Influence of feeding thermally peroxidized soybean oil on growth performance, digestibility, and gut integrity in finishing pigs

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ABSTRACT: Consumption of peroxidized lipids has been shown to reduce pig performance and energy and lipid digestibility. Objectives of the current study were to evaluate the effect of feeding soybean oil (**SO**) with different levels of peroxidation on growth performance, lipid, N, and GE digestibility, plasma Trp, and gut integrity in finishing pigs. Fifty-six barrows (46.7 ± 5.1 kg initial BW) were randomly assigned to one of four diets in each of two dietary phases, containing either 10% fresh SO (22.5 °C) or thermally processed SO (45 °C for 288 h, 90 °C for 72 h, or 180 °C for 6 h), each infused with of 15 L/min of air. Peroxide values were 2.0, 17.4, 123.6, and 19.4 mEq/kg; 2,4-decadienal values were 2.07, 1.90, 912.15, and 915.49 mg/kg; and 4-hydroxynonenal concentrations were 0.66, 1.49, 170.48, and 82.80 mg/kg, for the 22.5, 45, 90, and 180 °C processed SO, respectively. Pigs were individually housed and fed ad libitum for 81 d to measure growth performance, including a metabolism period to collect urine and feces for determination of GE, lipid, N digestibility, and N retention. Following the last day of fecal and urine collection when pigs were in the metabolism crates, lactulose and mannitol were fed and subsequently measured in the urine to evaluate gut permeability, while markers of oxidative stress were evaluated in plasma, urine, and liver. There were no differences observed in ADFI ($P = 0.91$), but average daily gain (ADG) and gain:feed G:F were decreased in pigs fed 90 °C SO diet ($P \leq 0.07$) compared to pigs fed the other SO diets. Pigs fed the 90 and 180 °C SO had the lowest ($P = 0.05$) DE as a % of GE compared to pigs fed the 22.5 °C SO, with pigs fed the 45 °C SO being intermediate. Lipid digestibility was similarly affected ($P = 0.01$) as energy digestibility, but ME as a % of DE was not affected by dietary treatment ($P = 0.16$). There were no effects of lipid peroxidation on N digested, N retained, or the urinary lactulose:mannitol ratio ($P \geq 0.25$). Pigs fed the SO processed at 90 and 180 °1C had lower concentrations ($P < 0.01$) of plasma Trp compared to pigs fed the 22.5 and 45 °C SO treatments. Pigs fed 90 °C SO had the greatest ($P < 0.01$) concentrations of F_2-isoprostane in plasma and urine thiobarbituric acid reactive substances compared to the other SO treatments. These results indicate that the change in FA composition and/or the presence of lipid peroxidation products in peroxidized SO may reduce ADG, G:F, and digestibility of GE and ether extract, but has little impact on N digestibility and balance or on gut permeability.

Key words: digestibility, finishing pigs, gastrointestinal integrity, oxidative stress, peroxided soybean oil

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INTRODUCTION

Refined, crude, or recycled through yellow grease, soybean oil (**SO**) is commonly added to swine diets as a concentrated energy source (Pettigrew

and Moser, 1991; Azain, 2001; Lin et al., 2013). SO contains a high concentration of MUFA and PUFA (NRC, 2012) which, unlike SFA, are more susceptible to lipid peroxidation due to double bonds in their structure (Holman, 1954). Lipid peroxidation is a free radical reaction that can be initiated by thermal processing of a lipid in the presence of oxygen, light, or metal ions (Labuza, 1971; Lundburg and Jarvi, 1971; Gray, 1978), with products generated and consumed being peroxides, lipid hydroperoxides, polar and nonpolar acids, ketones, aldehydes, and polymers (Gonzalez-Muñoz et al., 1998; Schaich, 2012). As lipid peroxidation progresses, the unsaturated fatty acid (**UFA**):SFA ratio (UFA:SFA) of a lipid will decrease and its FFA concentration may increase (Liu et al., 2014b), while antioxidants present in the lipid become depleted (Seppanen and Csallany, 2002). Because lipids may undergo thermal stress during their processing, lipid peroxidation and FA compositional changes have the potential to affect the caloric value of a lipid when added to feed formulations (Wiseman et al., 1998).

Consumption of peroxidized lipids have been shown to decrease lipid and energy digestibility (Liu et al., 2014c; Lindblom et al., 2018b) and growth performance parameters in swine (DeRouchey et al., 2004; Boler et al., 2012; Rosero et al., 2015), but there is a dearth of information as to which components of lipid peroxidation are most detrimental to lipid and energy digestibility, and ultimately animal performance. Therefore, the objective of this study was to evaluate the effect of feeding divergently thermally processed SO to finishing pigs on growth performance, plasma Trp, N, GE and lipid digestibility, intestinal integrity, and oxidative stress.

MATERIALS AND METHODS

All animal care and use procedures for this experiment were approved by the Institutional Animal Care and Use Committee at Iowa State University.

Dietary Treatments

There were four thermally processed SO treatments within each phase of growth, which included either 10% fresh SO (22.5 °C) or SO thermally processed at 45 °C for 288 h, 90 °C for 72 h, or 180 °C for 6 h. Except for the 22.5 °C temperature, each heating process was accompanied with constant air flow (15 L/min) using an air pump and a calibrated air flow controller with air forced into the tank using a 9.5-mm copper pipe. Immersion heaters were used to heat the SO to 45 and 90 °C, while a liquid propane heater was used to heat the SO to 180 °C. Oil temperatures were taken at regular intervals to ensure the predetermined temperatures were maintained. The stainless steel heating pots were 53 mm in circumference and 61 cm high and were filled 2/3 during the heating process. To generate enough SO for each treatment, multiple batches of SO were heated and subsequently pooled into a 210-liter container and stored at room temperature prior to feed mixing. Multiple batches of each phase of feed was mixed with no antioxidant added before or during diet preparation, each diet was sampled at the time of mixing, and then pooled across phase for a composite sample for subsequent analysis. Oils were also sampled at the time of mixing and pooled across all mixing times prior to analysis. Samples of the pooled oil and final mixed feed were stored at 0 °C prior to analysis. Diverse analyses of each SO treatment was conducted as outlined in Tables 1 and 2 to characterize the quality of each SO treatment. During the 81-d study, diets were formulated to contain 0.90% (days 1 to 49, Phase 1) or 0.68% (days 49 to 81, Phase 2) standardized ileal digestible Lys, with AA ratios, ME, and mineral content calculated to be adequate for 44 to 90 kg and 90 to 135 kg pigs, respectively, according to the NRC (2012) (Table 3). Diets were offered to the pigs ad libitum in meal form during the entire trial.

Experimental Design

Fifty-six finishing barrows (Geneticporc F25 females × B6.0 sires, Hendersonville, TN) with an

Table 1. Method of analysis for thermally processed SOs

Analyte	Method
Aldehydes[1]	Wang et al. (2016)
AnV[2]	AOCS Cd 18–90
FA[2]	AOCS Ce 1a-13
FFA[2]	AOCS Ca 5A-40
Free glycerin[2]	AOCS Ca 14–56
Insoluble impurities[2]	AOCS Ca 3–46
Moisture[2]	AOCS Ca 2c-25
OSI[2]	AOCS Cd 12b-92
OFA[2]	AOCS G 3–53
PV[2]	AOCS Cd 8b-90
PTAG[3]	AOAC 993.25
Thiobarbituric acid value[2]	AOCS Cd 19–90
Tocopherols[2]	AOCS Ce 8–89
TPC[2]	AOCS Cd 20–91
Unsaponifiable matter[2]	AOCS Ca 6a-40

[1]Analyzed by University of Minnesota, St. Paul, MN.
[2]Analyzed by Barrow-Agee, Memphis, TN (AOCS, 2011).
[3]Analyzed by the USDA-ARS, Peoria, IL (AOAC, 2002).

Table 2. Composition and peroxidation analysis of thermally processed SOs

Heating temperature, °C	22.5	45	90	180
Time heated, h[1]	0	288	72	6
FA, % of total fat[2,3]				
C8:0, caprylic	ND	ND	ND	0.07
C16:0, palmitic	10.69	10.74	11.70	11.21
C16:1, palmitoleic	0.09	0.08	0.09	0.08
C17:0, margaric	0.10	0.10	0.11	0.10
C18:0, stearic	4.19	4.13	4.52	4.42
C18:1, oleic	23.46	23.49	25.15	24.26
C18:2, linoleic	53.07	52.86	50.79	51.65
C18:3, linolenic	7.14	7.15	6.14	6.43
C19:0, nonadecanoic	0.26	0.25	0.23	0.35
C20:0, arachidic	0.32	0.32	0.35	0.34
C20:1, gadoleic	0.18	0.19	0.20	0.30
C22:0, behenic	0.35	0.35	0.49	0.41
C24:0, lignoceric	ND	0.12	ND	0.13
Other FA	0.15	0.22	0.23	0.27
UFA:SFA	5.28	5.23	4.73	4.88
IV[4]	131	131	126	127
FFA, %[2]	0.10	0.10	0.46	0.14
Free glycerin, %[2]	1.04	3.72	0.82	0.82
Moisture, %	0.02	0.02	0.04	0.04
Insoluble impurities, %	0.02	0.04	0.04	0.04
Unsaponifiable matter, %	0.51	0.39	0.41	0.47
Oxidized FA, %[2]	3.0	2.8	2.5	1.9
OSI @ 110 C, h[2]	6.65	4.65	2.70	3.65
AnV[2,5]	1.11	1.33	121	165
PV, mEq/kg[2]	2.0	17.4	123.6	19.4
Polar compounds, %[2]	3.61	3.28	20.25	11.58
PTAG[6], %	ND	ND	3.16	3.10
TBA value[2,5]	0.14	1.14	0.14	0.14
Aldehydes, mg/kg[7]				
DDE	2.07	1.90	912.15	915.49
HNE	0.66	1.49	170.48	82.80
Acrolein	6.15	6.06	27.12	44.60
2-Decenal	0.16	0.19	55.21	81.60
2,4-Heptadienal	0.47	1.19	268.62	151.65
2-Heptenal	2.33	1.82	254.48	90.68
Hexanal	2.01	2.02	33.69	6.28
2-Octenal	0.40	0.67	212.60	51.96
Pentanal	5.36	1.05	10.76	2.84
2,4-Undecadienal	0.06	0.07	43.73	53.34
2-Undecenal	0.19	0.19	50.29	110.38
Ratio[8]	0.12	0.21	0.58	1.14
TOC, mg/kg[2]	1,328	1,331	94	798
Alpha	98	97	<10	<10
Beta	<10	<10	<10	<10
Delta	196	209	15	169
Gamma	1,034	1,025	79	629

AnV, p-anisidine value; DDE, 2,4-decadienal; FA, fatty acid; FFA, free fatty acids; HNE, 4-hydroxynonenal; IV, iodine value; ND, not detected; OSI, oil stability index; PTAG, polymerized tryacylglycerides; PV, peroxide value; TBA, thiobarbituric acid; TOC, tocopherol; UFA:SFA, unsaturated:saturated fatty acid ratio.

[1]Thermally processed oils had constant air flow rate at 15 L/min.

[2]Analyzed by Barrow-Agee, Memphis, TN.

[3]No other FA were detected besides those listed.

[4]IVs were calculated using the FA profile data following the equation: VI = (16:1 × 0.95) + (18:1 × 0.86) + (18:2 × 1.732) + (18:3 × 2.616) + (20:1 × 0.795) + (20:2 × 1.57) + (20:3 × 2.38) + (20:4 × 3.19) + (20:5 × 4.01) + 22:4 × 2.93) + (22:5 × 3.68) + (22:6 × 4.64); Meadus et al. (2010).

[5]There are no units for AnV or TBA value.

[6]Analyzed by the USDA-ARS, Peoria, IL.

[7]Analyzed by University of Minnesota, St. Paul, MN.

[8]Ratio of 2-decenal, 2,4-hydroxynonenal, 2,4-undecadienal, and 2-undecenal as a percent of total aldehydes to acrolein, 2,4-heptadienal, and 2-heptenal as a percent of total aldehydes; Wang et al. (2016).

Table 3. Ingredient and calculated composition of treatment diets, as-is basis

Ingredient, %	Phase 1[1]	Phase 2[1]
Corn	66.45	73.23
Soybean meal, 46% CP	20.45	14.20
SO heat treatment[2]	10.00	10.00
Limestone	1.05	0.90
Monocalcium phosphate	0.85	0.64
Sodium chloride	0.35	0.35
Vitamin mix[3]	0.25	0.25
Trace mineral mix[4]	0.15	0.15
L-Lysine•HCl	0.30	0.21
L-Threonine	0.08	0.06
DL-Methionine	0.07	0.01
Total	100.00	100.00
Calculated composition		
ME, kcal/kg	3,800	3,800
CP, %	15.6	12.8
Lys, %	1.01	0.77
Ca, %	0.63	0.52
P, %	0.50	0.43
Analyzed composition[5]		
GE, kcal/kg	4,223	4,257
CP, %	15.1	12.6
Crude fat, %	12.1	11.9

[1]The Phase-1 diet was fed for 49 d (pig BW 46.7–98.2 kg) and the Phase-2 diet was fed for 32 d (pig BW 98.2–130.0 kg).

[2]Dietary treatments consisted of 10% refined SO that was either unheated (22.5 °C) or heated at 45 °C for 288 h, 90 °C for 72 h, or 180 °C for 6 h, with 15 L/min of air supplied by a bubbled into the heating vesicle, excluding the 22.5 °C temperature which was not aerated.

[3]Provided the following per kilogram of diet: vitamin A, 7,656 IU; vitamin D_3, 875 IU; vitamin E, 62.5 IU; vitamin K, 3.75 mg; vitamin B_{12}, 0.06 mg; riboflavin, 13.75 mg; niacin, 70 mg; and pantothenic acid, 33.75 mg.

[4]Provided the following per kilogram of diet: Cu (as $CuSO_4$), 16.5 mg; Fe (as $FeSO_4$), 165 mg; I (as $Ca(IO_3)_2$), 0.3 mg; Mn (as $MnSO_4$), 39 mg; Zn (as $ZnSO_4$), 165 mg; and Se (Na_2SeO_3), 0.3 mg.

[5]Average analysis across all four SO treatments.

initial BW of 46.7 ± 5.1 kg were randomly allotted into 56 pens equipped with partial slats, one 2-holed feeder, and a single nipple waterer in a room that was mechanically ventilated with a pull-plug manure storage system located at the Swine Nutrition Farm at Iowa State University (Ames, IA). Pigs were randomly assigned to one of four dietary treatments, resulting in 14 replications per treatment. Each pig was individually penned (1.8 × 1.9 m) and had ad libitum access to feed and water.

While pigs were being fed the Phase-1 diet, a metabolism experiment was conducted to evaluate N, acid hydrolyzed ether extract (**AEE**), and GE digestibility, and to collect urine to evaluate in vivo intestinal permeability and measures of oxidative stress. On day 21 and day 29, a group of 24 pigs each, representing 6 pigs from each treatment in each group, were weighed (day 21 BW = 68.3 kg, SD = 7.1 kg) and moved to individual stainless steel metabolism crates (1.2 × 2.4 m). For the next 3 d, pigs were fed twice daily at 0700 and 1900, 1.25 kg/meal which equates to 3.5% of BW/d, to acclimate them to the metabolism crate and feeding schedule, with water offered ad libitum through nipple waterers. Following this 3-d adaption period, a 3-d collection period occurred where stainless steel wire screens were placed under each metabolism crate for total fecal collection. Feces were collected twice daily and stored at 0 °C until the end of the collection period. At the end of the collection period, feces were dried in a 70 °C forced air oven, weighed, ground through a 2-mm screen, and a subsample was taken for nutrient analysis. Orts were subtracted from total feed offered to calculate net feed intake. Following this 3-d collection period, pigs were given 40 g lactulose and 4 g mannitol (Spectrum Chemical, Gardena, CA) in 100 g of feed at 2000 h, and once this feed was consumed, the remainder of their feed allotment was given. Chlorohexidine (5 mL) was added to each urine container, urine was collected for the next 12 h (ending at 0800 h), quantified, and a sample obtained for subsequent analysis to assess in vivo intestinal permeability (Wijtten et al., 2011). After this collection, the urine buckets were cleaned and replaced under the metabolism crates with 5 mL of chlorohexidine, and an additional collection of urine occurred for 7 h (ending at 1500 h), quantified, and a sample obtained for measures of in vivo oxidative stress. Pigs were returned to their individual pens for the remainder of the performance study, with feed consumption during the metabolism crated period added to the total feed intake for the performance trial. On day 78, while pigs were still on feed, each pig was bled (10 mL) at 0900 h via jugular venipuncture into heparinized tubes (158 USP units sodium heparin; BD Vacutainer, BD Diagnostics, Franklin Lakes, NJ), samples were centrifuged at 2,500 × *g* for 10 min at 4 °C, after which an aliquot of plasma was stored at −80 °C for subsequent analysis. On day 81, all pigs and feeders were individually weighted to determine average daily gain (ADG), ADFI, and G:F.

Calculations and Methodologies

Diets and feces were analyzed for AEE (Thermo Scientific Application Note 361, Thermo Fisher Scientific, Salt Lake City, UT) using an accelerated solvent extraction system (model 350, Dionex, Bannockburn, IL) and 100 mL stainless steel extraction cells to accomplish the lipid

extraction. GE of the SO, diets, feces, and urine was determined using an isoperibol bomb calorimeter (model 6400, Parr Instrument Co., Moline, IL) using benzoic acid as a standard. Nitrogen was analyzed by thermo-combustion (VarioMAX CNS, Elementar Analysensysteme GmbH, Hanau, Germany) where combustion gases are converted to individual gases and sorted into adsorption columns and are measured using a thermal conductivity detector. Apparent total tract digestibility coefficients for AEE, GE, and N were estimated using a time-based collection methodology. ME as a percent of DE was calculated by dividing ME intake by DE intake and N retention as a percent of N digested was calculated by dividing N retained by N digested, both reported as a percent. Detailed descriptions of metabolism experimental methods are provided elsewhere (Adeola, 2001; Kerr et al., 2013; Li et al., 2016). Urinary lactulose and mannitol concentrations were measured via HPLC as an in vivo indicator of small intestinal permeability and using the method as described by Kansagra et al. (2003). The ratio of lactulose:mannitol (**L:M**) was calculated back to the total amount of urine collected and reported on a recovery basis. For plasma Trp concentration, plasma was diluted 1:1 with 0.05 M potassium phosphate buffer, pH 6.0, and deproteinized with 2 M trichloroacetic acid. Plasma Trp levels were subsequently determined by separation on a 4-µm spherical silica gel particle column (Superspher 100 RP-18 LiChroCART, Millipore Sigma, Billerica, MA) by an automated HPLC system with a fluorescence detector (Jasco FP-1520, Jasco Analytical Instruments, Easton, MD).

Several markers of oxidative stress were evaluated in plasma, urine, and liver homogenates. Samples were diluted as needed in order for the measured values to fall within range of the standard curve. Assays were conducted in triplicate with an intra-assay CV of ≤5%. Thiobarbituric acid reactive substances (**TBARS**) and F_2-isoprostane (**ISP**) were analyzed in urine, plasma, and liver as indicators of lipid peroxidation. No preparation step was necessary prior to analyzing plasma or urine for TBARS or ISP. Liver tissue (100 mg) was homogenized in 1 ml of radioimmunoprecipitation assay (**RIPA**) buffer (Cayman Chemical Co., Ann Arbor, MI; #10010263) then centrifuged at 1600 × *g* for 10 min at 4 °C. The TBARS assay was conducted on the resulting supernatant. Analysis of ISP was measured in undiluted plasma and 1:10 solution of urine and deionized water. Both urinary TBARS and ISP were normalized to the volume of urine excreted during the collection period by multiplying the concentration of the analytes by the volume of urine. Protein carbonyls (**PC**) were measured in plasma and liver samples only, as protein is not excreted in the urine. Plasma samples were analyzed for PC using the protocol outlined by the kit (Cayman Chemical Co.; #10005020), whereas, liver tissue (200 mg) was homogenized in 1 mL of 50 mM phosphate buffer + 1 mM EDTA then centrifuged at 10,000 × *g* for 15 min at 4 °C. The resulting supernatant was then used to evaluate the concentration of PC, similar to the protocol employed for plasma. Under conditions of oxidative stress, DNA may also be damaged with the most oxidatively labile nucleic acid being guanine, and therefore its' oxidation product, 8-hydroxy-2′-deoxyguanosine (**8-OH-2dG**), is commonly assayed as a measure of DNA damage due to oxidative stress (Wu et al., 2004). Thus, urine and liver samples were assayed for 8-OH-2dG. For liver samples, DNA was extracted from 25 mg of tissue (ZR Genomic DNA- Tissue MiniPrep, Zymo Research, Irvine, CA) with yields of DNA evaluated using Gen 5 software on Cytation 5 Imaging Reader (BioTek, Winooski, VT); DNA yields ranged between 20 and 40 µg. Digestion of DNA was then conducted using nuclease (P1 nuclease, Sigma-Aldrich, St. Louis, MO) which converts double-stranded DNA to single-stranded DNA. Nucleotides were then converted to nucleosides by adding 1 unit of alkaline phosphatase (Roche Diagnostics, Mannheim, Germany) per 100 µg of DNA. The resulting supernatant was evaluated for 8-OH-2dG using a commercial kit (Cayman Chemical Co.; #589320) with no dilution being necessary. No DNA isolation step was required for urine samples prior to analysis, but urine was diluted 1:750 in sample buffer prior to assessment and normalized to the volume of urine excreted during the collection period by multiplying its concentration by the volume of urine.

The activity of endogenous antioxidant enzymes, such as glutathione peroxidase (**GPx**) and catalase (**CAT**), is often affected by oxidative stress (Lykkesfeldt and Svendsen, 2007; Royer et al., 2016). Therefore, GPx activity was assayed in both plasma and liver, and CAT activity was measured in liver samples. To measure GPx activity in liver and plasma, 100 mg of sample was first homogenized in 1 mL of 50 mM Tris-HCl + 5 mM EDTA + 1 mM 1,4-dithiothreitol is abbreviated (**DTT**). The homogenate was then centrifuged at 10,000 × *g* for 15 min at 4 °C. The resulting supernatant was diluted 1:20 in sample buffer prior to being assayed. Activity of CAT in liver was evaluated by first homogenizing 100 mg of tissue in 1 mL of 50 mM potassium

phosphate + 1 mM EDTA. The homogenate was then centrifuged at 10,000 × *g* for 15 min at 4 °C. The resulting supernatant was then diluted 1:10,000 in sample buffer and then assayed for CAT activity.

Statistical Analysis

Data were analyzed as a completely randomized design with individual pig as the experimental unit with initial BW serving as a covariate for analyses of growth performance data, using Proc MIXED procedure of SAS (version 9.4; SAS Institute, Cary, NC). Means were reported and separated using LSMEANS. In addition, relationships between lipid peroxidation measures with growth performance, digestibility variables, and measures of oxidative stress were evaluated by simple linear correlation (Pearson correlation coefficients) analysis. Differences were considered significant at $P \leq 0.05$, whereas values of $0.05 \leq P \leq 0.10$ were considered statistical trends.

RESULTS AND DISCUSSION

Compositional Changes of SO due to Thermal Processing

Lipid peroxidation is an extremely complex process, involving free radical formation and subsequent propagation of free radicals which ultimately bind to PUFA (Holman, 1954), where the development of lipid peroxidation products is based on the duration and intensity of thermal processing and presence of oxygen (St. Angelo et al., 1996). In general, thermal processing of lipids decreases lipid quality through hydrogenation of double bonds and the formation of peroxides in the initiation phase of lipid peroxidation, which can then be degraded into polar and nonpolar acids, ketones, and aldehydes in the propagation phase, ultimately forming indigestible polymers in the termination phase (Gray, 1978; Gonzalez-Muñoz et al., 1998). Similar to the peroxidation of SO as reported by Lindblom et al. (2018b), the current experiment induced peroxidation by processing SO at different temperatures and durations prior to being mixed in the diet. To generate SO with different degrees of peroxidation, SO was heated at 45 °C for 288 h, 90 °C SO for 72 h, and 180 °C for 12 h to reflect conditions noted in the livestock, rendering, and restaurant industries (Meeker and Hamilton, 2006) and to use temperatures which have been evaluated in previously research (Boler et al., 2012; Liu et al., 2014b; Kerr et al., 2015; Rosero et al., 2015; Hanson et al., 2016). The four SO were analyzed in detail prior to feed mixing for FA composition, lipid quality, and various lipid peroxidation products (Tables 1 and 2) which have been described in detail elsewhere (Shurson et al., 2015).

As shown in Table 2, the unprocessed SO (i.e., 22.5 °C) had a higher UFA:SFA than SO processed at 90 and 180 °C (5.28 vs. 4.73 and 4.88, respectively), a decrease which can be explained by the slight increases in C16:0 and C18:0 with slight decreases in C18:2 and C18:3. While UFA:SFA is an important measure for energy predictability in swine (Wiseman et al., 1998), it is fairly crude measure of the degree of unsaturation, and thus susceptibility to peroxidation. Because of this, we calculated iodine value (**IV**; Meadus et al., 2010) as an additional measure of FA unsaturation. For SO processed at 90 and 180 °C, there was a decrease in IV compared to SO not thermally processed (22.5 °C) or processed at 45 °C (126 and 127 vs. 131 and 131, respectively). The observed changes in both UFA:SFA and IV were expected because as lipid peroxidation progresses, there is a general hydrogenation of FA which decreases the number of double bonds available for peroxidation (Holman, 1954; Yin et al., 2011). The changes in FA composition, UFA:SFA, and IV in the current experiment are similar to that reported by others (DeRouchey et al., 2004; Liu et al., 2014b; Hanson et al., 2015; Kerr et al., 2015; Rosero et al., 2015; Lindblom et al., 2018b).

Even though numerous quality factors were measured in an effort to expand the basis of understanding of lipid peroxidation in livestock feeds (Table 2), only a few of the more common factors will be discussed. Peroxide value (**PV**), a measurement of peroxides and hydroperoxides formed in the initiation phase, was determined to be highest in the 90 °C processed SO followed by the 45 and 180 °C processed SO, and lowest in the fresh SO with values of 123.6, 17.4, 19.4, and 2.0 mEq/kg SO, respectively. Oxidized fatty acids (**OFA**), a measure of lipid hydroperoxides and peroxides and saturated epoxy-, keto-, and hydroxy-acids, did not differ greatly and were low across all SO treatments, but did slightly decline in SO processed at 90 and 180 °C. Although counterintuitive, this suggests that at 90 and 180 °C of thermal processing, these lipid peroxidation products have been further degraded into aldehydes and polymers. Lastly, p-anisidine value (**AnV**), a measure of high-molecular-weight saturated and unsaturated aldehydes, and total polar compounds (**TPC**), a measure of monoglycerides, diglycerides, and FFA, were highest in the 90 and 180 °C SO compared to the 22.5 and 45 °C SO, indicating that our method of thermal

processing generated SO to different degrees of peroxidation. The changes in PV, OFA, AnV, and TPC were expected based on the overall process of lipid peroxidation and are of similar directions that reported by others (Engberg et al., 1996; Kerr et al., 2015; Lindblom et al., 2018b).

Specific aldehydes were also measured because some (i.e., hexanal) are common measures of lipid peroxidation reported in the literature (Shurson et al., 2015), and also because acrolein (Kehrer and Biswal, 2000; Abraham et al., 2011), 2,4-decadienal (**DDE**; Chang et al., 2005), and 4-hydroxynonenal (**HNE**; Esterbauer et al., 1991) are considered highly damaging aldehydes to DNA, proteins, and lipids in vivo. Furthermore, a ratio among two clusters of aldehydes has been shown to be closely associated with the progression of SO peroxidation (Wang et al., 2016). However, only a few of these specific aldehydes or their ratio has been evaluated in respect to lipids fed to livestock and subsequent impacts on performance or digestive functions. In the current experiment, thermal processing of SO at 45 °C had little effect on acrolein, hexanal, DDE, or HNE concentrations, or a proposed aldehyde ratio, compared to the unprocessed SO (Table 2). In contrast, concentrations for each of these aldehydes and for the calculated aldehyde ratio were increased when SO was processed at 90 or 180 °C.

In addition to measuring lipid peroxidation compounds, we were interested in measuring the tocopherol concentrations status of SO. It is well known that tocopherols are natural antioxidants found in vegetable oils and function in protecting the lipid from degradation (Kamal-Eldin, 2006) so as lipid peroxidation progresses it would be expected that these compounds would be depleted. In the current study, the 90 °C processed SO had the lowest total tocopherol (**TOC**) concentration with 94 mg/kg oil followed by 180 and 45 °C, and fresh oil with 798, 1.331, and 1.328 mg/kg, respectively. Our data are similar to others (Miyagawa et al., 1991; Lindblom et al., 2018b) who reported that thermally processing a blend of SO and rapeseed oil or SO, respectively, would result in a degradation of tocopherols.

Growth Performance

Performance data were collected over the 81-d trial, including the 6 d while pigs were in the metabolism crates. During the experimental period, one pig fed the 22.5 °C SO, one pig fed the 45 °C SO, and one pig fed the 180 °C were removed from the trial due to health reasons unrelated to the SO treatment. As shown in Table 4, pigs fed the 90 °C SO had reduced ADG ($P = 0.07$) in comparison to the other three SO treatment groups, with no differences noted among pigs fed the 22.5, 45, and 180 °C SO treatments. Thermal processing of SO did not affect ADFI ($P = 0.91$), resulting in pigs fed diets containing the 90 °C processed SO having a reduced G:F ($P = 0.04$) compared to the other SO treatments, with no differences noted among pigs fed the other SO treatments. The reduction in ADG in pigs fed the 90 °C SO is in agreement with others (DeRouchey et al., 2004; Boler et al., 2012; Liu et al., 2014a; Rosero et al., 2015; Hanson et al., 2016; Lindblom et al., 2018b) who reported decreased ADG in pigs fed lipids with increased concentrations of lipid peroxidation products compared to pigs fed lipids with low levels of lipid peroxidation products. The reduction in ADG by 6% due to processing SO at 90 °C was similar to the reduction in growth compared to pigs fed fresh lipid sources as reviewed by Hung et al. (2017). Lipid peroxidation compounds have also been shown to affect palatability and feed intake in swine (Boler et al., 2012; Liu et al., 2014a; Rosero et al., 2015); consequently, we would have expected a decrease in ADFI with the different thermally processed SO. This was not, however, observed

Table 4. Growth performance and plasma Trp of pigs fed SO with differing peroxidation levels

	Processed SO[1,2]				Statistics	
Parameter	22.5	45	90	180	SEM	*P* value
ADG, kg	1.04[ab]	1.09[a]	0.98[b]	1.07[a]	0.03	0.07
ADFI, kg	2.77	2.77	2.72	2.80	0.07	0.91
G:F	0.38[ab]	0.39[a]	0.36[b]	0.38[ab]	0.01	0.04
Plasma Trp, μM/mL	62.6[a]	63.0[a]	50.4[b]	50.3[b]	1.65	0.01

[1]Data are least square mean of 13 observations for 22.5, 45, and 180; and 14 observations for 90. 22.5 = fresh oil; 45 = oil heated for 12 d at 45 °C with constant compressed air flow rate at 15 L/min; 90 = oil heated for 72 h at 90 °C with constant compressed air flow rate at 15 L/min; 180 = oil heated for 6 h at 180 °C with constant compressed air flow rate at 15 L/min. Performance data were collected over 81 d with initial average BW of 46.8 ± 5.2 kg ($P = 0.99$) and final average BW of 131.2 ± 11.5 kg ($P = 0.06$).

[2]Superscript letters reflect peroxidized SO treatment differences ($P \leq 0.07$).

in the current experiment (Table 4). While the lack of an effect on ADFI is in agreement with Hanson et al. (2016) who fed graded levels of peroxided corn oil to nursery pigs and Lindblom et al. (2018b) who fed thermally processed SO to growing pigs, these data are in disagreement with the review by Hung et al. (2017) who summarized that pigs fed peroxidized lipids had decreased ADFI by approximately 5% compared to pigs fed unperoxidized lipids. The reduction in G:F in the current experiment by 5% for pigs fed the 90 °C SO is supported by the review by Hung et al. (2017), where G:F was reduced by 2% compared to pigs fed fresh lipid sources, as well as by Hanson et al. (2016) who fed peroxidized corn oil to nursery pigs and Lindblom et al. (2018b) who fed peroxidized SO to growing pigs. In contrast, no effect on G:F was reported when nursery pigs were fed peroxidized choice white grease (DeRouchey et al., 2004), when finisher pigs were fed peroxidized corn oil (Boler et al., 2012), or when nursery pigs were fed peroxidized SO (Rosero et al., 2015). In the current experiment, the different thermal processing temperatures and times were selected to generate different concentrations of lipid peroxidation products from which to increase our understanding as to which lipid peroxidation products are most detrimental to pig growth. Even though thermally processing SO at 45 or 180 °C did generate different concentrations of lipid peroxidation products (Table 2), their consumption did not affect pig performance, similar to that reported by Lindblom et al. (2018b). Different lipid peroxidation product concentrations in the feed can also be accomplished by blending an unperoxidized lipid with a peroxided lipid, where it has been shown that consumption of low levels of lipid peroxidation products may have little to no measurable effect on pig performance (Rosero et al., 2015; Hanson et al., 2016), indicating that continued research efforts need to be made in this area.

Plasma Trp was measured because of its involvement in brain and nervous system function as well as many behavioral and physiological processes, including feed intake (Baranyiova, 1991; Seve, 1999). Although ADFI was not affected by SO peroxidation, pigs fed the SO processed at 90 and 180 °C had lower concentrations ($P < 0.01$) of plasma Trp compared to pigs fed the 22.5 and 45 °C SO treatments (Table 4). These data are supported by others (Chi Chen, University of Minnesota, personal communication) in mice and Lindblom et al. (2018b) in growing pigs that feeding peroxidized SO resulted in a decrease in plasma Trp. The reduction in plasma Trp may also be a consequence of the activation of the tryptophan-NAD+ pathway as others (Chi Chen, University of Minnesota, personal communication) suggested that consumption of peroxidized SO leads to an increased need of NAD+ and its metabolites because they are cofactors or substrates involved in detoxification of reactive aldehydes through aldehyde dehydrogenase which is vital for sustaining redox balance, DNA repair activities, and PPARα-mediated metabolic reactions.

Because measures of growth performance and plasma Trp were affected by SO processing, a correlation analysis of pig performance responses with various measures of lipid peroxidation products were conducted (Table 5). We elected to conduct correlations specifically for UFA:SFA and polymerized triacylglycerides (**PTAG**) because of their potential impact on energy digestibility (Marquez-Ruiz et al., 1992; Gonzalez-Muñoz et al., 1998; Wiseman et al., 1998), and peroxidation indices of PV, AnV, oil stability index (**OSI**), and hexanal because they are common measures of lipid peroxidation in the literature (Shurson et al., 2015). In addition, OFA and TPC were included because they are measureable components in the peroxidation process and the specific aldehydes of acrolein (Kehrer and Biswal, 2000; Abraham et al., 2011), DDE (Wang et al., 2016), and HNE (Esterbauer et al., 1991; Wang et al., 2016) because these aldehydes are considered highly damaging to DNA, proteins, and lipids in vivo, including a ratio of aldehydes associated with SO peroxidation (Wang et al., 2016). Lastly, TOC levels were evaluated as they assist in providing an accurate depiction of the antioxidant status naturally occurring in SO (Seppanen and Csallany, 2002; Kamal-Eldin, 2006).

Irrespective of diet, UFA:SFA and TOC exhibited a positive correlation to ADG, G:F, and plasma Trp. In contrast, PV, TPC, hexanal, and HNE all exhibited negative correlations with ADG, G:F, and plasma Trp (Table 5). Interestingly, there were no correlations between ADG and G:F with AnV, OFA, PTAG, acrolein, DDE, or the aldehyde ratio. Likewise, there were no significant correlations noted between ADFI and any of the lipid quality indices, and thus not listed in Table 5. Because the difference in FFA and TBARS values among the SO treatments were considered to be insignificant, we did not attempt to correlate these parameters to pig performance, even though it is known that FFA and PTAG can affect the energy value of a lipid (Marquez-Ruiz et al., 1992; Gonzalez-Muñoz et al., 1998; Wiseman et al., 1998) and TBARS has been shown to be correlated with ADG and ADFI in swine (Hung et al., 2017). The positive correlation between ADG and

Table 5. Pearson correlation coefficients among lipid composition and peroxidation measures with performance and plasma Trp[1]

	ADG		G:F		Trp	
Lipid quality indices	*R* value[1]	*P* value[1]	*R* value	*P* value	*R* value	*P* value
UFA:SFA	0.23	0.10	0.26	0.06	0.72	0.01
PV	−0.32	0.02	−0.33	0.01	−0.46	0.01
AnV	–	–	–	–	−0.73	0.01
OFA	–	–	–	–	0.63	0.01
TPC	−0.29	0.04	−0.32	0.02	−0.66	0.01
PTAG	–	–	–	–	−0.74	0.01
OSI	–	–	–	–	0.63	0.01
Hexanal	−0.33	0.01	−0.35	0.01	−0.49	0.01
Acrolein	–	–	–	–	−0.69	0.01
DDE	–	–	–	–	−0.74	0.01
HNE	−0.29	0.04	−0.32	0.02	−0.66	0.01
Ratio	–	–	–	–	−0.64	0.01
TOC	0.30	0.03	0.33	0.02	0.64	0.01

ADG, average daily gain; AnV, p-anisidine value; DDE, 2,4-decadienal; FA, fatty acid; HNE, 4-hydroxynonenal; IV, iodine value; ND, not detected; OFA, oxidized fatty acids; OSI, oil stability index; PTAG, polymerized tryacylglycerides; PV, peroxide value; TBA, thiobarbituric acid; TOC, tocopherol; TPC, total polar compounds; UFA:SFA, unsaturated:saturated fatty acid ratio.

Ratio, ratio of aldehydes as described by Wang et al. (2016).

[1]Correlation (*R* value) and correlation significance (*P* value). If no value is given, it was not found to be significant (−) at $P \leq 0.10$. There were no correlations between ADFI and any lipid quality indices.

G:F with TOC is in contrast to Hung et al. (2017) who reported no such relationship.

Energy and Lipid Digestibility

As shown in Table 6, DE as a percentage of GE was greatest in pigs fed the fresh SO (91.13%), lowest in pigs fed the 90 and 180 °C SO (89.51% and 89.37%, respectively), and intermediate in pigs fed the 45 °C SO (90.96%, $P = 0.03$). There were no differences noted among the SO treatments for ME as a percent of DE ($P = 0.16$). Similar to energy digestibility, pigs fed the fresh SO had the greatest AEE digestibility (84.60%), was lowest in pigs fed the 90 and 180 °C SO (80.99% and 80.59%, respectively), and intermediated in pigs fed the 45 °C SO (82.54%, $P = 0.01$). These results are in agreement with DeRouchey et al. (2004), Rosero et al. (2015), and Lindblom et al. (2018b) who reported a reduction in energy and lipid digestibility when pigs were fed peroxidized lipids relative to unperoxidized lipids. In contrast, Liu et al. (2014c) did not report changes in DE as a percentage of GE or ME as a percentage of DE when pigs were fed various peroxidized lipids.

Multiple positive correlations were observed between DE as a percent of GE, ME as a percent of DE (even though there was not a treatment effect noted for this measure), and AEE digestibility with

Table 6. Apparent total tract energy and lipid digestibility, nitrogen balance, and intestinal permeability in finishing pigs fed various levels of peroxidized SO

	Processed SO[1,2]				Statistics	
Parameter	22.5	45	90	180	SEM	*P* value
DE, % of GE	91.13[a]	90.96[ab]	89.51[b]	89.37[b]	0.52	0.03
ME, % of DE	98.35	98.19	97.95	97.96	0.15	0.16
AEE digestibility, %	84.60[a]	82.54[ab]	80.99[b]	80.59[b]	0.86	0.01
Nitrogen digested, %	90.53	90.17	90.24	90.09	0.53	0.94
Nitrogen retained, %[3]	80.45	75.98	76.12	75.37	1.96	0.25
Urinary L:M ratio[4]	0.086	0.065	0.086	0.069	0.012	0.51

[1]Data are least square mean of 14 observations for 22.5 and 90; and 13 observations for 45 and 180. 22.5 = fresh oil; 45 = oil heated for 12 d at 45 °C; 90 = oil heated for 72 h at 90 °C; 180 = oil heated for 6 h at 180 °C. All oil groups had a constant compressed air flow rate at 15 L/min.

[2]Superscript letters reflect peroxidized SO treatment differences ($P \leq 0.05$).

[3]Nitrogen retained as a percent of N digested.

[4]Urinary L:M ratio.

Table 7. Pearson correlation coefficients among SO composition and peroxidation measures with digestibility responses[1]

	DE:GE		ME:DE		EE digestibility		N retention	
Lipid quality indices	*R* value[1]	*P* value[1]	*R* value	*P* value	*R* value	*P* value	*R* value	*P* value
UFA:SFA	0.43	0.01	0.32	0.03	0.45	0.01	–	–
PV	−0.25	0.10	–	–	−0.29	0.07	–	–
AnV	−0.44	0.01	−0.31	0.04	−0.45	0.01	–	–
OFA	0.40	0.01	0.28	0.06	0.44	0.01	–	–
TPC	−0.38	0.01	−0.29	0.05	−0.38	0.01	–	–
PTAG	−0.44	0.01	−0.32	0.03	−0.45	0.01	–	–
OSI	0.38	0.01	0.33	0.03	0.49	0.01	0.26	0.08
Hexanal	−0.27	0.08	–	–	−0.28	0.08	–	–
Acrolein	−0.42	0.01	−0.29	0.05	−0.43	0.01	–	–
DDE	−0.44	0.01	−0.32	0.03	−0.45	0.01	–	–
HNE	−0.38	0.01	−0.29	0.05	−0.39	0.01	–	–
Ratio	−0.41	0.01	−0.28	0.28	−0.43	0.01	–	–
TOC	0.37	0.01	0.28	0.06	0.37	0.02	–	–

AnV, p-anisidine value; DDE, 2,4-decadienal; FA, fatty acid; HNE, 4-hydroxynonenal; IV, iodine value; OFA, oxidized fatty acids; OSI, oil stability index; PTAG, polymerized tryacylglycerides; PV, peroxide value; TBA, thiobarbituric acid; TOC, tocopherol; TPC, total polar compounds; UFA:SFA, unsaturated:saturated fatty acid ratio.

Ratio, ratio of aldehydes as described by Wang et al. (2016).

[1]Correlation (*R* value) and correlation significance (*P* value). If no value is given, it was not found to be significant (−) at $P \leq 0.10$. There were no correlations observed between N digested or L:M and any lipid indices.

UFA:SFA, OFA, OSI, and TOC (Table 7). In contrast, these energy and lipid digestibility measures were negatively correlated with measures associated with lipid peroxidation (PV, TPC, AnV, acrolein, DDE, hexanal, HNE, and the aldehyde ratio). These correlations would be expected because when SO is peroxidized, there are small, but consistent changes in the FA profile which are known to affect energy and lipid digestibility (Wiseman et al., 1998). Although confounded with the FA profile changes in the current experiment, the generation of lipid peroxidation products may also be independently responsible for decreased digestibility, but no known data are available to support this statement. The reduction in energy and AEE digestibility in pigs fed the 90 °C SO also helps explain the reduction in ADG and G:F noted in this SO treatment.

Nitrogen Digestibility and Balance

There were no differences noted among the SO treatments for N digestibility ($P = 0.94$) or N retained as a percent of N digested ($P = 0.25$; Table 6). In addition, there were no correlations observed between SO treatments with N digestibility, and N retention as a percent of N digested was only correlated with OSI ($R = 0.28$, $P = 0.08$; Table 7). These results are supported by DeRouchey et al. (2004) who did not report any statistical differences among N digestibility in nursery pigs fed thermally processed choice white grease, and Liu et al. (2014c) who did not report any differences in N digestibility or N retention due to feeding various peroxidized lipids. In contrast, Lindblom et al. (2018b) reported a decrease in N digestibility and N retention in growing pigs fed SO thermally processes at 90 °C for 72 h, and also reported that N retention as a percent of N digested was correlated with many lipid quality indices. Differences in N digestibility and retention suggest that lipid peroxidation may affect in vivo metabolism, but further research is needed to confirm this statement.

Intestinal Barrier Function

Measuring the effects of lipid peroxidation products on intestinal permeability is of interest because it has been shown that intestinal permeability is increased when consuming a diet high in saturated FA (Laugerette et al., 2012; Liu et al., 2014d; Mani et al., 2012), but little data are available on the effects of feeding peroxidized lipids on intestinal permeability. In the current study, there were no differences in the urinary L:M ratio among SO treatment groups with urinary ratios of L:M on a recovery basis averaging 0.077 ($P = 0.51$; Table 6). Likewise, there were no correlations observed between the L:M ratio with any lipid peroxidation product. While we might have expected an increase in intestinal permeability in pigs fed 90 and 180 °C SO because of the changes in lipid saturation, this was not the case. Our findings are, however, in agreement with Liu et al. (2014d) and Lindblom

et al. (2018b) who reported no significant differences in urinary L:M ratios among lipid peroxidation levels, and as expected, no correlations with any lipid quality indices.

Oxidative Status of Plasma, Liver, and Urine

Oxidative stress is defined as a disturbance in the balance between the production of reactive oxygen species (**ROS**) and the available system of antioxidant defenses (Betteridge, 2000). If the pool of antioxidant defenses is insufficient, ROS are left unchecked and may cause damage to tissues. One of the most routinely used markers of oxidative stress are TBARS, which is a measure of compounds produced from lipid oxidation (Dalle-Donne et al., 2006). Previous studies involving feeding peroxidized fat sources have reported increased TBARS in blood and tissues (Ringseis et al., 2007; Boler et al., 2012; Varady et al., 2012). However, in the present study, there was no effect ($P \geq 0.39$) of SO peroxidation status noted on plasma TBARS, PC, or GPx (Table 8). This is supported by Hanson et al. (2015) who reported a similar result in comparing serum TBARS of pigs fed either peroxidized- or fresh-dried distillers grains with solubles. In the current experiment, plasma ISP of pigs fed 90 °C SO was 1.4 to 1.6 times greater ($P < 0.01$) than pigs fed the other SO treatments, with no difference ($P \geq 0.32$) noted in plasma ISP among pigs fed the 22.5, 45, or 180 °C SO. Because TBARS are a nonspecific metric of lipid peroxidation that detects numerous compounds and ISP is specifically a product of the oxidation of arachidonic acid (Morrow et al., 1995), we elected to also measure ISP because it is a preferred measure of lipid damage (Dalle-Donne et al., 2006). The discernable difference in plasma ISP among SO treatments in the current experiment, where other markers were not indicative of oxidative stress, may be due to the stability and specificity of the ISP molecule as an indicator of lipid damage.

It was hypothesized that the most pronounced oxidative stress would be observed in the livers of pigs fed oxidized oils. Although there was liver hypertrophy observed with oxidized oil feeding of these pigs, there was no effect ($P \geq 0.12$) of SO noted on TBARS, PC, 8-OH-2dG, GPx activity, or CAT activity (Table 8). In light of the enlarged livers in pigs fed peroxidized SO, we had expected there to be accompanying evidence of cytotoxic damage, especially in the form of DNA damage. DNA is particularly susceptible to oxidative damage (Wu et al., 2004) and 8-OH-2dG concentrations are known to increase in livers damaged by toxicity or oxidative stress, such as with hepatitis and cirrhosis

Table 8. Oxidative stress biomarkers in urine, plasma, and liver of pigs fed SO with differing peroxidation levels

	Oil treatment[2,3]				Statistics	
Item[1]	22.5 °C	45 °C	90 °C	180 °C	SEM	*P* value
Plasma, number of observations	14	14	14	14	–	–
TBARS, µM	11.4	11.1	11.4	12.0	0.9	0.91
ISP, pg/mL	44. 9[b]	46.4[b]	72.0[a]	51.2[b]	4.7	0.01
PC, nmol/mL	38.1	36.0	47.1	35.9	5.2	0.39
GPx, nmol/min/mL	2,991	2,870	3,238	2,999	378	0.92
Liver, number of observations	13	14	14	13	–	–
TBARS, µM	94.3	94.9	83.1	109.4	11.9	0.48
PC, nmol/mg	292.8	318.3	268.5	302.3	15.1	0.12
8-OH-2dG, pg/mg	1,459	1,435	1,637	1,547	134	0.68
GPx, nmol/min/mg	35.1	33.9	43.7	39.4	3.9	0.24
CAT, nmol/min/mg	721.7	812.4	908.0	879.8	105.4	0.59
Urine, number of observations	12	12	12	12	–	–
TBARS, µM	6.3[b]	7.7[b]	20.6[a]	11.3[b]	2.8	0.01
ISP, pg	1,956[b]	1,969[b]	4,839[a]	3,534[ab]	628	0.01
8-OH-2dG, µg	96.3	78.0	91.6	118.7	162	0.36

CAT, catalase; GPx, glutathione peroxidase; ISP, F2-isoprostane; TBARS, thiobarbituric acid reactive substances; PC, protein carbonyls.

[1]Units are per milliliters of plasma and per mg of liver sample while urine data are corrected for urine output by multiplying the analyte concentration by the volume of urine collected during the metabolism experiment.

[2]22.5 = fresh oil; 45 = oil heated for 12 d at 45 °C; 90 = oil heated for 72 h at 90 °C; 180 = oil heated for 6 h at 180 °C. All oil groups had a constant compressed air flow rate at 15 L/min.

[3]Superscript letters reflect peroxidized SO treatment differences ($P \leq 0.05$).

Table 9. Pearson correlation coefficients among SO composition and lipid peroxidation products with measures of oxidative status[1]

	Criterion				
Lipid quality indices	Plasma ISP	Liver PC	Liver GPx	Urine TBARS	Urine ISP
UFA:SFA	−0.49	–	−0.27	−0.47	−0.49
	(0.01)	–	(0.05)	(0.01)	(0.01)
PV	0.54	−0.27	–	0.50	0.44
	(0.01)	(0.05)	–	(0.01)	(0.01)
AnV	0.32	–	–	0.34	0.41
	(0.02)	–	–	(0.02)	(0.01)
OFA	–	–	–	–	−0.29
	–	–	–	–	(0.04)
TPC	0.54	−0.27	0.28	0.50	0.50
	(0.01)	(0.05)	(0.04)	(0.01)	(0.01)
PTAG	0.42	–	–	0.41	0.47
	(0.01)	–	–	(0.01)	(0.01)
OSI	−0.45	–	–	−0.44	−0.44
	(0.01)		–	(0.01)	(0.01)
Hexanal	0.56	−0.29	–	0.50	0.46
	(0.01)	(0.03)	–	(0.01)	(0.01)
Acrolein	–	–	–	–	0.35
	–	–	–	–	(0.01)
DDE	0.41	–	–	0.41	0.46
	(0.01)	–	–	(0.01)	(0.01)
HNE	0.54	–	0.28	0.50	0.50
	(0.01)	–	(0.04)	(0.01)	(0.01)
Ratio	–	–	–	–	0.31
	–	–	–	–	(0.03)
TOC	−0.54	0.27	−0.28	−0.50	−0.50
	(0.01)	(0.05)	(0.04)	(0.01)	(0.01)

AnV, p-anisidine value; CAT, catalase; DDE, 2,4-decadienal; FA, fatty acid; GPx, glutathione peroxidase; HNE, 4-hydroxynonenal; ISP, F2-isoprostane; IV, iodine value; ND, not detected; OFA, oxidized fatty acids; OSI, oil stability index; PTAG, polymerized tryacylglycerides; PV, peroxide value; TBA, thiobarbituric acid; TBARS, thiobarbituric acid reactive substances; TOC, tocopherol; TPC, total polar compounds; UFA:SFA, unsaturated:saturated fatty acid ratio.

Ratio, ratio of aldehydes as described by Wang et al. (2016).

[1]Top value represents correlation (*R* value) and bottom value in parenthesis represents significance (*P* value). If no value is given, it was not found to be significant (–) at $P < 0.05$. There were no correlations observed between SO quality indices and plasma TBARS, plasma PC, plasma GPx, liver TBARS, liver 8-OH-2dG, liver CAT, or urinary 8-OH-2dG so they were removed from the table.

(Shimoda et al., 1994), as well as in non-alcoholic fatty liver disease (Seki et al., 2002).

Amounts of TBARS detected in urine of pigs fed 90 °C were 1.8 to 3.3 times greater ($P < 0.03$) than pigs fed the other SO treatments, with no difference ($P > 0.22$) in urinary TBARS among pigs fed 22.5, 45, or 180 °C SO (Table 8). Excretion of TBARS, in particular malondialdehyde, is reported to increase with the onset of oxidative stress (Draper et al., 2000). Because the excretion of TBARS in urine is dependent on the quantity of fat consumed, FA profile and other factors, it is, therefore, not considered a reliable marker of oxidative stress in clinical settings (Draper et al., 2000; Dalle-Donne et al., 2006). In this study, however, the pigs were fed similar diets (except for the thermal treatment imposed on the SO) and were raised under controlled conditions. Consequently, the increased urinary TBARS observed in pigs fed 90 °C SO should be considered an indication of lipid damage caused by oxidative stress. However, due to the number of factors that can influence urinary TBARS, they should not be relied upon as the sole marker of oxidative stress. The increased urinary TBARS is supported by the 2.5 times greater ($P < 0.01$) amounts of urinary ISP in pigs fed 90 °C SO compared with pigs fed either 22.5 or 45 °C SO, with 180 °C being intermediate.

Because oxidative stress can be attributed, at least in part, to specific or combinations of physicochemical characteristics of the oils, we conducted a correlation analyses to examine the relationships among several SO quality indices with markers of oxidative stress (Table 9). There were no

correlations observed between SO quality indices and plasma TBARS, plasma PC, plasma GPx, liver TBARS, liver 8-OH-2dG, liver CAT, or urinary 8-OH-2dG, so they were omitted from the table. In addition, the correlations among liver PC and liver GPx activity and the SO quality indices listed were largely weak and inconsistent. In contrast, plasma ISP, urinary TBARS, and ISP were correlated with numerous SO quality measures (Table 9). The consistent correlation of SO quality measures with ISP in both the urine and plasma may be reflective of the compounds stability (Dalle-Donne et al., 2006), and lends support for using ISP as a viable marker of oxidative stress in finishing pigs, especially if the induction of oxidative stress is by feeding the animal a peroxidized lipid. Among the SO quality indices evaluated, the most consistently correlated SO quality indices with the measures of oxidative stress were PV, TPC, hexanal, HNE, and TOC (Table 9). It must be remembered, however, that correlations do not specify a cause and effect relationship, but the current study only suggests that these parameters may need scrutiny in future research. While not in perfect agreement, it is noteworthy that Lindblom et al. (2018a, 2018b) also reported that PV, TPC, and hexanal or HNE were SO traits with the highest correlations to pig performance and markers of oxidative stress in growing pigs. Although it is unclear why such disparity occurred between these two studies, even though they were conducted under similar experimental conditions, the more mature finishing pigs used in the present study may have had a more developed antioxidant defense systems than the growing pigs, which may have ameliorated the effect of consuming peroxidized SO in their diet. It should be noted, however, that while significant, many of the correlation (*R*) values in the current study as well as that reported by Lindblom et al. (2018a, 2018b), whether they were positive or negative, were relatively small to make a leap to a causal relationship.

CONCLUSION

In conclusion, thermal processing of SO reduced UFA:SFA and IV, but increased numerous lipid peroxidation compounds including PV, AnV, TPC, and several aldehydes. The combination of changes in FA profile and the formation of lipid peroxidation products were found to be greatest in the SO thermally processed at 90 °C for 72 h in comparison to other SO treatment groups, which resulted in reduced ADG, G:F, and GE and AEE digestibility. In contrast, peroxidation of SO did not affect N digestion, N retention, or intestinal permeability as measured by urinary L:M. There were minimal effects of feeding peroxidized SO on measures of oxidative stress, although plasma ISP and urinary TBARS and ISP were found to be greatest in pigs fed the SO thermally processed at 90 °C for 72 h in comparison to other SO treatment groups. Using simple correlation coefficients, PV, TPC, hexanal, HNE, and TOC were the most common indices of lipid peroxidation products correlated with growth and digestibility, and oxidative stress, which may provide insight on which lipid peroxidation products should be further assessed in determining lipid quality impacts on animal productivity.

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