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

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ABSTRACT: Consumption of highly peroxidized oils has been shown to affect pig performance and oxidative status through the development of compounds which differ according to how oils are thermally processed. The objective of this study was to evaluate the effect of feeding varying degrees of peroxidized soybean oil (SO) on parameters of growth performance; lipid, N, and GE digestibility, gut integrity in growing pigs, and plasma Trp. Fifty-six barrows (25.3 ± 3.3 kg initial BW) were randomly assigned to one of four diets 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 with an air infusion of 15 L/min. Peroxide values for the 22.5, 45, 90, and 180 °C processed SO were 2.0, 96, 145, and 4.0 mEq/kg, respectively; 2,4-decadienal values for 22.5, 45, 90, and 180 °C processed SO were 2.11, 5.05, 547.62, and 323.57 mg/kg, respectively; and 4-hydroxynonenal concentrations of 0.05, 1.05, 39.46, and 25.71 mg/kg with increasing SO processing temperature. Pigs were individually housed and fed ad libitum for a 49 d period to determine the effects of SO peroxidation status on growth performance, including a metabolism period for assessing GE and N digestibility, and N retention. In

vivo urinary lactulose to mannitol ratio was also assessed to evaluate potential changes in small intestinal integrity. Although there were no differences observed in ADFI ($P = 0.19$), ADG was decreased in pigs fed 90 °C SO diet ($P = 0.01$), while G:F was increased ($P = 0.02$) in pigs fed 45 °C SO diet compared to the other SO diets. Pigs fed the 90 °C processed SO had the lowest ($P = 0.01$) DE as a percentage of GE, whereas ME as a percentage of DE was lowest ($P = 0.05$) in pigs fed the 180 °C SO and 90 °C SO followed by 45 °C SO and fresh SO. Ether extract (EE) digestibility was lowest ($P = 0.01$) in pigs fed 90 °C SO followed by pigs fed 180 °C SO, 45 °C SO, and fresh SO. The percent of N retained was greatest ($P = 0.01$) in pigs fed fresh SO followed by pigs fed 45 °C SO, 180 °C SO, and 90 °C, respectively. There were no differences observed among SO treatments for urinary lactulose to mannitol ratio ($P = 0.60$). Pigs fed SO processed at 90 °C and 180 °C had lower concentrations ($P < 0.01$) of serum Trp compared to pigs fed the 22.5 °C and 45 °C SO treatments. The presence of lipid peroxidation products, namely several aldehydes, contained in the 90 °C SO diet reduced ADG, GE and EE digestibility, and N balance, but had no impact on gut permeability.

Key words: digestibility, gastrointestinal integrity, growing pigs, performance, peroxidized soybean oil

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INTRODUCTION

Lipids are added to swine diets as a concentrated energy source (Pettigrew and Moser, 1991; Azain, 2001; Lin et al., 2013). In the United States, soybean oil (SO) is a common lipid source added in swine diets that contains a high concentration

of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (NRC, 2012). In contrast to saturated fatty acids (SFA), MUFA and PUFA are more susceptible to lipid peroxidation due to the double bonds in their conformation (Holman, 1954). Thermally processed lipids may be an economical alternative to increase dietary energy concentration compared to refined lipid sources, at the expense of lipid quality. Commonly caused by thermal processing in the presence of oxygen, lipid peroxidation occurs as a free radical chain reaction. Products formed through lipid peroxidation include peroxides, aldehydes, polymers, and polar compounds (Gonzalez-Muñoz et al., 1998). In contrast, as lipid peroxidation products are formed, antioxidants in the lipid become depleted (Seppanen and Csallany, 2002). In addition to the compounds formed during lipid peroxidation, the unsaturated:saturated fatty acid ratio (U:S) decreases and FFA concentration increases (Liu et al., 2014b), which may also affect the energy value (Wiseman and Salvador, 1991).

Consumption of peroxidized lipids have been shown to decrease growth performance in swine (Boler et al., 2012; Rosero et al., 2015) and poultry (Dibner et al., 1996; Anjum et al., 2004; Tavárez et al., 2011). Furthermore, feeding peroxidized lipids have been linked to decreased digestibility (Liu et al., 2014c) and plasma Trp (Chi Chen, University of Minnesota, personal communication). However, limited information is available regarding the effects of feeding various degrees of thermally processed SO in swine nutrition. Therefore, the objective of this study was to evaluate the effects of feeding divergently thermally processed SO on growth performance, intestinal integrity, and digestibility of N, GE, and lipids in growing pigs.

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

Treatments consisted of four diets containing either 10% fresh SO or SO thermally processed at 1) 45 °C for 288 h, 2) 90 °C for 72 h, or 3) 180 °C for 6 h. Except for the 22.5 °C temperature, each heating process was conducted in stainless steel tanks that were 53 cm in circumference and 61 cm high. Each tank was filled with 80 kg of the same batch

of refined SO and was accompanied with a constant air flow (15 L/min) during the entire heating process 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 °C and 90 °C, whereas a liquid propane heater was used to heat the SO to 180°. After thermal processing and before feed mixing, processed oils were stored at -20 °C, and no antioxidant was added before or during diet preparation. Diverse analyses including FA profile, oil quality, lipid peroxidation products, and total tocopherols were conducted on each SO treatment (Table 1) and summarized in Table 2 to characterize the quality of each SO treatment. Diets (Table 3) were formulated to contain 1.30% standardized ileal digestible Lys, with AA ratios, ME, and mineral content adequate for 25 kg pigs according to the NRC (2012).

Experimental Design

A total of 56 barrows (initial BW 25.28 ± 3.31 kg) were housed at the Swine Nutrition Farm at Iowa State University (Ames, IA) for the duration of the study. 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) for 49 d and had ad libitum access to feed and water. Performance data were observed for 49 d with amount of feed added to feeders recorded throughout the experiment, and pigs and feeders

Table 1. Method of analysis for thermally processed soybean oils

Analyte	Method
Aldehydes ¹	Wang et al., 2016
p-Anisidine value ²	AOCS Cd 18-90
Fatty acids ²	AOCS Ce 1a-13
Free fatty acids 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
Oil stability index ²	AOCS Cd 12b-92
Oxidized fatty acids ²	AOCS G 3-53
Peroxide value ²	AOCS Cd 8b-90
Polymerized triacylglycerides ³	AOAC 993.25
Thiobarbituric acid value ²	AOCS Cd 19-90
Tocopherols ²	AOCS Ce 8-89
Total polar compounds ²	AOCS Cd 20-91
Unsaponifiable matter ²	AOCS Ca 6a-40

¹Analyzed by University of Minnesota, St. Paul, MN.

²Analyzed by Barrow-Agee, Memphis, TN.

³Analyzed by the USDA-ARS, Peoria, IL.

weighed on d 0, 21, d 49 to determine ADG, ADFI, and G:F.

During the performance portion of the experiment, 20, 20, and 16 pigs were moved on d 21, 25, and 29, respectively, to individual metabolism crates for 5 d to collect urine and feces to evaluate N, ether extract (EE), and GE digestibility, and an additional urine collection to evaluate in vivo intestinal permeability. During this period, pigs were

fed an amount of diet equivalent to 4% of their average BW twice daily (2% at 0700 h and 2% at 1700 h) with constant access to water. After 2 d for crate adaptation, a 3 d fecal and urine collection period occurred for GE digestibility and for N balance study. To limit microbial growth and ammonia loss in the collected urine, 15 mL of 6 N HCl was added to the collection containers during urine collection. Urine was collected twice daily and stored

Table 2. Composition and peroxidation analysis of thermally processed soybean oils

Heating temperature, °C	22.5	45	90	180
Time heated ¹ , h	0	288	72	6
Fatty acids, % of total fat ^{2,3}				
C8:0, Caprylic	ND [§]	ND	0.07	ND
C14:0, Myristic	0.08	0.07	0.09	0.08
C15:0, Pentadecanoic	ND	ND	0.07	ND
C16:0, Palmitic	10.80	10.94	12.25	11.26
C16:1, Palmitoleic	ND	0.08	0.09	ND
C17:0, Margaric	0.11	0.10	0.12	0.11
C18:0, Stearic	3.83	3.88	4.35	4.03
C18:1, Oleic	22.08	22.29	24.28	22.78
C18:2, Linoleic	54.05	53.74	50.96	53.02
C18:3, Linolenic	7.83	7.50	6.27	7.07
C19:0, Nonadecanoic	0.24	0.27	0.20	0.39
C20:0, Arachidic	0.30	0.31	0.34	0.32
C20:1, Gadoleic	0.18	0.19	0.21	0.30
C22:0, Behenic	0.36	0.35	0.23	0.38
C22:5, Docosapentaenoic	ND	0.11	0.12	ND
Other FA ⁴	0.13	0.15	0.35	0.20
U:S ⁴	5.35	5.27	4.64	5.02
IV ⁵	133	132	126	130
Free fatty acids ² , %	0.04	0.07	0.35	0.14
Free glycerin ² , %	2.68	3.50	2.27	2.44
Moisture, %	0.02	0.02	0.10	0.02
Insoluble impurities, %	0.02	0.02	0.04	0.02
Unsaponifiable matter, %	0.29	0.35	0.53	0.30
Oxidized FA ² , %	1.3	2.5	3.1	1.4
OSI ^{2,4} @ 110 °C, h	7.15	3.65	2.70	3.35
p-Anisidine value ^{2,6}	1.19	8.38	261	174
Peroxide value ² , mEq/kg	2.0	95.6	145.3	4.0
TPC, % ²	2.67	7.01	22.65	10.19
PTAGS ^{4,7} , %	ND	ND	6.39	2.06
TBA value ^{2,6}	0.10	0.14	0.14	0.09
Aldehydes, mg/kg ⁸				
2,4-decadienal	2.11	5.05	547.62	323.57
4-hydroxynonenal	0.05	1.05	39.46	25.71
Acrolein	3.88	3.31	15.82	45.39
Decenal	0.24	0.35	28.17	19.81
Heptadienal	0.12	4.40	85.50	61.86
Heptanal	0.22	3.60	89.12	40.08
Hexanal	2.97	2.71	21.20	16.84
Octenal	0.19	1.10	59.86	21.31
Pentanal	0.28	0.31	1.82	2.50
Undecadienal	0.10	0.18	26.02	15.35
Undecenal	0.27	0.29	27.57	23.38

continued

Table 2. Continued

Ratio ⁹	0.16	0.14	0.64	0.57
Total tocopherols ² , mg/kg	772	620	405	609
Alpha	56	14	343	197
Beta	<10	<10	<10	<10
Delta	206	190	15	75
Gamma	510	416	47	337

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

²Analyzed by Barrow-Agee, Memphis, TN.

³No other FA were detected besides those listed.

⁴Abbreviations: ND, not detected; FA, fatty acid; U:S, unsaturated:saturated fatty acid ratio; TBA, thiobarbituric acid; OSI, oil stability index; TPC, total polar compounds; PTAGS, polymerized tryacylglycerides; IV, iodine value.

⁵Iodine values were calculated using the FA profile data following the equation proposed by Meadus et al., 2010: $VI = (16:1 \times 0.95) + (18:1 \times 0.86) + (18:2 \times 1.732) + (18:3 \times 2.616) + (20:1 \times 0.795) + (20:2 \times 1.57) + (20:3 \times 2.38) + (20:4 \times 3.19) + (20:5 \times 4.01) + 22:4 \times 2.93 + (22:5 \times 3.68) + (22:6 \times 4.64)$.

⁶There are no units for p-anisidine value or TBA value.

⁷Analyzed by the USDA-ARS, Peoria, IL.

⁸Analyzed by University of Minnesota, St. Paul, MN.

⁹Ratio 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-fed basis

Item	Percent
Corn	54.15
Soybean meal	32.40
Soybean oil ¹	10.00
Limestone	1.13
Monocalcium phosphate	0.90
Titanium dioxide	0.50
Sodium chloride	0.35
Vitamin mix ²	0.25
Trace mineral mix ³	0.15
L-Lys-HCl	0.13
DL-Met	0.04
TOTAL ⁴	100.00

¹Soybean oil was either fresh oil, heated for 12 d at 45 °C, heated for 72 h at 90 °C, or heated for 6 h at 180 °C. All oil groups had a constant compressed air flow rate at 15 L/min.

²Provided the following per kilogram of diet: vitamin A, 6,125 IU; vitamin D₃, 700 IU; vitamin E, 50 IU; vitamin K, 30 mg; vitamin B₁₂, 0.05 mg; riboflavin, 11 mg; niacin, 56 mg; and pantothenic acid, 27 mg.

³Provided the following per kilogram of diet: Cu (as CuSO₄), 22 mg; Fe (as FeSO₄), 220 mg; I (as Ca(IO₃)₂), 0.4 mg; Mn (as MnSO₄), 52 mg; Zn (as ZnSO₄), 220 mg; and Se (Na₂SeO₃), 0.4 mg.

⁴Diets were calculated to contain 3,770 kcal ME/kg, 1.05% standardized ileal digestible Lys, 0.70% total Ca, and 0.33% standardized total tract digestible P.

at −10 °C until subsequent analysis. Titanium dioxide (0.5%) was added in the feed as an indigestible marker for digestibility calculations. A subsample of feces were collected during the collection period and stored at −10 °C. At the end of the collection period, feces were dried at 70 °C for 48 h, weighed, ground through a 2 mm screen, and a subsample from each pig was collected for digestibility analysis.

To determine in vivo intestinal permeability, all pigs were subjected to an oral lactulose and mannitol challenge. Following a 12 h fast on the evening of d 4 in the metabolism crates, each pig was fed 100 g of their assigned treatment diet which contained 500 mg/kg BW lactulose and 50 mg/kg BW mannitol (Spectrum Chemical, Gardena, CA). After each pig consumed this portion (within 15 min), they were then fed the remaining portion of their respective diet. Plastic containers containing 5 mL chlorhexidine were placed under each metabolism crate to eliminate microbial growth and urine was collected for the next 12 h (overnight), volume quantified, subsampled, and stored at −20 °C for analysis. Immediately after urine collection, approximately 8 mL of blood was obtained via jugular venipuncture using a 10 mL vacuum serum tube (BD Diagnostics, Franklin Lakes, NJ). Blood samples were centrifuges at $2,500 \times g$ for 15 min at 4 °C and serum was harvested. Serum samples were immediately frozen and stored at −80 °C until analysis of serum Trp concentrations. Following the metabolism experiment, pigs were returned back to their pens for the remainder of the performance portion of the trial, with feed consumption during the metabolism crated period recorded, and added to the total feed intake for the performance trial.

Calculations and Methodologies

Gross energy of the diet, feces, and urine was determined using an isoperibol bomb calorimeter (model 1281, Parr Instrument Co., Moline, IL) using benzoic acid as a standard. Analyses performed on

diets and fecal samples were done in duplicates and urine analyses were performed in triplicates. For urine analysis, 1 mL was filtered and added to 0.5 g of dried cellulose and dried for 24 h at 50 °C and repeated two times to determine of urinary GE. In addition, GE of cellulose was determined in order to calculate GE of urine by subtracting the GE content of cellulose from the GE content of urine samples. Treatment diets and feces were analyzed for EE as described previously (Luthria et al., 2004) for EE digestibility. Briefly, samples were mixed with sand (Fisher #S23-3) to avoid compaction and were added to a 10 mL stainless steel cell. The cell was extracted three times in the extraction system at 120 °C and pressure using petroleum ether as the solvent. The petroleum ether was added to provide extract for the sample and was collected into a preweighed glass vial which was placed in an evaporation system (Multivap Model 118, Organomation Associates, Berlin, MA). The vial was then weighed to determine the residual EE.

Nitrogen was analyzed by thermocombustion (VarioMAX CNS, Elementar Analysensysteme GmbH, Hanau, Germany). During combustion, gases are converted to individual gases and sorted into adsorption columns and are measured using a thermal conductivity detector. Digestibility coefficients were estimated by marker methodology using titanium dioxide, with DE, EE, and N digestibility calculated as a percentage using the equation $[1 - (Ti_{diet} \times GE_{feces}, EE_{feces}, \text{ or } N_{feces}) / (Ti_{feces} \times GE_{diet}, EE_{diet}, \text{ or } N_{diet})] \times 100$. Metabolizable energy as a percent of DE was calculated by dividing ME intake/d and DE intake/d. Nitrogen retention was calculated by subtracting excreted N from digested N then taking the ratio of N retained to N digested to report as a percent.

Urinary lactulose and mannitol concentrations were measured via HPLC as an in vivo indicator of small intestinal permeability and using the method that has been previously described by Kansagra et al., (2003). The ratio of lactulose:mannitol was calculated back to the total amount of urine collected and reported on a recovery basis. For serum Trp concentration, serum was diluted 1:1 with 0.05 M potassium phosphate buffer, pH 6.0, and deproteinized with 2 M trichloroacetic acid. The free Trp levels were 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). Serum Trp concentrations were expressed on a µM basis.

Statistical Analysis

Data were analyzed as a completely randomized design with individual pig as the experimental unit, using Proc GLM procedure of SAS (version 9.4; SAS, 2009) with means reported and separated using LSMEANS. Despite the fact that lipid peroxidation measures can be correlated to each other (Liu et al., 2014b), relationships between lipid peroxidation measures with growth performance, plasma Trp, and GE, EE, and N digestibility variables were evaluated by simple linear correlation (Pearson correlation coefficients) analysis to provide direction as to which peroxidation measures may need to be evaluated in future research. 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 a complex process that is typically achieved through thermal processing of a lipid (St. Angelo et al., 1996). It is a chain reaction involving free radical formation and propagation of free radical binding to PUFA (Holman, 1954), where its progression is based on the duration and intensity of thermal processing and presence of oxygen. Products formed through thermal processing of lipids decreases the quality of the lipid by increasing peroxides in the initiation phase, which are readily degraded into aldehydes and acids in the propagation phase, ultimately forming polar compounds and indigestible polymers (Gonzalez-Muñoz et al., 1998) in the termination phase. Consequently, understanding lipid quality requires the measurement of multiple lipid peroxidation products.

The current experiment induced differing levels of lipid peroxidation of SO by thermally processing at variable temperatures and durations prior to being mixed in the pig diets. Three different SO processing temperatures were selected to generate different types and concentrations of lipid peroxidation products (Kerr et al., 2015), but also have relevance to temperatures observed in the livestock, rendering, or food industries. The 45 °C SO was heated for 288 h (12 d) which is our estimate of the temperature that feed in a bulk bin could reach during summer months. The 90 °C SO was processed for 72 h and was chosen as a doubling of

45 °C and was also used to resemble the rendering of animal fats where heating temperatures in that industry average 115 to 145 °C for approximately 40 to 90 min (Meeker and Hamilton, 2006), and is similar to Liu et al., (2014b) who heated various lipids at 95 °C for 72 h. Likewise, Kerr et al. (2015) thermally processed corn oil at 95 °C for 72 h which resulted in increased aldehyde production, including 4-hydroxynonenal (**HNE**) and 2,4-decadienal (**DDE**) concentrations. The 180 °C SO was chosen to resemble a temperature used for frying in the restaurant industry. In addition, thermally processing corn oil at 190 °C has been shown to yield the greatest concentrations of HNE, DDE, and thio-barbituric acid reactive substances (**TBARS**) when heated for 6 h (Kerr et al., 2015).

The four SO utilized in this study were thoroughly analyzed prior to feed mixing for fatty acid composition along with several lipid peroxidation products (Table 2). Although a detailed description of lipid peroxidation products is provided elsewhere (Halliwell and Chirico, 1993; Frankel, 2005; Schaich, 2005); a few specific ones will be described herein because of their potential importance and periodic measurement in the livestock industry. Peroxide value (**PV**) is a measurement of hydroperoxides formed in the initiation phase of lipid peroxidation and was determined to be highest in the 90 °C processed SO followed by the 45 °C, 180 °C processed SO, and fresh SO with values of 145.3, 95.6, 4.0, and 2.0 mEq/kg SO, respectively. We suspect that thermally processing SO at 180 °C results in unstable hydroperoxides that have already begun forming secondary and tertiary peroxidation compounds which is in agreement with Wang et al., (2016). Anisidine value (**AnV**), a unit-less measure of high molecular weight saturated and unsaturated aldehydes, was determined to be highest in the 90 °C and 180 °C processed SO (261 and 174, respectively), and lowest in the 22.5 °C and 45 °C processed SO (1.19 and 8.38, respectively). In contrast, **TBARS**, which is an indirect measure of malondialdehyde, differed little among the thermally processed SO. Oxidized fatty acids (**OFA**), a measure of lipid hydroperoxides and peroxides and saturated epoxy-, keto-, and hydroxy-acids, was not greatly different among the SO treatments, regardless of processing temperature. Lastly, polymerized triacylglycerides (**PTAGS**) were not detected in the 22.5 °C and 45 °C processed SO but were slightly elevated in SO thermally processed at 90 °C and 180 °C (6.39%, and 2.06%, respectively). In the current experiment, concentrations of DDE and HNE were greatest (547.62, and 39.46 mg/kg

respectively) at 90 °C. Many other aldehydes were measured to show the complexity of lipid peroxidation although we will focus our discussion on DDE and HNE. With the degeneration of SO through heating and the noted changes in the various lipid peroxidation products as described above, it was not surprising that the U:S and iodine value of the thermally processed SO decreased with increasing lipid peroxidation.

In addition to measuring lipid peroxidation compounds, we were interested in measuring the antioxidant status of SO. Tocopherols are natural antioxidants found in vegetable oils and function in protecting the lipid from degradation (Kamal-Eldin, 2006) so as lipid peroxidation progresses, antioxidant status becomes diminished. In the current study, the 90 °C processed SO had the lowest total tocopherol (**TOC**) concentration with 405 mg/kg oil followed by 180 °C, 45 °C, and fresh oil with 609, 620, and 772 mg/kg respectively. Miyagawa et al. (1991) reported that thermally processing a blend of SO and rapeseed oil at frying temperature (185 °C) resulted in a rapid degradation rate for gamma tocopherol followed by delta- and alpha-tocopherol, respectively. In the current experiment, the 90 °C SO had the lowest gamma tocopherol concentration suggesting that it had the highest degradation rate in comparison to the other SO treatments. This trend was also noted for delta tocopherol. Alpha-tocopherol concentration was greatest in the 90 °C SO, which is again in agreement with Miyagawa et al. (1991) who reported the greatest retention rate for alpha-tocopherol in thermally processed oil.

Growth Performance

Performance data were collected over the 49 d trial, including 5 d while pigs were in metabolism crates. During the experimental period, one pig fed the 45 °C SO and one pig fed the 180 °C SO died due to causes unrelated to dietary SO treatment; therefore, data are reported using 14 observations for 22.5 °C and 90 °C SO and 13 observations for 45 °C and 180 °C SO. As shown in Table 4, pigs fed the 90 °C SO had reduced ADG ($P = 0.01$) by about 7% in comparison to the other three SO treatment groups over the 49 d test period, with no differences noted among pigs fed the 22.5 °C, 45 °C, and 180 °C SO treatments. Interestingly, thermal processing of SO did not affect ADFI ($P = 0.19$). Pigs fed 45 °C processed SO had an increased G:F ($P = 0.02$) by about 3% compared to the other SO treatments, with no differences

noted among pigs fed the other SO treatments. The reduction in ADG by 7% for pigs fed the SO thermally processed at 90 °C in the current experiment is comparable to Hung et al. (2017) who reported that ADG was reduced by 6% in pigs fed peroxidized lipids compared to pigs fed unperoxidized lipids. In contrast, we did not observe reductions in ADFI or G:F due to consumption of peroxidized lipids as reported by Hung et al. (2017), who reported a reduction in ADFI and G:F by 5% and 2%, respectively.

Serum Trp concentration has been shown to affect brain and nervous system function, and serves as the immediate precursor for serotonin synthesis, where Trp-induced serotonergic activity in the brain has been implicated in the regulation of many behavioral and physiological processes, including feed intake (Baranyiova, 1991; Seve, 1999). Because it has been reported that feeding peroxidized SO to mice resulted in a reduction in plasma Trp and subsequent increases in Trp metabolites in the urine such as kynurenic acid, nicotinamine, and nicotinamide N-oxide (Chi Chen, University of Minnesota personal communication), we felt it important to determine if there was a relationship between consumption of peroxidized SO and plasma Trp in growing pigs. Even though we did not observe reductions in ADFI, pigs fed the 90 °C and 180 °C processed SO had lower concentrations ($P < 0.01$) of serum Trp compared to pigs fed the 22.5 °C and 45 °C SO treatments as reported in Figure 1; and there was a significant correlation of ADFI to serum Trp ($r = 0.27$, $P = 0.07$).

Because growth performance and serum Trp were affected by dietary SO processing, a correlation analysis among various measures of lipid

peroxidation products and these phenotypic responses were conducted (Table 5). However, because we considered the U:S, FFA, and TBARS differences among the SO treatments to be insignificant we elected not to report or discuss these relationships, even though there were significant correlations between U:S, FFA, and TBARS with various growth performance and serum Trp. We did this despite the fact that U:S and FFA can affect the energy value of a lipid (Wiseman and Salvador, 1991; Marquez-Ruiz et al., 1992; Gonzalez-Muñoz et al., 1998; Wiseman et al., 1998) and TBARS have been shown to be correlated to ADG and ADFI in swine (Hung et al., 2017). Correlations between PV, AnV, and hexanal and growth performance and serum Trp were conducted because these are common measures of lipid peroxidation in the literature (Shurson et al., 2015); OFA and total polar compounds (TPC) because these measures should help clarify which classification of compounds are present in a peroxidized oil and the stage of peroxidation a lipid is in; oil stability index (OSI) because it is a routinely used assay and is a measure to predict how much potential peroxidation exists in an oil (Shurson et al., 2015; Kerr et al., 2017). Individual aldehydes including 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 they are considered highly damaging to DNA, proteins, and lipids; a ratio of 10 aldehydes associated with SO peroxidation which has been suggested to be a good measure of SO peroxidation (Wang et al., 2016); and total tocopherols as they would provide an accurate

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

Parameter	Processed soybean oil ¹				Statistics	
	22.5	45	90	180	SEM	P value
ADG, kg	1.02 ^a	1.05 ^a	0.96 ^b	1.03 ^a	0.02	0.01
ADFI, kg	2.05	2.00	1.94	2.09	0.05	0.19
G:F	0.50 ^b	0.53 ^a	0.49 ^b	0.50 ^b	0.01	0.02

¹Data 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 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 was collected over 49 d with initial ABW of 25.28 ± 3.31 kg ($P = 0.36$) and final average BW of 70.80 ± 5.73 kg ($P = 0.11$).

²Superscripts reflect peroxidized soybean oil treatment differences (ab, $P \leq 0.05$).

Figure 1. Effect of thermally peroxidized soybean oil on serum Trp concentrations in growing pigs. 22.5C = fresh oil; 45C = oil heated for 12 d at 45 °C; 90C = oil heated for 72 h at 90 °C; 180C = oil heated for 6 h at 180 °C. All processed soybean oil had constant compressed air flow rate of 15 L/min. Pigs were blocked in groups of 20, 20, and 16 and blood was taken on d 21, 25, and 29, respectively. Peroxidation effect $P = 0.01$ with superscripts reflecting peroxidized soybean oil treatment differences (ab, $P \leq 0.05$).

Table 5. Pearson correlation coefficients among SO composition and peroxidation products with performance, digestibility, and gut integrity responses¹

SO quality indices ²	ADG	ADFI	G:F	Trp
PV	-0.33 (0.01)	-0.28 (0.04)	-	-0.38 (0.01)
AnV	-0.39 (0.01)	-	-0.32 (0.02)	-0.67 (0.01)
OFA	-0.33 (0.02)	-0.28 (0.04)	-	-0.39 (0.01)
TPC	-0.44 (0.01)	-	-	-0.63 (0.01)
PTAGS	-0.47 (0.01)	-	-0.28 (0.04)	-0.63 (0.01)
OSI	-	-	-	0.55 (0.01)
Hexanal	-0.36 (0.01)	-	-0.34 (0.02)	-0.67 (0.01)
Acrolein	-	-	-0.26 (0.06)	-0.41 (0.01)
DDE	-0.41 (0.01)	-	-0.32 (0.02)	-0.67 (0.01)
HNE	-0.39 (0.01)	-	-0.32 (0.02)	-0.67 (0.01)
Ratio	-0.33 (0.02)	-	-0.35 (0.01)	-0.66 (0.01)
TOC	0.39 (0.01)	-	-	0.62 (0.01)

¹Correlation (*r* value) is top value and correlation significance (*P* value) is below in parentheses. If no value is given, it was not found to be significant (-) at *P* ≤ 0.10.

²PV, peroxide value; AnV, p-anisidine value; OFA, oxidized fatty acids; TPC, total polar compounds; PTAGS, polymerized triacylglycerides; OSI, oxygen stability index; DDE, 2,4-decadienal; HNE, 4-hydroxynonenal; Ratio, ratio of aldehydes as described by Wang et al., 2016; TOC, total tocopherols.

depiction of the antioxidant status naturally occurring in SO (Seppanen and Csallany, 2002; Kamal-Eldin, 2006).

Irrespective of SO treatment, PV, AnV, OFA, TPC, PTAGS, hexanal, HNE, DDE, and the aldehyde ratio all had negative correlations with ADG (*P* < 0.01), while total tocopherols had a positive correlation with ADG (*P* < 0.01) as shown in Table 5. The current experiment did not observe a correlation between TBARS in the SO with ADG (data not shown), which is in contrast to Liu et al. (2014a) who reported a negative correlation between oil TBARS and ADG. The reduction in ADG in pigs fed the 90 °C SO is in agreement with Boler et al. (2012) who reported decreased ADG in finishing pigs fed corn oil processed at 95 °C compared to pigs fed a diet containing fresh corn oil, and further confirmed by Liu et al. (2014a) who reported reduced ADG in pigs fed diets containing

peroxidized oil (canola oil, corn oil, poultry fat, and tallow) compared pigs fed diets containing fresh oil. Furthermore, Hung et al. (2017) conducted a detailed review of eight experiments in swine which were fed various peroxidized lipids and reported that pigs fed thermally processed lipids reduced growth by 6% compared to pigs fed fresh lipid sources.

Secondary lipid peroxidation compounds, including many aldehydes, can produce a rancid odor and flavor and have been shown to affect palatability and feed intake in swine and poultry (Dibner et al., 1996; Boler et al., 2012; Liu et al., 2014a). Therefore, we would have expected a decrease in ADFI as SO processing increased. However, in the current experiment this was not observed (Table 4), where ADFI was negatively correlated (*P* = 0.04) only with PV (*r* = -0.28), TBARS (*r* = -0.28, correlation not shown), and OFA (*r* = -0.28). These data are in partial agreement with a review by Hung et al. (2017) who summarized that pigs fed peroxidized lipids had decreased ADFI (5%) and reported that ADFI was only correlated with dietary TBARS (*r* = -0.46, *P* = 0.11).

Interestingly, in the current experiment, pigs fed the 45 °C SO had the greatest feed efficiency. This is due to 45 °C SO fed pigs having numerically higher ADG, but a similar ADFI compared to other treatment diets, resulting in increased G:F compared to other SO treatments. Negative correlations (*P* ≤ 0.08) with AnV, PTAGS, hexanal, acrolein, DDE, HNE, and the aldehyde ratio were observed in relation to G:F as shown in Table 5. According to the review by Hung et al. (2017), G:F of pigs fed peroxidized lipids was reduced by 2% compared to pigs fed fresh lipid sources and was negatively correlated to TBARS.

Even though there was little to no effect of lipid peroxidation on ADFI, serum Trp was negatively correlated (*P* < 0.01) to all lipid peroxidation measures in the SO (except for TBARS, data not shown, Table 5). One could speculate that even though there was a decrease in serum Trp, the decrease was not great enough to elicit a depression in ADFI. Whether this decrease in serum Trp has other metabolic consequences is unknown. The decrease in serum Trp is supported by Dr. Chen at the University of Minnesota (personal communication) who stated that feeding peroxidized SO to mice resulted in a decrease in plasma Trp and subsequent increases in Trp metabolites in the urine such as kynurenic acid, nicotinamine, and nicotinamide N-oxide.

Energy and Lipid Digestibility

Digestibility of lipids is dependent on several factors, one of which being their degree of saturation. Unsaturated fatty acids are typically more digestible because their ability to form a micelle is greater in comparison to saturated fatty acids, with a lower U:S negatively impacting digestibility (Wiseman and Salvador, 1991). In the current experiment, U:S was lowest in the 90 °C SO (4.64) followed by 180 °C SO (5.02), 45 °C SO (5.27), and fresh oil (5.35), which confirms that thermal processing increased the saturation of the lipid in the current experiment (Table 2). This is further supported by others (DeRouchey et al., 1997; Liu et al., 2014b) who have also shown that lipid peroxidation hydrogenates lipids making them more saturated. In addition, polymers generated during lipid peroxidation have been reported to affect digestibility (Marquez-Ruiz et al., 1992; Gonzalez-Muñoz et al., 1998), and in the SO processed at 90 °C and 180 °C, these concentrations were higher than in the fresh SO (22.5 °C) or 45 °C processed oil.

In the current experiment, DE as a percentage of GE was greatest in the fresh SO group and lowest in the 90 °C SO group, with the 45 °C and 180 °C SO groups being intermediate ($P = 0.01$), Table 6. While U:S ratios and FFA concentrations affect lipid digestibility (Wiseman and Salvador, 1991), because these differences were small and considered to be insignificant, we elected not to discuss any significant correlations. Energy digestibility differences may also be due to the increase in lipid peroxidation products as measured by increased lipid peroxidation products in the thermally processed SO shown in Table 2. This is supported by the negative correlations of PV, OFA, TPC, and PTAGS with DE:GE, $P \leq 0.03$ (Table 7). Changes in EE digestibility were in a similar direction as DE:GE among treatments, with fresh SO having the greatest EE digestibility followed by 45 °C SO, 180 °C SO, and 90 °C SO with values of 83.03%, 82.11%, 81.20%, and 78.35% respectively, $P = 0.01$ (Table 6). Likewise, multiple negative correlations were observed between DE and EE digestibility with all lipid peroxidation products ($P \leq 0.02$) except acrolein. The reduction in energy and EE digestibility in pigs fed the 90 °C SO helps to explain the reduction in ADG, likely resulting from the increase in lipid peroxidation products, and in the concentration of saturated fatty acids.

The ME as a percentage of DE also differed among the SO thermal processing procedures, with fresh SO having the greatest ME as a percentage of

DE (99.18%), the 90 °C and 180 °C SO the lowest (98.76 and 98.64%, respectively), and the 45 °C SO (99.04%) being intermediate ($P = 0.05$), Table 6. In contrast to a greater number of correlations between SO composition and quality indices with DE or EE digestibility, there were fewer correlations between SO composition and quality indices with ME as a percentage of DE. Interestingly, AnV, hexanal, acrolein, HNE, and the aldehyde ratio were negatively correlated ($P \leq 0.09$) to ME as a percentage of DE. Our results are in agreement with DeRouchey et al. (1997) and Rosero et al. (2015) who reported reductions in nutrient digestibility when pigs were subjected to dietary lipid peroxidation in choice white grease and SO, respectively. In contrast, Liu et al. (2014c) did not report changes in DE, ME, and EE digestibility in pigs fed various peroxidized lipids.

Nitrogen Balance

There was a tendency ($P = 0.10$) for N digestibility to be highest for both the fresh SO and the 180 °C SO (88.72% and 88.65%, respectively) and lowest for the 45 °C SO (87.05%), with the 90 °C SO (88.19%) being intermediate (Table 6). These results are in contrast to DeRouchey et al. (1997) who did not report any statistical differences among N digestibility in nursery pigs fed thermally processed choice white grease compared to unprocessed choice white grease. In addition, Liu et al. (2014c) did not report differences in N digestibility in pigs fed peroxidized lipids in comparison to pigs fed fresh lipids. In the current experiment, N digestibility was not correlated to any key lipid peroxidation products (Table 7).

Dietary treatment affected N retention ($P = 0.01$) with fresh SO having the highest N retention followed by 45 °C SO, 180 °C SO, and 90 °C SO (90.56%, 88.98%, 86.94%, and 86.04%, respectively). In the current experiment, N retention was negatively correlated to all lipid peroxidation measures ($P \leq 0.06$) and positively correlated with OSI and TOC ($P = 0.01$). In contrast, Liu et al. (2014c) did not report any differences in N retention. Furthermore, the differences in N digestibility and retention may suggest that lipid peroxidation affects muscle accretion, although further research is needed to confirm this statement.

Intestinal Barrier Function

Changes in intestinal integrity have been shown to be associated with changes in absorption of nutrients and resistance to pathogens (Wijten

et al., 2011). Therefore, we were interested in measuring the effects of lipid peroxidation products in variably processed dietary SO on intestinal permeability. It has been shown that intestinal permeability is increased when consuming a diet high in

SFA (Laugerette et al., 2012; Mani et al., 2012; Liu et al., 2014d). The ratio of lactulose to mannitol in urine is commonly used as an *in vivo* indicator of small intestinal paracellular permeability (Wijtten et al., 2011). In the current study, there

Table 6. Energy and lipid digestibility, nitrogen balance, and intestinal permeability in growing pigs fed various levels of peroxidized soybean oil

Parameter	Processed soybean oil ¹				Statistics	
	22.5	45	90	180	SEM	<i>P</i> value ²
DE, % of GE	88.63 ^a	87.84 ^{bc}	87.26 ^c	88.44 ^{ab}	0.24	0.01
ME, % of DE	99.18 ^a	99.04 ^{ab}	98.76 ^b	98.64 ^b	0.15	0.05
EE digestibility, %	83.03 ^a	82.11 ^{ab}	78.35 ^c	81.20 ^b	0.57	0.01
Nitrogen digested, %	88.72 ^x	87.05 ^y	88.19 ^{xy}	88.65 ^x	0.51	0.10
Nitrogen retained ³ , %	90.56 ^a	88.98 ^{ab}	86.06 ^c	86.94 ^b	0.95	0.01
Urinary lactulose:mannitol	0.05	0.06	0.05	0.04	0.01	0.60

¹Data 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.

²Superscripts reflect peroxidized soybean oil treatment differences (abc, $P \leq 0.05$; xy, $P \leq 0.10$).

³Nitrogen retention was calculated by subtracting excreted N from digested N then taking the ratio of N retained to N digested to report as a percent.

Table 7. Pearson correlation coefficients among SO composition and peroxidation products with digestibility responses¹

SO quality indices ²	DE:GE	ME:DE	EE digestibility	N retention
PV	-0.40 (0.01)	-	-0.36 (0.01)	-
AnV	-	-0.24 (0.09)	-0.47 (0.01)	-0.43 (0.01)
OFA	-0.39 (0.01)	-	-0.36 (0.01)	-
TPC	-0.33 (0.02)	-	-0.50 (0.01)	-0.40 (0.01)
PTAGS	-0.30 (0.03)	-	-0.51 (0.01)	-0.39 (0.01)
OSI	-	-	0.32 (0.02)	0.37
Hexanal	-	-0.25 (0.07)	-0.45 (0.01)	-0.43 (0.01)
Acrolein	-	-0.27 (0.06)	-	-0.26 (0.06)
DDE	-	-	-0.48 (0.01)	-0.42 (0.01)
HNE	-	-0.24 (0.09)	-0.47 (0.01)	-0.43 (0.01)
Ratio	-	-0.26 (0.06)	-0.43 (0.01)	-0.42 (0.01)
TOC	0.33 (0.01)	-	0.47 (0.01)	0.39 (0.01)

¹Correlation (*r* value) is top value and correlation significance (*P* value) is below in parentheses. If no value is given, it was not found to be significant (-) at $P \leq 0.10$. There were no correlations observed between SO composition with N digestibility or with the urinary lactulose:mannitol ratio so they were removed from the table.

²PV, peroxide value; AnV, p-anisidine value; OFA, oxidized fatty acids; TPC, total polar compounds; PTAGS, polymerized triacylglycerides; OSI, oxygen stability index; DDE, 2,4-decadienal; HNE, 4-hydroxynonenal; Ratio, ratio of aldehydes as described by Wang et al., 2016; TOC, total tocopherols.

were no differences ($P = 0.60$) in the urinary lactulose to mannitol ratio among SO treatment groups with urinary ratios of lactulose to mannitol on a recovery basis, averaging 0.05, Table 6. Due to the creation of lipid peroxidation compounds and the increased saturation of thermally processed SO, we would have expected increased intestinal permeability in pigs fed 90 °C and 180 °C SO. Despite this, our findings are in agreement with Liu et al. (2014d) who also found no significant differences in urinary lactulose to mannitol ratios among lipid peroxidation level. Given the relationship between serum Trp with the immune system and gastrointestinal inflammation (Izcue and Powrie, 2012; Palego et al., 2016; Routh et al., 2016) and the fact that serum Trp was depressed in pigs fed the 90 °C and 180 °C SO, we may have expected some changes in urinary lactulose:mannitol ratios. Even though there were no significant differences in urinary lactulose:mannitol ratios, a simple correlation matrix was conducted and not surprisingly, no significant correlations were noted.

In conclusion, thermal processing resulted in slight changes in the U:S ratio and FFA concentrations, but more moderate increases in several lipid peroxidation products. The combination of changes in the formation of lipid peroxidation products was found to be greatest in the SO thermally processed at 90 °C for 72 h in comparison to other SO treatment groups. This resulted in reduced ADG, energy and EE digestibility, and N retention in pigs fed the 90 °C SO, but intestinal permeability as measured by urinary lactulose to mannitol ratio was not affected. Other thermal processing temperatures, 45 °C for 288 h and 180 °C for 6 h, had minimal effects. While not a cause and effect relationship, correlations noted between the various lipid peroxidation products measured suggest that several key lipid quality indices that may need to be measured and evaluated in future research relative to their impact on pig performance and energy, nitrogen, and lipid digestibility include PV, AnV or TPC, OFA, and hexanal, DDE, or HNE.

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