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

P. Liu,* C. Chen,* B. J. Kerr,† T. E. Weber,† L. J. Johnston,‡ and G. C. Shurson*

*University of Minnesota, St. Paul 55108; †USDA-ARS-National Laboratory for Agriculture and the Environment, Ames, IA 50011; and ‡West Central Research and Outreach Center, Morris, MN 56267

ABSTRACT: 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 expression patterns. Lipid oxidation analysis indicated that compared with the OL, SO and RO of lipids had a markedly increased concentrations of primary and secondary peroxidation products, and the increased lipid peroxidation products in CN and CA were greater than those in PF and TL. After a 28-d ad libitum feeding period, pigs fed RO lipids tended to have reduced ADFI (P = 0.09) and ADG (P < 0.05) compared with pigs fed OL, and pigs fed CA had reduced G:F (P < 0.05) compared with pigs fed all other lipids. Pigs fed RO lipids tended to have increased relative liver weight (P = 0.09) compared with pigs fed OL. Liver triglyceride concentration (LTG) in pigs fed OL was greater (P < 0.05) than in pigs fed SO lipids 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-methylglutaryl-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 with pigs fed TL. Liver cholesterol concentration in pigs fed CN was less (P < 0.05) than in pigs fed PF and tended to be less (P = 0.06) than in pigs fed TL, whereas pigs fed CA had a reduced (P < 0.05) liver cholesterol compared with pigs fed PF or TL. In conclusion, feeding thermally oxidized lipids negatively affected growth performance and LTG of young pigs, which was associated with an upregulation of fatty acid catabolism pathways.

Key words: 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 consid-
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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

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

To generate oxidized lipids, vesicles containing the required amount of each of the 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. Using an air pump, both heating processes were accompanied with constant flow of compressed air of 12,000 cm³/min and a temperature between 22 and 24°C. Before feed mixing, the OL and 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 2 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 and SO and RO lipids).

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 greatest 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 the NRC (1998). Other lipid supplemented diets were then formulated by replacing 10% CA with the other lipids. The control diet, with no added lipid, was formulated by factoring out the lipid supplement and increasing all other ingredients on a proportional basis. Phase 1 lipid-supplemented diets (Table 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 postweaning. Due to slower than expected growth of pigs during Phase 1, Phase 2 diets (Table 1) were formulated based on the NRC (1998) recommendation for pigs weighing 13 to 20 kg and were fed to pigs from d 24 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 1 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 0 and 29 to calculate ADG, ADFI, and G:F. From d 29 to 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; ThermoScientific, 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 pento-barbital sodium solution (390 mg/mL; Fatal-Plus Powder;
frozen liver tissue from the same region of the liver 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 at 20°C and was then transferred to a new glass tube. After drying under N₂, the lipid fraction was reconstituted in n-butanol for further analysis of TG and CH. Triglyceride and CH concentrations were determined enzymatically by conducting colorimetric assays (Pointe Scientific, Canton, MI) in a 96-well plate reader (SpectraMAX 250, Molecular Devices, Sunnyvale, CA).

**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). For determination of mRNA concentration, a threshold cycle and amplification efficiency were 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 glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene due to its relatively stable expression (Kerr et al., 2005; Paczkowski et al., 2011). The sequences of customized primers used in the reverse transcriptase-PCR reactions (Integrated DNA Technologies, Coralville, IA) are listed in Table 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 and SO and RO lipids), 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

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

<table>
<thead>
<tr>
<th>Ingredients, %</th>
<th>Phase 1</th>
<th>Phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Lipid</td>
</tr>
<tr>
<td>Corn</td>
<td>48.09</td>
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</tr>
<tr>
<td>Soybean meal (46%)</td>
<td>28.16</td>
<td>25.34</td>
</tr>
<tr>
<td>Fish meal, menhaden</td>
<td>10.67</td>
<td>9.60</td>
</tr>
<tr>
<td>Whey powder</td>
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<td>10.00</td>
</tr>
<tr>
<td>Limestone</td>
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<tr>
<td>Dicalcium phosphate</td>
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<td>0.06</td>
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<tr>
<td>NaCl</td>
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<td>0.18</td>
</tr>
<tr>
<td>t-Lys HCl</td>
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<td>–</td>
</tr>
<tr>
<td>dl-Met</td>
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<td>0.03</td>
</tr>
<tr>
<td>Premix²</td>
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</tr>
<tr>
<td>Mecadox³</td>
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<td>0.50</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

Calculated nutrients,⁴ %

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<th>Lipid</th>
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</thead>
<tbody>
<tr>
<td>CP</td>
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<td>22.50</td>
</tr>
<tr>
<td>Total P</td>
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<tr>
<td>Available P</td>
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<td>Ca</td>
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<td>0.87</td>
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<td>SID² Lys</td>
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<tr>
<td>SID Met</td>
<td>0.80</td>
<td>0.72</td>
</tr>
<tr>
<td>SID Thr</td>
<td>0.88</td>
<td>0.79</td>
</tr>
<tr>
<td>SID Trp</td>
<td>0.26</td>
<td>0.23</td>
</tr>
</tbody>
</table>

⁴Calculated 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, respectively. The calculated ME of canola oil, corn oil, poultry fat, and tallow supplemented diet: vitamin A, 7,716 IU; vitamin D₃, 1,929 IU; vitamin E, 39 mg; vitamin B₁₂, 120 mg; Zn (oxide), 300 mg; and Se (Na₂SeO₃), 0.3 mg. Mecadox is the trade name for carbadox (Phibro Animal Health Corporation, Ridgefield Park, NJ).

**Serum and Liver Cholesterol and Triglycerides**

Total lipids from the liver were extracted using the modified method of Folch et al. (1957). Briefly, 250 mg of

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**Footnotes:**

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 postweaning. 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 24 to 39 of the experiment.

2. Vitamin and mineral premix provided the following per kilogram of lipid supplemented diet: vitamin A, 7,716 IU; vitamin D₃, 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; and Se (Na₂SeO₃), 0.3 mg.


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, respectively.

5. SID = standardized ileal digestible.
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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 of \( 0.05 \leq P \leq 0.10 \) were considered statistical trends.

RESULTS

Characterization of Experimental Lipids

The various characteristics of the experimental lipids have been described in detail previously (Liu et al., 2013). In brief, the concentration of crude fat, moisture, insolubles, and unsaponifiables were similar among the 12 experimental lipids. As expected, CN and CA had greater concentrations of unsaturated fatty acids than TL, with PF being intermediate. Averaged among lipid sources, both SO and RO of lipids decreased the linoleic acid and linolenic acid concentrations compared with the OL, but changes in concentration of other major fatty acids were not observed. Lipid peroxidation tests indicated that all of the OL were relatively unoxidized, but SO and RO of lipids 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 greater than that in PF and TL (Liu et al., 2013).

Growth 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, 1 pig from the first group fed original CA and 1 pig from the second group fed SO PF died. For the growth performance portion of the experiment, there were 3 observations per treatment consisting of 3 pens with 8 to 9 total pigs. For all other data, there were 8 or 9 observations per treatment.

During the first 28 d, no lipid source or lipid source × peroxidation interactions were observed for ADFI and ADG (Table 3). In contrast, peroxidation level affected both ADFI (\( P = 0.09 \)) and ADG (\( P = 0.04 \)). Compared with 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 lipids and OL 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 (\( P < 0.05 \)).

Liver Weight

No lipid source or lipid source × peroxidation level interaction was noted for liver weight as a percentage of BW (Fig. 1). Liver weight tended to be greater for pigs fed RO lipids compared with 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 (Fig. 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

<table>
<thead>
<tr>
<th>Gene1</th>
<th>Forward primer (from 5′ to 3′)</th>
<th>Reverse primer (from 5′ to 3′)</th>
<th>Product length, bp</th>
<th>Temperature, °C</th>
<th>NCBI GenBank</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACO</td>
<td>GCTGATTACACACATGCACGGCGCA</td>
<td>ACCCGTAAAGGCTGCTCCGGTA</td>
<td>132</td>
<td>59</td>
<td>AF185048</td>
</tr>
<tr>
<td>CPT-1</td>
<td>AGGCTGCGGAAATGGGTTGCG</td>
<td>AGGCGCTGGTTCGCCGTTG</td>
<td>143</td>
<td>59</td>
<td>AF288789</td>
</tr>
<tr>
<td>GAPDH</td>
<td>AGCCACACGTTACGAGGCAGTCGTT</td>
<td>TCTCTCCCCCTCAACCGCAGCA</td>
<td>135</td>
<td>60</td>
<td>AF288789</td>
</tr>
<tr>
<td>mHMGC-CoA-S</td>
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<td>TCGCTCGATGCACTGGTCTTT</td>
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<td>60</td>
<td>U90884</td>
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<td>PPARα</td>
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<td>GGCGGAGGAGGACTCCTGGGAA</td>
<td>141</td>
<td>59</td>
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<tr>
<td>SCD</td>
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<td>58</td>
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<tr>
<td>SREBP-1</td>
<td>GCCTGCACTTTTCTGACCGCT</td>
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<td>AAGTGGAGACCGACAGCCCG</td>
<td>112</td>
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</table>

1ACO = acyl-CoA oxidase; CPT-1 = carnitine palmitoyltransferase 1; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; mHMGC-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.

2NCBI = National Center for Biotechnology Information.
Table 3. Growth performance of pigs fed vegetable oils and animal fats of differing oxidation status

<table>
<thead>
<tr>
<th>Day</th>
<th>Corn oil</th>
<th>Canola oil</th>
<th>Poultry fat</th>
<th>Tallow</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>1–29</td>
<td>OL SO RO OL SO RO OL SO RO OL SO RO SEM</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ADFI, g</td>
<td>463</td>
<td>411</td>
<td>391</td>
<td>430</td>
<td>385</td>
</tr>
<tr>
<td>ADG, g</td>
<td>320</td>
<td>293</td>
<td>246</td>
<td>276</td>
<td>206</td>
</tr>
<tr>
<td>G:F</td>
<td>0.69</td>
<td>0.71</td>
<td>0.63</td>
<td>0.63</td>
<td>0.53</td>
</tr>
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</table>

*aPigs fed RO lipids tended to have reduced ADFI (P = 0.09) compared with pigs fed OL lipids.
*bPigs fed RO lipids had a reduced ADG (P = 0.03) compared with pigs fed OL lipids.
*cPigs fed canola oil supplemented diets had a reduced G:F compared with pigs fed other source of lipids (P = 0.05).

1 Data are least square mean of 3 observations per treatment. OL = original lipids (lipids were stored as received without antioxidants or heating); SO = slow oxidation (SO lipids were heated for 72 h at 95°C with constant compressed air flow rate at 12 L/min); RO = lipids (RO lipids were heated for 7 h at 185°C with constant compressed air flow rate at 12 L/min). Average initial and final BW were 6.67 (SD = 0.03) and 13.87 (SD = 2.01) kg, respectively.

2 SOU = lipid source; PER = peroxidation level; SOU × PER = lipid source × oxidation level interaction.

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 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 ketogenesis), stearoyl-CoA desaturase (SCD; a key enzyme in fatty acid metabolism that 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 CH 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 4). However, a peroxidation level effect in liver mRNA level of these genes was observed (P < 0.10). Pigs fed either SO or RO supplemented diets had greater mRNA levels of ACO, CPT-1, and mHMG-CoA-S than pigs fed the OL supplemented diets (P < 0.05). Oxidation level tended to increase the mRNA expression level of PPARα (P = 0.08),...
Figure 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 (RO decreased [P < 0.05] and SO lipids tended to decrease [P = 0.07] the liver triglyceride concentration compared with OL). The P-value for lipid source effect = 0.03 (pigs fed either corn or canola oil tended to have increased liver triglyceride concentration compared with 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).

Table 4. Expression levels of mRNAs in livers of pigs fed vegetable oils and animal fats

<table>
<thead>
<tr>
<th>Gene 2</th>
<th>OL</th>
<th>SO</th>
<th>RO</th>
<th>OL</th>
<th>SO</th>
<th>RO</th>
<th>OL</th>
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<th>SO</th>
<th>RO</th>
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<td>1.50</td>
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<td>0.98</td>
<td>1.17</td>
<td>0.98</td>
<td>0.95</td>
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<td>1.03</td>
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<td>1.23</td>
<td>1.23</td>
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</table>

1 Data are least square mean of 8 observations per treatment; OL original lipids (lipids were stored as received without antioxidants or heating); SO = slow oxidation (SO lipids were heated for 72 h at 95°C with constant compressed air flow rate at 12 L/min); RO = lipids (RO lipids were heated for 7 h at 185°C with constant compressed air flow rate at 12 L/min). Average BW (d 39) was 15.90 kg (SD = 2.39).

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 SOU = lipid source; PER = oxidation level; SOU × PER = lipid source × oxidation level interaction.
but no differences mRNA abundance of PPARα among OL and SO and RO lipids 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**

Because lipids with various levels of peroxidation, measured using different peroxidation tests, resulted in differences in growth performance, liver weight, liver TG concentrations, and expression of certain liver genes (PPARα, ACO, CPT-1, and mHMG-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 associations between various lipid peroxide measures and different biological responses (Table 5). Due to the limited number of animals used in the current experiment, only correlations where the \( P \)-value was 0.20 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 peroxide value (PV) and oxidative stability index (OSI) tests, negative correlations were found between p-anisidine value (AnV) and liver weight and \( (P = 0.06, \ r = 0.19) \), between TBARS and liver weight \( (P = 0.11, \ r = 0.16) \), between hexanal and liver weight \( (P = 0.17, \ r = 0.14) \), between 2,4-decadienal (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 measures of lipid oxidation were correlated with the gene expression of PPARα, ACO, CPT-1, and mHMG-CoA-S as shown in Table 5. No correlation was found between TG and any measure 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. (2013).

**Pig Performance**

After the 28-d ad libitum feeding, the growth performance results observed in the current study are con-
sistent with those reported in other studies (Alexander et al., 1987; Behniwal et al., 1993; Dibner et al., 1996) 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 lipids and OL were not different, potentially due to our use of only three 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). Second, impaired growth may be a consequence of the toxic effects of the lipid peroxidation products. Secondary lipid peroxidation products, such as α,β-unsaturated hydroxyaldehydes, 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 feed efficiency compared with those fed the other three lipid sources, which may be due to CA having the greatest total secondary oxidation products as indicated by the greatest 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 \( r = -0.29 \) 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 greatest 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 trend for an increase in relative liver weight may be a result of increased secretion of stress hormones or greater metabolic activity or may be 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 AnV \( r = 0.19 \), between liver weight and TBARS \( r = 0.11 \), between liver weight and hexanal \( r = 0.14 \), between liver weight and HNE \( r = 0.24 \), and between liver weight and AOM \( r = 0.22 \). Although there were significant positive correlations between liver weight and HNE and AOM, they explained only 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**

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., 2004). However, studies conducted to determine the differential effect of thermally oxidized oils and animal fats on the lipid profiles in pigs are limited. In this study, pigs fed both SO or RO lipids had reduced liver TG concentrations compared with pigs fed OL, regardless of the lipid source. Subsequent analysis of gene expression levels of the enzymes or transcription factors involved in fatty acid metabolism in the liver, including ACO, CPT-1, and mHMG-CoA-S, suggested that SO and RO of lipids caused activation of PPARα in the liver. Considering the central role of PPARα in fatty acid transportation, uptake, oxidation, and ketogenesis, it is possible that activation of PPARα contributes to reduced liver TG in pigs treated with oxidized lipids. Activation of PPARα caused by feeding oxidized lipids had been reported from studies in both rats and pigs (Chao et al., 2001, 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). In addition, the correlations between gene expression levels and lipid oxidation levels suggested that the measurements of PV, AnV, TBARS, hexanal, DDE, HNE, AOM, and OSI might predict the activities of PPARα pathways. Besides the PPARα-mediated fatty acid catabolism pathway, fatty acid de novo synthesis was also examined by measuring the expression levels of SREBP-1 and its target genes. The effect of feeding oxidized fat on gene expression of lipogenic enzymes is controversial. Luci et al. (2007) reported an upregulation effect of oxidized sunflower oil on lipogenic pathways in pigs, while others reported a downregulation 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 relative expression 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 of lipids. The exact mechanisms behind these different observations are unknown. Overall, 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, as the PPARα-mediated upregulation of catabolic metabolism, such as fatty acid oxidation lead to decreased lipid availability for adipose tissue accretion and protein synthesis.

Besides the effect of oxidized lipids, the effects of feeding lipids from plant and animal sources were compared in this study. The pigs fed either CN or CA had increased liver TG concentrations compared with 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. The different fatty acid profiles of the lipids evaluated in this study may have contributed to 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 upregulation of SREBP-2, a transcription factor that 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 levels of peroxidized 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 with pigs fed PF or TL. Pigs fed CN diets had lower liver CH concentration compared with pigs fed PF and tended to have lower liver CH than pigs fed TL. Pigs fed CA had a lower liver CH concentration compared with 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 polyunsaturated 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 RO lipids had reduced growth performance and a trend for increased relative liver weight compared with those fed the OL. Measures of lipid peroxidation may provide helpful information for predicting various biological responses in pigs. In addition, both SO and RO of lipids, regardless of lipid source, decreased liver TG presumably by the activation of the PPARα pathway.

**LITERATURE CITED**


