Title: Evaluation of lipid source and peroxidation level on digestible and metabolizable energy concentration, and the impact of lipid peroxidation on intestinal barrier function **- NPB #10-013**

Revised

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Industry Summary:

BACKGROUND: A better understanding of methods to assess quality and feeding value of feed fats and oils is needed because of variability in composition and quality. Commonly used lipid quality measures such as moisture-insolubles-unsaponables, titer and free fatty acid content are used to insure that the lipid products meet trading specifications. However, these measures provide limited information regarding the feeding value of feed fats and oils and provide essentially no information regarding the degree of degradation, called peroxidation, of a given lipid source. Methods specific for evaluating lipid peroxidative stability can be divided into indicative and predictive tests. Indicative tests are used to measure the presence of peroxidation products in lipids and include: peroxide value (**PV**), thiobarbituric acid reactive substances (**TBARS**), p-anisidine value (**AnV**), conjugated dienes, hexanal value, 2, 4-decadienal (**DDE**), and 4-hydroxynonenal (**HNE**). Predictive tests measure the stability or susceptibility of lipids to peroxidation and include active oxygen method (**AOM**) and oxygen stability index (**OSI**). In predictive tests, the lipid is subjected to specific conditions that accelerate peroxidation and a peroxidation endpoint is defined to determine the degree of peroxidation damage. However, assessment of the degree of lipid peroxidation is complex because the process consists of three phases: (1) an initiation phase which involves the formation of free lipid radicals and hydroperoxides as primary reaction products, (2) a propagation phase where the hydroperoxides formed are decomposed into secondary peroxidation products, and (3) a termination phase involving the formation of tertiary peroxidation products.

LIPID PEROXIDATION: In the current study, analysis of the slowly and rapidly peroxidized lipids compared to the original lipids showed that a high PV accurately indicated a high degree of lipid peroxidation, but a moderate or low PV may be misleading. This is due to the unstable characteristics of hydroperoxides as indicated by the unchanged PV of rapidly peroxidized corn and canola oil compared to their original, unoxidized state. Additional tests which measured secondary peroxidation products (AnV, TBARS, hexanal, HNE and DDE) showed peroxidation differences between the lipid treatments, but similar to PV analysis, these tests also may not provide irrefutable information regarding the amount of peroxidation because secondary peroxidation products are extremely volitile. For the predictive tests, AOM stability accurately reflected the increased lipid peroxidation caused by the slow and rapid peroxidation treatments as indicated by the increased AOM stability value in corn and canola oil, but not in poultry fat and tallow, which indicates a potential

disadvantage of the AOM stability test. The OSI assay successfully showed the increased lipid peroxidation caused by slow or rapid peroxidation treatments in all lipids, but it too may have disadvantages similar to AnV, TBARS, hexanal, HNE and DDE, because the OSI assay directly depends on quantification of the volatile secondary peroxidation products. Thus, relative to peroxidation assays utilized in the current study, no individual indicative or predictive test appears to provide a complete assessment of the degree of lipid peroxidation. Therefore, 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 points during the peroxidation process using more than one test.

ANIMAL EXPERIMENTATION: Results from this study showed no effect of lipid peroxidation (slow or rapid peroxidation) in corn oil, canola oil, poultry fat or tallow on digestible energy or metabolizable energy content or on apparent total tract digestibility of dry matter, gross energy, ether extract, nitrogen, carbon, and sulfur when fed to nursery pigs. Feeding rapidly oxidized lipids (corn oil and canola oil as well as poultry fat and tallow heated at 185°C for 7 h) to nursery pigs for 28 days tended to reduce average daily feed intake and average daily gain compared to pigs fed non-oxidized lipids suggesting that lipid peroxidation can negatively impact pig growth performance. Relative to metabolic oxidation status, feeding slow and rapid peroxidized lipids to nursery pigs increased plasma TBARS concentration. However, feeding peroxidized corn oil, canola oil, poultry fat, or tallow to nursery pigs had no effect on intestinal barrier function as measured by the lactoluse-mannitol protocol utilized in the current study. Lastly, feeding thermally oxidized lipids altered *in vivo* lipid metabolism by activating the peroxisome proliferator-activated receptor α (**PPARα**) via up-regulation of some target genes in PPARα, such as acyl CoA oxidase, catalase, and carnitine palmitoyltransferase-1.

Overall the data suggested that feeding thermally-peroxidized lipids to young pigs has little influence on gut barrier function or serum immunity parameters, but may decrease liver triglyceride concentrations, impair metabolic oxidative status, and reduce growth performance, especially if fed lipids containing high concentration of polyunsaturated fatty acids. However, at this point in time, we do not have a clear relationship relating the change in pig performance relative to a defined level of lipid peroxidation. For further information, contact Dr. Brian Kerr, USDA-ARS-NLAE, Ames, IA, by phone (515-294-0224) or email (brian.kerr@ars.usda.gov).

Keywords: gene expression, lipid peroxidation, metabolic oxidation, nutrient digestibility, pig

Scientific Abstract
Four lipids were obtained and peroxidized by a slow or rapid method, and subsequently characterized by various methods. Analysis showed that a high peroxide value accurately indicated the high degree of lipid peroxidation, but a moderate or low peroxide value may be misleading due to the unstable characteristics of hydroperoxides as indicated by the unchanged peroxide value of rapidly oxidized corn and canola oil compared to their original, unoxidized state. Additional tests which measured secondary peroxidation products (p-anisidine , thiobarbituric acid reactive substances, hexanal, 4-hydroxynonenal, and 2, 4-decadienal) were suggested to provide a better indication of lipid peroxidation than peroxide value for lipids subjected to a high degree of peroxidation. Similar to peroxide value analysis, it was suggested that 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, active oxygen method stability accurately reflected the increased lipid peroxidation caused by the slow and rapid peroxidation treatments as indicated by the increased active oxygen method stability value in corn and canola oil, but not in poultry fat and tallow. This indicates a potential disadvantage of the active oxygen method stability test. The oxidative stability index assay successfully showed the increased lipid peroxidation caused by slow or rapid peroxidation treatments in all lipids, but it too may have disadvantages similar to anisidine , thiobarbituric acid reactive substances, hexanal, 4-hydroxynonenal, and 2, 4-decadienal, because the oxidative stability index assay directly depends on quantification of the volatile secondary peroxidation products. It was concluded that to

accurately analyze the peroxidation damage in lipids, measurements may need to be determined at various time intervals by more than one test and include different types of peroxidation products simultaneously. At this time, however, we do not have data to recommend at what specific times these measurements should be taken or which peroxidation products should be measured.

Pigs fed diets containing lipids that were rapidly oxidized tended to have reduced average daily feed intake and body weight gains compared to pigs fed the unoxidized lipids. Pigs fed diets containing the rapidly oxidized lipids tended to have increased liver weight compared to pigs fed diets containing the unoxidized lipids. In contrast, liver triglyceride concentration in pigs fed the unoxidized lipids were greater than pigs fed the rapidly oxidized lipids, and tended to be greater than in pigs fed the lipids that had been slowly oxidized. The reduced liver triglycerides were consistent with increased mRNA expression of peroxisome proliferator activated receptor-alpha factor target genes in pigs fed lipids that had been slowly or rapidly oxidized compared with pigs fed the unoxidized lipids. Lipid peroxidation had little effect on digestible or metabolizable energy values determined for any of the lipids tested, and did not appreciably affect total tract digestibility of dietary dry matter, lipid, nitrogen or carbon. Serum α-T concentration were decreased when pigs were fed corn and canola oil that had been slowly or rapidly oxidized, but not in pigs fed diets containing poultry fat or tallow. Lipid peroxidation had no effect on serum endotoxin, haptoglobin, immunoglobulin A, or immunoglobulin G, or on urinary thiobarbituric acid reactive substances and lactulose to mannitol ratio.

Overall the data suggested that feeding thermally-oxidized lipids to young pigs has little influence on gut barrier function or serum immunity parameters, but may decrease liver triglyceride concentrations, impair metabolic oxidative status, and reduce growth performance, especially for lipids containing high concentration of polyunsaturated fatty acids.

Introduction:

Supplemental fats and oils are commonly added to swine diets to increase energy density of the diet. Fats and oils have generally been considered to be 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). However, their source and level may affect nitrogen digestibility and retention, and amino acid absorption (Lowrey et al., 1962; Cera et al., 1988b, 1989a,b; Li et al., 1990; Li and Sauer, 1994; Jorgensen and Fernandez, 2000). In addition, the apparent digestibility of various lipids in nursery pigs has been shown to increase with age (Hamilton and McDonald, 1969; Frobish et al., 1970) with digestibility of the lower digestible animal fat sources (lard and tallow) increasing to a greater extent with age compared to digestibility of vegetable oils (Cera et al., 1988a,b; 1989a, 1990). The NRC (2012) estimates of DE content of various fat sources are based on the classic research by Wiseman et al. (1990) and Powles et al. (1993, 1994, 1995), with ME subsequently calculated as 98% of DE, and net energy calculated as 88% of ME. However, the value of using these equations to accurately predict the energy content for all types and qualities of fats is limited. Although recent research (Jorgensen and Fernandez, 2000; Kerr et al., 2009; Silva et al., 2009; Anderson et al., 2010) has shown that the DE and ME content of various refined lipids were similar to values reported in the NRC (2012), the effect of quality (peroxidation) on energy value among fat sources has not been established. Only recently has it been shown that increasing the rancidity of choice white grease will decrease feed intake, but fatty acid digestibility was not affected and no DE or ME determination was made (DeRouchey et al., 2004). Because lipid sources may vary substantially in quality, particularly in the extent of peroxidation, their DE and ME content may be reduced. In addition, lipid peroxidation may cause negative effects on gut health and the immune system, further reducing the value of these lipids as an energy source. To date, no studies have been conducted determining the effects of feeding peroxidized animal fats or vegetable oils on energy content, gut health, and metabolic oxidation status.

Fats and oils are highly susceptible to peroxidation (Mayes 1996), especially when subjected to heat during further processing, which likely affects energy digestion and utilization of fat sources. The estimated energy content of a fat source (NRC, 2012) accounts for only the total fatty acid content and the unsaturated:saturated

fatty acid ratio, and not other potential factors (peroxidation products) which may affect the DE and ME value. It is also known that consumption of unsaturated and peroxidized fats may increase oxidative stress within the animal. While feeding increased levels of antioxidants may alleviate some of the oxidative stress associated with dietary unsaturated fats, some of the physiological effects of feeding unsaturated fats may not be mitigated by antioxidant supplementation. For example, feeding diets containing corn oil (CN) increased circulating endotoxin levels in mice (Cani et al., 2007). Likewise, the consumption of CN by young pigs increased blood urea nitrogen, decreased nitrogen retention (Cera et al., 1988a), and negatively impacted intestinal architecture (Cera et al., 1988c), suggesting that perturbations in nitrogen metabolism and intestinal function occur when unsaturated fats are fed. Whether the decreased nitrogen retention in pigs fed CN is due to increased intestinal permeability, and thus, increased circulating endotoxin, or due to increased oxidative stress remains unknown.

The heating/peroxidation of unsaturated fat sources leads to the production of reactive aldehyde compounds. (Seppanen and Csallany, 2002). Of these compounds, HNE (Uchida, 2003) is best characterized for its adverse physiological effects. The consumption of fat sources containing HNE is likely to add to the oxidative load because HNE readily conjugates to glutathione, thus depleting this important antioxidant (Uchida, 2003). Treating cells directly with HNE increases the activation of stress pathways (Biasi et al., 2006; Yun et al., 2009) and alters immune signaling pathways. Treatment of macrophages with HNE increases the expression of the inflammatory mediator, cyclooxygenase-2 by inducing the p38 mitogen activated protein kinase (Kumagai et al., 2004). The antibody IgA, which plays an important role in mucosal defense, is directly bound by HNE which decreases the ability of IgA to bind bacterial antigens (Kimura et al., 2006). In addition, HNE has been found to block macrophage signaling by binding to toll-like receptor-4, an important bacterial pathogen recognition receptor (Kim et al., 2009). Therefore, it is likely that consumption of an oxidized unsaturated fat source directly alters the mucosal immune system through the direct action of compounds such as HNE that are produced by the lipid peroxidation process.

At the level of the small intestine, feeding a peroxidized fat source to growing pigs has been shown to increase markers of oxidative stress (Ringseis et al., 2007). However, no information has been reported regarding the impact of feeding peroxidized fat on intestinal architecture, barrier function, or immune function. In young chickens, it was observed that feeding a peroxidized fat source decreased small intestinal villus length (Dibner et al., 1996). Studies conducted in broiler chickens (Takahashi and Akiba, 1999) found that feeding peroxidized fat decreased *ex vivo* primary antibody production to a bacterial pathogen. Taken together, these data suggest that peroxidized fat likely has negative effects on intestinal function. However, the impact of increasing the content of peroxidized fat, and presumably dietary HNE, in swine diets on nutrient utilization, intestinal barrier function, and inflammatory status remains largely unknown. In order to optimize pig health and growth performance, it is important to know the physiological impacts of feeding peroxidized fat sources to growing pigs.

Objectives of Research Project:
The contribution of dietary peroxidized fats to total energy intake has markedly increased with the higher inclusion rates of corn co-products and various other supplemental fat sources, which may cause suboptimal pig performance and negatively affect intestinal health. Only recently, have data been published on the impact of fat rancidity (peroxidation) on fat digestibility and pig growth performance. However, no data are available on the impact of dietary peroxidized fat on DE or ME content, nitrogen retention, or its impact on oxidative stress and intestinal barrier function in growing pigs. Thus, there is a *critical need* to understand the impact of fat type and peroxidation level on dietary energy (DE and ME) and nitrogen utilization, as well as its intestinal and physiological effects. There are limited data suggesting that feeding peroxidized fat to swine increases oxidative stress, but the impact of dietary peroxidized fat on intestinal barrier function and mucosal immunity is unknown. Therefore, the objectives of the proposed research are to: 1) determine the variation in DE and ME content of four dietary fat sources ranging from saturated animal fat (tallow, (TL) and poultry fat (PF)) and two unsaturated vegetable oils containing either low or high amounts of linoleic acid (canola oil (CA) and CN,

respectively); 2) determine the impact of lipid peroxidation on DE and ME content and nitrogen retention; and 3) determine the impact of dietary fat source and lipid peroxidation on indicators of oxidative stress and intestinal barrier function.

Materials & Methods

Lipid Preparation: Four lipids, each with 3 degrees of peroxidation were evaluated. Lipid sources were: CN (ADM, Decatur, IL), CA (ConAgra Foods, Omaha, NE), PF (American Protein, Inc., Hanceville, AL), and TL (Darling International, Wahoo, NE). Peroxidation levels were: original lipids (**OL**), slow-peroxidation (**SO**), and rapid-peroxidation (**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 and 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. Lipids were analyzed for 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. Peroxide value (method Cd 8-53; AOCS, 1993), TBARS (Buege and Aust, 1978), and HNE (Zanardi et al., 2002; Fitzmaurice et al., 2006) were analyzed at the University of Minnesota. Active oxygen method (PV at 20 h of Method Cd 12-57; AOCS, 2009), OSI (Method Cd 12b-92; AOCS, 1997), 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 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).

Animal Experimentation: All animal use procedures were reviewed and approved by the University of Minnesota Institutional Animal Care and Use Committee. 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. 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 (CN, CA, PF, and TL) and peroxidation 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). Other lipid supplemented diets were then formulated by replacing 10% CA with the other lipids. 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. 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 gain:feed. 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 body weight daily (2% at 0700 h and 2% at 1900 h). Pigs were allowed *ad libitum* access to water.

During the balance study, 5 d (d 29 to d 34) was allowed for crate and diet feeding regimen adaptation, following which a 3-d total urine and fecal collection period occurred. 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.

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**), cholesterol (**CH**). serum α-tocopherol (**α-T**), TBARS, endotoxin, haptoglobin, IgG, and IgA analysis. After blood sampling, 2 mL chlorhexidine 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 TBARS 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 and 2 g of mannitol as markers of intestinal permeability. Plastic containers with 2 ml chlorhexidine 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 and 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. Spleen and kidney were also excised and weighed to calculate their organ weight as a percentage of BW.

Metabolism-Balance Study Analysis: Gross energy of lipids, diets, feces, and urine samples were determined using an isoperibol bomb calorimeter using benzoic acid used as a standard. 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 using an accelerated solvent extraction system, while C, N, and S were analyzed by thermocombustion. Apparent total tract digestibility (**ATTD**) 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 dry matter (**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.

Serum and Liver Cholesterol and Triglyceride Analysis: Total lipids from the liver were extracted using the modified method of Folch et al. (1957). 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. 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). 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).

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

For lipid peroxidation data, 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). All other data were analyzed using the MIXED procedure of SAS with a two-way ANOVA 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 body weight 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

Nutrient Balance and Digestibility: 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.

Serum Lipid Parameters-Liver Gene Expression-Animal Performance: 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.

Metabolic Oxidation-Gut Barrier Function: 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 ($P < 0.01$).

DISCUSSION

Analysis of the lipids indicate they were clearly peroxidized by the two heating methods, but that a single method of analysis (either indicative or predictive) may be misleading due to the unstable characteristics of primary and secondary peroxidation products. As such, it is suggested that 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. At this time, however, we do not have data to recommend at what specific times these measurements should be taken or which peroxidation products should be measured.

Feeding the peroxidized lipids did not affect the ATTD of various nutritional components and had no effect on subsequent DE or ME of the lipids evaluated. This suggests that peroxidized lipids have little effect on these measures, levels of peroxidation were not great enough to have an effect, or that these measures are not sensitive enough to measure potential peroxidation effects. Furthermore, 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. In contrast, feeding weaned pigs diets containing 10% thermally-oxidized lipids for 38 d, especially vegetable oils containing high concentrations of PUFA, impaired 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 peroxidation. Pigs fed rapidly oxidized lipids had reduced growth performance and increased liver weight compared to those fed the original lipids. In addition, lipids that were peroxidized, 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 TG and CH concentrations, which indicate that feeding lipids that are markedly different in fatty acid profiles will lead to different liver TG and CH concentrations. Overall, measures of lipid peroxidation may provide helpful information for predicting various biological responses in pigs, but it remains a complicated and confusing field of science.

Therefore, 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 points during the peroxidation process using more than one test.