, especially CN and CA which contain high concentration of polyunsaturated fatty acids, impairs their peroxidative status by increasing the production of lipid peroxidation products and depleting α-T in serum.

The gastrointestinal tract not only serves to digest and absorb nutrients from the intestinal lumen, but it also acts as the first protective barrier between the intestinal lumen and the body. Changes in gastrointestinal tract structure, such as gut barrier function, can be associated with changes in its nutrient absorption and pathogen resistance function. Poor gut barrier function may reduce the resistance of an animal to infectious agents such as endotoxins or pathogenic bacteria, and may cause activation of the immune system. Therefore, the effect of thermally-oxidized lipids, containing various levels of peroxidation products, on intestinal barrier function of young pigs was investigated.

One of the most popular methods used to evaluate gut barrier function is to measure intestinal permeability, which is generally dictated by paracellular permeability (Bjarnason et al., 1995). Paracellular permeability can be determined *in vivo* by urinary

recovery of inert markers (Bjarnason et al., 1995; Wijtten et al., 2001a,b). The principle of the test is based in the fact that the orally administered lactulose can only be absorbed through the paracellular route when the barrier function is compromised. While the monosaccharide, such as mannitol, can be absorbed both through paracellular or transcellular routes, and therefore provides an assessment of the absorptive surface. Therefore, lactulose and mannitol are used commonly together to minimize the influence of pre- and post-intestinal factors on recovery of the paracellular markers because these two markers empty similarly from the stomach, are not metabolized in the small intestine, and are cleared in the same manner from the kidneys (Bjarnason, et al., 1995). In the current experiment, lipid source, peroxidation level, and their interaction did not influence the paracellular permeability as measured by the lactulose to mannitol ratio. To date, few experiments have been conducted to evaluate the effect of thermally-oxidized lipids on intestinal barrier function of pigs. Oxidized lipids containing secondary peroxidation products such as MDA and HNE, can negatively influence cells directly by causing membrane perturbations which contribute to poor membrane permeability. Previously, Dibner et al. (1996) reported that feeding oxidized PF to broilers resulted in intestinal structural injury as indicated by a decreased half-life of enterocytes. Assimakopoulos et al. (2004) suggested that intestinal oxidative stress was a key factor resulting in intestinal physical injury as indicated by decreased villous density and total mucosal thickness. In addition, feeding thermally-oxidized sunflower oil to growing pigs increased markers of oxidative stress in the small intestine (Ringseis et al. 2007). Therefore, consumption of thermally-oxidized lipids may promote intestinal oxidative stress and subsequently, cause intestinal injury and gut barrier dysfunction in pigs. In the

current study, we noted that feeding 10% thermally-oxidized lipids to nursery pigs for 38 d caused metabolic oxidative stress by depleting serum α-tocopherol and increasing serum TBARS. However, no impaired gut permeability was observed in pigs fed thermally-oxidized lipids. The lack of an intestinal barrier function effect observed in the current study might be explained by the different degrees of oxidative stress caused by the peroxidized lipids, duration of feeding period, as well as the animal species considered.

In pigs, little information regarding feeding diets with and without lipids on gut permeability has been reported. In the current experiment, pigs fed lipid supplemented diets had tighter paracellular permeability compared to pigs fed the control diet as indicated by a lower lactulose to mannitol ratio. Fasting can be one of the major reasons resulting in poor intestinal integrity and permeability due to malnutrition (Sundqvist et al., 1982; Fan et al., 2008). In the current study, all pigs had a 29-h fast before the gut permeability test to insure that pigs consumed all gut permeability markers. As a result, we speculate that the improved gut permeability of pigs fed lipid supplemented diets may have been associated with the fact that the added lipid may help prevent malnutrition by delaying gastric digesta emptying and subsequently improving nutrient absorption (Hunt and Knox, 1968; Li and Sauer, 1994).

Endotoxins are combinations of lipid and polysaccharide side chains on the cell wall of gram-negative bacteria. Under normal conditions, there is none, or a minimal amount of endotoxins appearing in serum because of a tight intestinal barrier. Therefore, serum endotoxins can be an important indicator of the intestinal barrier function (Lichtman, 2001). In the current experiment, no lipid source or peroxidation level effects,

or their interaction was observed in serum endotoxin concentrations which are consistent with the results of the gut permeability measurement. However, the fact that pigs fed diets containing lipids had a higher serum endotoxin concentration than pigs fed control diets is in contrast to the finding that pigs fed lipid supplemented diets had tighter paracellular permeability than pigs fed the control diet. This contrast between intestinal permeability marker and serum endotoxin concentration or bacterial translocation has also been reported previously (Wijtten et al., 2011b), where an increase in intestinal lactulose permeability of pigs was associated with a decrease in bacterial translocation. Because both intestinal permeability and intestinal lumen toxin or bacterial concentrations are important factors determining the serum endotoxin concentration or bacterial translocation, it is speculated that the disassociation between gut permeability markers and serum endotoxin analyses is related to the different concentrations of intestinal lumen toxin or bacterial concentration (Wijtten et al., 2011b). As such, more research is needed to understand the effects of feeding lipid supplemented diets on intestinal toxin or bacterial concentrations of pigs.

Previous studies suggest that consumption of specific lipid peroxidation products may influence the immune response of animals, because treating cells with 4-HNE can increase activation of stress pathways (Biasi et al., 2006; Yun et al., 2009) and increase the expression of macrophage inflammatory mediators (Kumagai et al., 2004). Activation of stress pathways or overexpression of inflammatory mediators not only causes redistribution of nutrients away from growth processes in support of the immune system (Liu et al., 2010), but may also inhibit IGF-1 mRNA expression (Thissen and Verniers, 1997), of which both can contribute to poor growth performance. Haptoglobin, a

representative acute phase protein produced by hepatocytes during inflammatory conditions (Kent, 1992), has been found in swine serum and has been shown to be increasingly produced during non-specific bacterial infections and chemical stimulation (Shim et al., 1971; Hall et al., 1992). Thus, serum haptoglobin can be considered a sensitive indicator in evaluating the activation of the immune system in pigs (Petersen et al., 2004). In the current experiment, no lipid source or peroxidation level effect, nor lipid source × peroxidation level interaction were found for serum haptoglobin concentration, and no difference in serum haptoglobin was found between pigs fed lipid supplemented diets and pigs fed the control diet.

Immunoglobulin levels are determined for evaluation of the humoral immune status, with low levels of immunoglobulins being associated with humoral immunodeficiencies (Buckley 1986) and high immunoglobulin concentrations being associated with inflammatory and pathological conditions (Haye and Kornegay, 1979; Redman, 1979; Parreno et al., 1999). Previous studies conducted in broilers (Takahashi and Akiba, 1999) found that feeding oxidized fat decreased *ex vivo* primary antibody production in response to a bacterial pathogen. In the current experiment, no changes in serum IgA and IgG suggest that the different lipid peroxidation levels used in the current experiment did not influence the synthesis of antibodies. In agreement with serum endotoxin analysis, pigs fed the lipid supplemented diets tended to have increased serum IgA and IgG compared to pigs fed the control diet. And the increased the synthesis of antibodies in pigs fed lipids diet helped to improve defense system for eliminating the endotoxin (Zou et al., 2010).

The spleen is an important organ for red blood cells and the immune system and is found in virtually all vertebrate animals. The spleen functions to remove old red blood cells and holds a reserve of blood in case of hemorrhagic shock while also recycling iron. The spleen also plays a significant role in the synthesis antibodies and clearing antibody-coated bacteria along with antibody-coated blood cells by way of blood and lymph node circulation (Mebius and Kraal, 2005). The enlargement of the spleen is also known as splenomegaly, and one of the major causes of splenomegaly is a bacterial infection (Jackson et al., 2010). To our knowledge, this is the first report regarding the effect feeding thermally-oxidized lipids on spleen weight in pigs. We noted that pigs fed TL diets had increased spleen weight compared with pigs fed CN or PF. However, further investigations of mechanism responsible for the increased spleen weight in pigs fed TL are needed.

In conclusion, feeding weaned pigs diets containing 10% thermally-oxidized lipids for 38 d, especially vegetable oils containing high concentrations of PUFA, impairs their metabolic oxidative status by depleting serum α-T and increasing serum TBARS. However, the unchanged gut barrier function and immune response between pigs fed thermally-oxidized lipids and unheated lipids suggest that pigs are relatively resilient to certain levels of lipid oxidation.

Table 5.1. Serum and urine chemical analyses in pigs fed different experimental lipids[1]

	Control	CN			CA			PF			TL				*P* value[2]			
		OL	SO	RO	OL	SO	RO	OL	SO	RO	OL	SO	RO	SEM	S	O	S×O	C vs L
Observations	6	9	9	9	8	8	8	8	8	8	9	8	8					
Serum parameters[3]																		
α-T, ug/mL	0.96	2.86[a]	0.83[b]	0.54[b]	1.80[a]	0.88[b]	1.08[b]	1.1	0.81	0.77	1.16	1.12	1.04	0.28	< 0.01	< 0.01	< 0.01	< 0.01
TBARS, ug/L	30.00	32.03	38.78	36.78	32.08	39.41	38.62	29.05	31.08	30.03	30.32	31.25	30.92	1.81	< 0.01	< 0.01	0.28	0.17
Endt, EU/mL	551	834	1181	961	867	1219	1166	1060	931	794	1250	1019	1012	220	0.40	0.79	0.29	0.06
HPT, mg/mL	0.36	0.58	0.49	0.30	0.78	0.54	0.81	0.41	0.52	0.54	0.61	0.39	0.91	0.18	0.41	0.25	0.44	0.47
IgA, µg/mL	466	634	569	718	5623	858	656	870	588	503	779	878	644	398	0.60	0.57	0.24	0.10
IgG, µg/mL	2,653	2,992	3,706	4,145	3,817	3,955	4,888	3,689	4,170	3,433	4,280	5,672	3,696	2,467	0.20	0.27	0.28	0.09
Urine parameters[4]																		
TBARS, ug/L	15.51	16.62	16.46	17.12	16.51	18.07	17.34	15.3	16.98	15.17	16.63	16.8	16.00	1.80	0.69	0.51	0.99	0.64
L:M	0.45	0.07	0.05	0.05	0.06	0.04	0.07	0.26	0.05	0.13	0.19	0.09	0.05	0.17	0.09	0.12	0.89	< 0.01
Organ weight																		
Spleen, %	0.32	0.33	0.29	0.32	0.34	0.33	0.38	0.33	0.34	0.32	0.39	0.38	0.38	0.03	0.02	0.80	0.92	0.51

[1]Data are least square means (for control n = 6, for lipid diets n = 8 or 9); OL = lipids were stored as received without antioxidants or heating; SO = lipids heated for 72 h at 95°C with constant compressed air flow rate at 12 L/min; RO = lipids heated for 7 h at 185°C with constant compressed air flow rate at 12 L/min.

[2]S = lipid source; O = peroxidation level; S×O = lipid source by peroxidation level interaction; C vs L = control versus lipids.

[3]α-T = α-tocopherol; TBARS = thiobarbituric acid reactive substances; Endt=endotoxin; HPT = haptoglobin.

[4]L:M = lactulose to mannitol ratio.

[ab]Means with different superscript differ ($P < 0.05$) .

Table 5.2. The correlation coefficient between tests of lipid oxidation (**TLO**) and the analysis of serum α-tocopherol (**α-T**) and serum TBARS[1]

	TLO[2]							
	PV	*AnV*	*TBARS*	*Hex*	*DDE*	*HNE*	*AOM*	*OSI*
Serum α-T	-0.22	-0.37	-0.21	-0.30	-0.30	-0.35	-0.32	0.14
	0.03	< 0.01	0.03	< 0.01	< 0.01	< 0.01	< 0.01	0.15
Serum TBARS	0.43	0.33	0.49	0.43	0.45	0.42	0.52	-0.32
	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

[1]Top value represents correlation (r value) and bottom value represents significance (*P* value).

[2]α-T = α-tocopherol, PV = peroxide value, AnV = p-ansidine value, TBARS = thiobarbituric acid reactive substances, HEX = hexanal, DDE = 2, 4-decadinal, HNE = 4-hydroxy nonenal, AOM = active oxygen method, and OSI = oxidative stability index.

CHAPTER 6

IMPLICATIONS

Measurements of lipid peroxidation provide important information in assessment of lipid quality which is important because of the potential impact on animal health and performance due to the effects of secondary and tertiary peroxidation products . Unfortunately, the assessment of the degree of lipid peroxidation is challenging because of the drawbacks of each method used. Our results from chapter 2 suggest that there is no single method that seems to adequately describe or predict lipid peroxidation due to the complexity of lipid composition and the phases involved in lipid peroxidation. To accurately analyze the amount of lipid damage caused by peroxidation, it may be advantageous to determine the degree of lipid peroxidation at several time intervals using more than one test. However, a high value of peroxide value, p-anisidine value, thiobarbituric reactive substances, hexanal, 2, 4-dicadienal, 4-hydroxylnoneal, or active oxygen method as well as a low value of oxidative stability index indicate a high degree of lipid peroxidation. If a lipid undergoes a mild degree of lipid peroxidation and most of the hydroperoxides formed had not been decomposed, it is economical and feasible to use peroxide value as a primary measure of peroxidation because there is less concern about decomposition of hydroperoxides and peroxide value can accurately reflect the degree of lipid peroxidation. However, if a lipid is subjected to a high degree of lipid peroxidation, and most of the hydroperoxides formed have already been decomposed to yield secondary or tertiary lipid peroxidation products, a single measure of secondary oxidation products, such as p-anisidine value or thiobarbituric acid substances may be more

acceptable and economical because the production of secondary lipid peroxidation products can be measured more accurately than a single peroxide value analysis. Furthermore, for better evaluation of lipid peroxidation of lipids that have been subjected to extreme peroxidation, future research should focus on development of measures used to assess lipid peroxidation based on quantification of the triacylglycerol dimers or triacylglycerol polymers. The amount of dimers and polymers formed during lipid peroxidation keeps increasing as the heating time is increased (Sanchez-Muniz et al., 1993; Takeoka et al., 1997).

Two major uses of absorbed triglycerides include incorporation into adipose tissue to function as stored energy, or providing energy for maintaining essential life processes including lean tissue synthesis. Understanding the effect of oxidized lipids on lipid metabolism in pigs is essential for improving our ability to optimize pork production. Our data from chapter 3 suggest that feeding thermally-oxidized lipids to pigs may be related to the activation of PPARα pathway, which caused the reduced liver triglyceride concentrations. Reduced liver triglyceride concentrations may lead to decreased lipid availability for adipose tissue accretion and protein synthesis, and subsequently result in decreased growth performance. In addition, regardless of lipid peroxidation level, lipids derived from different origins will contribute to different hepatic triglyceride and cholesterol concentrations, which indicate that feeding lipids that are markedly different in fatty acid profiles will lead to different liver triglyceride and cholesterol concentrations. Thus, the results from chapter 2 indicate that inclusion of thermally-oxidized lipids in diets fed to young pigs should be of concern. In order to avoid the negative effects of

thermally-oxidized lipids on lipid metabolism, the dietary inclusion rate and feeding length of thermally-oxidized lipids should be further defined.

Energy is one of the most expensive components of swine dietsand lipids are important energy sources due to their higher energy content compared to carbohydrates and proteins (Pettigrew and Moser, 1991). Lipids used in the animal feeds vary greatly in fatty acid composition, quality, and cost. Better knowledge of the energy value of lipids based on the degree of peroxidation and fatty acid profile will increase the ability of nutritionists to optimize the use of lipids in swine diets. Our results from chapter 4 indicate that the increase in lipid peroxidation products produced by heating of lipids in two common unsaturated vegetable oils (corn and canola oil) and two commonly used saturated animal fats (tallow and poultry fat) did not affect ATTD of various nutritional components and had no impact on subsequent DE or ME of the lipids evaluated. In addition, results from chapter 4 support the notion that nutrient and energy digestibility, and consequently DE and ME values, are mainly dependent on their fatty acid composition rather than their level of peroxidation. Thus, our results from chapter 4 indicate that measures of lipid peroxidation may not be as sensitive as the unsaturated to saturated fatty acid ratio in predicting the lipid digestibility and subsequent DE and ME values.

The degree of metabolic oxidative stress not only impairs animal growth performance, but also influences animal health. Results from chapter 5 support the concept that feeding thermally-oxidized lipids, especially the vegetable oils with high concentration of polyunsaturated fatty acids, to pigs contributed to metabolic oxidative

stress based on the depletion of serum α-tocopherol and the.increased production of secondary lipid peroxidation products. However, the amount of lipid peroxidation products produced by the rapid and slow peroxidation processes used in this study were not sufficient to adversely affect gut barrier function and immune response. Although several measurements used in this study showed that metabolic oxidative stress does occur when pigs are fed diets containing thermally-oxidized lipids, others did not. The results from this study will be useful in future research to better understand and characterize the effects of lipid peroxidation on pig health and growth performance. Furthermore, research is needed to determine if supplemental dietary antioxidants are needed in swine diets when consuming thermally-oxidized lipids, especially lipids which contain a high concentration of polyunsaturated fatty acids.

CHAPTER 7

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