Influence of feeding thermally peroxidized soybean oil on oxidative status in growing pigs

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ABSTRACT: The objectives of this study were to determine whether feeding thermally processed peroxidized soybean oil (SO) induces markers of oxidative stress and alters antioxidant status in pig tissue, blood, and urine. Fifty-six barrows (25.3 ± 3.3 kg initial BW) were randomly assigned to dietary treatments containing 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 constant air infusion rate of 15 liters/minute. Multiple indices of lipid peroxidation were measured in the SO including peroxide value (2.0, 96, 145, and 4.0 mEq/kg for 22.5, 45, 90, and 180 °C processed SO, respectively) and p-anisidine value (1.2, 8.4, 261, and 174 for 22.5, 45, 90, and 180 °C processed SO, respectively); along with a multitude of aldehydes. Pigs were individually housed and fed ad libitum for 49 d which included a 5 d period in metabolism crates for the collection of urine and serum for measures of oxidative stress. On day 49, pigs were euthanized to determine liver weight and analyze liver-based oxidative stress markers. Oxidative stress markers included serum, urinary, and liver thiobarbituric acid reactive substances (TBARS), and urinary F₂-isoprostanes (ISP) as markers of lipid damage; serum and liver

protein carbonyls (PC) as a marker of protein damage; and urinary and liver 8-hydroxy-2'-deoxyguanosine (8-OH-2dG) as a marker of DNA damage. Superoxide dismutase (SOD), and catalase activity (CAT) were measured in liver, glutathione peroxidase activity (GPx) was measured in serum and liver, and ferric reducing antioxidant power (FRAP) was measured in serum and urine as determinants of antioxidant status. Pigs fed 90 °C SO had greater urinary ISP ($P = 0.02$), while pigs fed the 45 °C SO had elevated urinary TBARS ($P = 0.02$) in comparison to other treatment groups. Pigs fed 45 °C and 90 °C SO had increased serum PC concentrations ($P = 0.01$) and pigs fed 90 °C SO had greater ($P = 0.01$) liver concentration of 8-OH-2dG compared to pigs fed the other SO treatments. Furthermore, pigs fed 90 °C SO had reduced serum GPx activity in comparison to pigs fed fresh SO ($P = 0.01$). In addition, pigs fed 180 °C SO had increased liver CAT activity ($P = 0.01$). Liver GPx and SOD or serum and urinary FRAP were not affected by dietary treatment. These results indicate that dietary peroxidized soybean oil induced oxidative stress by increasing serum PC while diminishing serum GPx, increasing urinary ISP and TBARS, and increasing 8-OH-2dG and CAT in liver.

Key words: growing pigs, oxidative stress, peroxidized soybean oil

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INTRODUCTION

Soybean oil (SO) is an energy source added to swine diets and is rich in linoleic acid, making it highly susceptible to lipid peroxidation during thermal processing (Holman, 1954). Lipid peroxidation is a free radical chain reaction that progressively forms and degrades peroxides, acids, and aldehydes, while diminishing antioxidants (Gonzalez-Muñoz et al., 1998). Research suggests that consumption of lipid peroxidation products induces oxidative stress in swine and poultry (Tavárez et al., 2011; Lu et al., 2014); these experiments, however, have focused on individual markers of oxidative stress, namely thiobarbituric acid reactive substances (TBARS). Feeding peroxidized oils to pigs has also been shown to negatively impact growth performance (Boler et al., 2012; Rosero et al., 2015).

Endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) protect the body from reactive oxygen species and free radicals; however, oxidative stress can result when free radical production overwhelms antioxidant compounds leading to modification of lipids, proteins, and DNA. Thiobarbituric acid reactive substances and F₂-isoprostane (ISP) concentrations are often measured as markers of lipid damage due to lipid peroxidation (Montuschi et al., 2004). Free radicals also bind to proteins altering protein function and can be accessed via protein carbonyl (PC) content (Dalle-Donne et al., 2003). Lastly, free radicals target DNA, which upon oxidation a hydroxyl group is added to a guanosine molecule to produce 8-hydroxy-2'-deoxyguanosine (8-OH-2dG; Kalyanaraman, 2013). Oxidative stress has also been shown to affect immune (Turek et al., 2003) and brain function (Adibhatla and Hatcher, 2010). Because oxidative stress is not a singular metabolic event, the objectives of this study were to determine the impacts of feeding thermally processed SO on multiple measures of oxidative stress and antioxidant status in pig liver, serum, and urine.

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.

Experimental Design

Methodologies regarding lipid peroxidation of SO, formulation of diets, and animal management

have been described previously (Lindblom et al., 2018). In brief, 56 barrows (initial average BW 25.3 ± 3.31 kg) were randomly assigned to one of four dietary treatments that included 10% fresh SO or 10% SO thermally processed at either: 1) 45 °C for 288 h, 2) 90 °C for 72 h, or 3) 180 °C for 6 h. A single batch of refined SO was selected to minimize initial lipid composition, with the different thermal processing temperatures and time selected to generate different lipid peroxidation products and concentrations. Lipid peroxidation products and quality of SO were assessed and reported previously (Lindblom et al., 2018). All diets 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). Each pig was individually penned with ad libitum access to feed and water for a 49-d experimental period. Pigs were moved in groups of 20, 20, and 16 to metabolism crates on days 21, 25, and 29, respectively, for a 5-d period for collections of urine and serum for oxidative stress analyses. During this period, pigs were fed their treatment diet equivalent to 4% of their average BW twice daily (2% at 0700 h and 2% at 1700 h) with constant access to water. On day 5 of the metabolism period, urine was collected for 5 h into plastic containers containing 5 ml chlorhexidine to eliminate microbial growth. Following the collection, urine volume was quantified and stored at -80 °C until subsequent analysis of markers of oxidative stress. Immediately following this urine collection (5 h after morning feeding), approximately 8 ml of blood was obtained via jugular venipuncture using a 10-ml vacuum serum tube, which was then centrifuged at $2,500 \times g$ for 15 min at 4 °C and serum was harvested. Serum samples were then aliquoted and immediately frozen at -80 °C and stored until subsequent oxidative stress analyses. Thereafter, pigs were removed from metabolism crates and returned to their assigned individual pen where they remained for the duration of the experiment. On day 49, all pigs (final average BW 70.80 ± 5.73 kg) were euthanized by barbiturate overdose followed by exsanguination. Livers were excised, weighed, and a sample taken and snap frozen in liquid N, transported on dry ice, and stored at -80 °C until subsequent analyses of oxidative stress markers. Based on pig performance, brain tissue samples (~1 g) were collected from regions including the hippocampus, striatum, and prefrontal cortex in the right hemisphere of each pig fed the 22.5 °C and 90 °C SO treatments, and snap frozen in liquid N, transported on dry ice, and

stored at -80°C until subsequent analyses of catecholamine concentrations.

Oxidative Stress Markers

Multiple oxidative stress markers were measured in urine, serum, and liver homogenates. Analyses were measured using commercially available assay kits and are reported in Table 1 along with the dilution factors used. Assay kits were purchased from Cayman Chemical Company (Ann Arbor, MI) and were performed according to the recommendations from the manufacturer, with assays run in triplicate in 96-well microplates and intra-assay CV of $\leq 5.0\%$. Thiobarbituric acid reactive substances and ISP concentrations were measured as indicators of lipid damage. After thawing, urine, serum, and liver samples were assessed for TBARS where 100 mg of liver tissue was homogenized per mL of RIPA buffer (Cayman Chemical Co., Ann Arbor, MI; #10010263), centrifuged at $1,600 \times g$ for 10 min at 4°C then the supernatant was used to run the assay. No dilutions were necessary and data were reported as μM of MDA. F_2 -isoprostanes were only measured in urine with urine diluted 1:10 in sample buffer provided in the assay kit prior to performing the assay. Urinary ISP was reported on a recovery basis of ISP in the total urine collected so data is reported as total pg ISP excreted. Urine TBARS were normalized to the volume of urine excreted by multiplying the concentration of TBARS from the assay by the quantity of urine collected during the collection period. Because proteins are not excreted in urine, protein damage was measured via PC concentration in serum and liver samples only. Briefly, 200 mg of liver tissue was homogenized per milliliter in 50 mM phosphate buffer containing 1 mM EDTA and centrifuged at $10,000 \times g$ for 15 min at 4°C then supernatant was assayed to determine

PC concentration. Both serum and liver PC concentration data are expressed as nmol/mL.

The nucleic acid guanine is the base that is most prone to DNA oxidative damage and 8-OH-2dG, the form of oxidized guanine, is most commonly studied (Wu et al., 2004; Mateos and Bravo, 2007). Urine and liver 8-OH-2dG were assessed in which DNA was extracted from 25 mg of liver using ZR Genomic DNA-Tissue MiniPrep (Zymo Research, Irvine, CA). Following extraction, DNA yields were determined using Gen5 software on Cytation 5 Imaging Reader (BioTek, Winooski, VT) and DNA yields ranged from 20–40 μg DNA. The DNA was then digested by nuclease P1 (Sigma-Aldrich, St. Louis, MO) to convert double-stranded DNA to single-stranded DNA, then 1 unit of alkaline phosphatase (Roche Diagnostics, Mannheim, Germany) was added per 100 μg of DNA to convert nucleotides to nucleosides. The supernatant was assayed on the Cayman Chemical assay kit to determine the 8-OH-2dG of liver and no dilution was required; however, urine was diluted 1:750 in sample buffer prior to assessment. Both urine and liver 8-OH-2dG concentrations were expressed in pg/mL.

Antioxidant enzymes in the body act to detoxify oxidatively damaged molecules, with the most common enzymatic antioxidants measured to evaluate oxidative status being SOD, GPx, and CAT. Superoxide dismutase and CAT were measured in liver homogenates and GPx was measured in serum and liver. For SOD activity, 100 mg of liver tissue was homogenized in 1 mL of 20 mM HEPES buffer containing 1 mM EGTA, 210 mM mannitol, and 70 mM sucrose, centrifuged at $1,500 \times g$ for 5 min at 4°C . The supernatant was diluted 1:10 in sample buffer before being assayed and expressed as U/mL. For CAT activity, 100 mg of liver tissue was homogenized in 1 mL of 50 mM potassium phosphate containing 1 mM EDTA and centrifuged at $10,000 \times g$ for 15 min at 4°C and the supernatant

Table 1. Assay kits performed to determine oxidative status in serum, urine, and liver¹

	Catalog	Wavelength	Serum	Urine	Liver
Assay Kit ²	Number	nm	Dilution factor	Dilution factor	Dilution factor
TBARS	700870	535	ND	ND	ND
ISP	516351	410	NA	1:10	NA
PC	10005020	370	ND	NA	ND
8-OH-2dG	589320	410	NA	1:750	ND
SOD	706002	450	NA	NA	1:10
GPx	703102	340	1:20	NA	1:20
CAT	707002	540	NA	NA	1:10,000

¹All assay kits were purchased from Cayman Chemical Company (Ann Arbor, MI).

²TBARS = thiobarbituric acid reactive substances; ISP = F_2 -isoprostanes; PC = protein carbonyls; 8-OH-2dG = 8-hydroxy-2'-deoxyguanosine; SOD = superoxide dismutase; GPx = glutathione peroxidase activity; CAT = catalase activity; ND = no dilution; NA = not applicable.

was diluted 1:10,000 in sample buffer and assayed to determine CAT activity ($\text{nmol min}^{-1}\text{ml}^{-1}$). Glutathione peroxidase activity ($\text{nmol min}^{-1}\text{ml}^{-1}$) was measured in liver and serum where 100 mg of liver tissue was homogenized in 1 ml of 50 mM Tris-HCl containing 5 mM EDTA and 1 mM DTT, centrifuged at $10,000 \times g$ for 15 min at 4 °C. Serum and liver homogenates were diluted 1:20 in sample buffer prior to being assayed. To further assess the effects of peroxidized lipids on antioxidant status in the pigs, total antioxidant capacity was measured in urine and serum via ferric reducing antioxidant power (FRAP) assay. Briefly, the FRAP assay colorimetrically measures the reduction of the ferric ion (Fe^{+3}) to ferrous ion (Fe^{+2}) by the reaction of ferrous-tripyridyltriazine complex in relation to antioxidant based ascorbic acid standards (Benzie and Strain, 1996; Gabler et al., 2005). Urine samples required a 1:10 dilution before being assayed, but serum was run without diluting, both expressed as μM (FRAP value).

Neutrophil Isolation and Neutrophil Extracellular Trap (NET) Assay

Whole blood was collected by venipuncture into vacutainer collection tubes containing acid citrate dextrose, (ACD, Fisher Scientific, Hampton, NH). The ACD anticoagulated blood was subsequently mixed with additional ACD at 1 ml ACD per 5 ml of blood. Blood was further processed with modifications to the protocol as previously described (Heit et al., 2006). Briefly, dextran sedimentation (equal v/v mixture of ACD/blood to 6% dextran in 0.9% NaCl solution) was used to sediment erythrocytes. After sedimentation, supernatant with leukocytes was collected and centrifuged at $300 \times g$ for 12 min. This was followed by two rounds of lysis (10.6 mM Na_2HPO_4 , 2.7 mM NaH_2PO_4) and restoration (10.6 mM Na_2HPO_4 , 2.7 mM NaH_2PO_4 , 462 mM NaCl) to remove remaining erythrocytes. Cells were resuspended in phosphate buffered saline (PBS), overlaid on Histopaque-1077 (Millipore Sigma, St. Louis, MO), and centrifuged at $450 \times g$ for 30 min. Mononuclear cells at the buffy coat were discarded and the cell pellet containing neutrophils was resuspended in PBS and centrifuged at $450 \times g$ for 5 min to pellet neutrophils. Cells were resuspended in 2 ml Hanks' Balanced Salt Solution (HBSS) without calcium and phenol red (ThermoFisher Scientific, Waltham, MA). Viable neutrophils were enumerated using Muse Count and Viability Assay and Instrument according to manufacturer's recommendations (EMD Millipore, Billerica, MA).

Isolated neutrophils were used in a NET assay as previously described (Loving et al., 2013). Briefly, neutrophils were resuspended at 5×10^6 per ml in RPMI 1640 (ThermoFisher Scientific) supplemented with 2% heat-inactivated fetal bovine serum (FBS; Omega Scientific, Tarzana, CA). The resuspended cells (0.1 ml) were seeded in duplicate in nontissue culture plates (Corning, Corning, NY) for each animal per stimulant. Neutrophils were either stimulated with 0.1 ml of RPMI alone (no stimulation), phorbol myristate acid (PMA, 10 ng/ml; Millipore Sigma) and ionomycin (1 μM ; Millipore Sigma), opsonized zymosan (Millipore Sigma) or lipopolysaccharide (LPS, 1 $\mu\text{g/ml}$; Sigma Aldrich, St. Louis, MO). Zymosan was opsonized in-house as previously described (Roth and Kaeberle, 1981). After seeding cells and adding respective treatments, plates were centrifuged at $500 \times g$ for 5 min and incubated at 37 °C in 5% CO_2 for 30 min. Cells were then washed, and incubated with 50 $\mu\text{l/well}$ of Sytox Orange (5 $\mu\text{g/ml}$; ThermoFisher Scientific). After 10 min of incubation, cells were washed with PBS. Fluorescence was detected using excitation 530 nm, emission 570 nm, and cutoff 550 nm (Synergy HT Microplate Reader, BioTek, Winooski, VT). The stimulation index was determined by dividing the average fluorescence of stimulated cells by the average fluorescence of the nonstimulated cells for each animal and each stimulus.

Brain Catecholamine Concentrations

Tissue samples were collected from brain regions including the hippocampus, striatum, and prefrontal cortex in the right hemisphere of each pig. Absolute quantification, ng/mg tissue, of the following catecholamines were conducted in duplicate using liquid chromatography methods: dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine, epinephrine, 5-hydroxytryptamine (serotonin) and 5-hydroxyindoleacetic acid (HIAA). In brief, high-performance liquid chromatography (HPLC) methods (Application Note 228; Dionex, 2009) were used for rapid and sensitive separation and quantification using electrochemical detection. Method B of this procedure simultaneously quantified all the aforementioned biochemicals in brain samples, and extraction procedures were optimized for extraction of catecholamines from pig brain tissue samples. Catecholamine concentrations that could not be quantified in four or more subjects for both dietary groups were considered to be below

the detectable limit, and thus, data for these outcomes were not reported.

Statistical Analysis

Data were analyzed as a completely randomized design with individual pig as the experimental unit, using Proc MIXED 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 products with oxidative stress markers and relationships between growth performance and digestibility measures with oxidative stress markers were evaluated by simple linear correlation (Pearson correlation coefficients) analysis. Brain catecholamine concentrations were subjected to a simple one-way ANOVA to statistically differentiate the effects of two dietary treatments that were provided to pigs. For all data, differences were considered significant at $P \leq 0.05$, whereas values of $P \leq 0.10$ were considered statistical trends.

RESULTS AND DISCUSSION

Lipid Peroxidation and Pig Performance

The composition of experimental SO have been described in detail previously (Lindblom et al., 2018) and highlighted in Table 2. In brief, thermal processing decreased the unsaturated:saturated fatty acid ratio (U:S) of the dietary FA

Table 2. Compositional and peroxidation analysis of soybean oil (adapted from Lindblom et al., 2018)

Heating temperature, °C	22.5	45	90	180
Time heated, hours ¹	0	288	72	6
U:S ²	5.35	5.27	4.64	5.02
Free fatty acids, %	0.04	0.07	0.35	0.14
Peroxide value, mEq/kg	2.0	95.6	145.3	4.0
p-Anisidine value ³	1.19	8.38	261	174
TBARS ^{2,3}	0.10	0.14	0.14	0.09
Hexanal, mg/kg	2.97	2.71	21.20	16.84
Acrolein, mg/kg	3.88	3.31	15.82	45.39
2,4-decadienal, mg/kg	2.11	5.05	547.62	323.57
2-hydroxynonenal, mg/kg	0.05	1.05	39.46	25.71
Total tocopherols, mg/kg	772	620	405	609

¹Thermally processed oils had constant air flow rate at 15 liters/minute.

²U:S, unsaturated to saturated fatty acid ratio; TBARS, thiobarbituric acid reactive substances.

³There are no units for p-anisidine value or TBARS value.

with a corresponding increase in the production of lipid peroxidation products including peroxide value (PV), p-anisidine value (AnV), and multiple aldehydes, including 2,4-decadienal (DDE), and 4-hydroxynonenal (HNE). The production of these lipid peroxidation products was generally greatest in the 90 °C processed SO which was further confirmed with the reduced concentration of total tocopherols. 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. The effects of SO peroxidation on pig performance, digestibility, and intestinal integrity were previously reported (Lindblom et al., 2018), and in general, pigs fed the 90 °C SO had reduced ADG and N retention, and reduced energy and ether extract digestibility.

In the present study, dietary treatment impacted liver weight as a percentage of BW (Fig. 1). Liver weights as a percentage of BW were greatest in pigs fed the 90 °C processed SO ($P = 0.01$) followed by pigs fed the 180 °C SO, 45 °C SO, and 22.5 °C with values of 2.88%, 2.49%, 2.41%, and 2.31%, respectively. This is in agreement with others who reported an increase in liver weight as a percentage of BW when feeding various peroxidized lipids (Eder, 1999; Anjum et al., 2004; Liu et al., 2014a; Lu et al., 2014). The increase in liver weight relative to BW may result from the increase in consumption of lipid peroxidation products including peroxides,

Figure 1. Effect of thermally peroxidized soybean oil on liver weight as a percentage of BW in growing pigs. 22.5 = fresh soybean oil as 22.5 °C; 45 = soybean oil heated for 288 h at 45 °C; 90 = soybean oil heated for 72 h at 90 °C; 180 = soybean oil heated for 6 h at 180 °C. All processed soybean oil had constant compressed air flow at a rate of 15 liters/minute. Average final BW was 70.80 ± 5.73 kg ($P = 0.11$). Peroxidation effect $P = 0.01$, with superscripts reflecting peroxidized soybean oil treatment differences at $P \leq 0.05$.

acids, and aldehydes (Wang et al., 1997). Because a function of the liver is to detoxify pro-oxidants, an increase in consumption of lipid peroxidation products would result in hypertrophy of hepatocytes and therefore increased liver weight.

Oxidative Status in Serum, Urine, and Liver

Pro-oxidants target lipids, proteins, and DNA, and when the antioxidant system reaches capacity and can no longer maintain redox balance, oxidative stress occurs. Previous studies have shown that consumption of peroxidized oils induces oxidative stress in blood and tissue samples primarily measured by TBARS (Ringseis et al., 2007; Boler et al., 2012; Liu et al., 2014c; Rosero et al., 2015). Consumption of lipid peroxidation products can also result in the accumulation of aldehydes in the gastric lumen which are then absorbed through the small intestine where they are metabolized in the liver (Kanazawa and Ashida, 1998). This is supported by Cardoso et al. (2013) who reported that high fat diets fed to mice enhanced reactive oxygen species release from liver mitochondria. Considering the above literature and because a main function of the liver is detoxification of pro-oxidants, we chose

to measure oxidative balance in liver tissue in addition to serum and urine (Table 3).

Consumption of peroxidized oils has been shown to increase TBARS in bodily fluids in livestock (Tavárez et al., 2011; Boler et al., 2012; Liu et al., 2014c; Lu et al., 2014). In the current experiment, no differences were noted for serum TBARS ($P = 0.51$) among pigs fed the variable thermally processed SO. In contrast, others (Boler et al., 2012; Liu et al., 2014c; Lu et al., 2014) have shown that feeding peroxidized lipids increases plasma TBARS concentration. Pigs fed the 45 °C processed SO treatments tended ($P = 0.08$) to have elevated liver TBARS concentration compared to pigs fed the 22.5 °C SO, with intermediate concentrations for pigs fed the 90 °C SO and 180 °C SO treatments. These data are in agreement with Lu et al. (2014) who observed a similar increase in liver TBARS in pigs fed peroxidized SO for 55 d. Likewise, urinary TBARS was greatest in pigs fed the 45 °C SO and intermediate in pigs fed the 90 °C SO compared to pigs fed the other SO treatment groups ($P = 0.02$). Because we observed an increase in liver and urinary TBARS and a lack of an effect in the serum, our results suggest that the malondialdehyde (which is measured in the TBARS assay) is being rapidly

Table 3. Oxidative status in serum, urine, and liver of pigs fed soybean oil with differing peroxidation levels

Parameter ²	Processed soybean oil ¹				Statistics	
	22.5	45	90	180	SEM	P value
Serum ³						
TBARS, µM/ml	8.3	7.3	7.3	7.5	0.6	0.51
PC, nmol/ml	19.5 ^b	26.3 ^a	28.2 ^a	21.4 ^b	1.5	0.01
GPx, nmolmin ⁻¹ ml ⁻¹	1,954 ^a	1,805 ^{ab}	1,430 ^c	1,529 ^{bc}	116	0.01
FRAP, µM/ml	188.3	198.1	193.9	205.6	13.6	0.83
Urine ⁴						
TBARS, µM	9.0 ^c	18.1 ^a	11.1 ^b	8.6 ^c	2.31	0.02
ISP, pg	5,150 ^b	3,848 ^b	17,812 ^a	4,947 ^b	3,646	0.02
8-OH-2dG, µg	43	67	54	37	10	0.15
FRAP, µM	1,080	1,588	1,378	1,316	210	0.41
Liver ⁵						
TBARS, µM	99 ^y	127 ^x	116 ^{xy}	115 ^{xy}	8	0.08
PC, nmol	286	274	277	259	20	0.82
8-OH-2dG, pg	4,170 ^b	5,810 ^b	10,269 ^a	6,536 ^b	1,068	0.01
SOD, U	3,255	2,556	3,361	2,971	291	0.22
GPx, µmol min ⁻¹	46	50	47	47	4	0.84
CAT, µmol min ⁻¹	124 ^b	113 ^b	99 ^b	193 ^a	17	0.01

¹Processing temperatures, °C: 22.5, fresh oil; 45, SO heated for 288 h at 45 °C; 90, SO heated for 72 h at 90 °C; 180, SO heated for 6 h at 180 °C. all processed oil treatments were heated with constant compressed air flow rate at 15 liters/minute. Superscripts reflect peroxidized soybean oil treatment differences (abc, $P \leq 0.05$; xy, $P \leq 0.10$).

²TBARS = thiobarbituric acid reactive substances; PC = protein carbonyls; GPx = glutathione peroxidase activity; ISP = F₂-isoprostanes; 8-OH-2dG = 8-hydroxy-2'-deoxyguanosine; SOD = superoxide dismutase; CAT = catalase activity.

³Serum obtained after a 17 h fast.

⁴Urine collected and quantitated for 5 h following a 12 h fast.

⁵Liver obtained on d 49 from pigs in a fed state.

absorbed and metabolized in the liver and excreted out in urine without affecting circulating levels of TBARS. While the measurement of TBARS is the most common measurement of lipid damage due to lipid peroxidation, this assay detects aldehydes that are not specific to lipid peroxidation (namely 2-alkenals and 2,4-alkedienals), thereby often resulting in overestimations of lipid peroxidation (Halliwell and Chirico, 1993; Monaghan et al., 2009). For this reason, we also measured urine ISP concentrations which measures specific products produced in response to lipid peroxidation and because ISP is stable and concentrated in urine (Montuschi et al., 2004) and is a noninvasive and repeatable assay. In the current experiment, urinary ISP was nearly four times greater in pigs fed the 90 °C SO in comparison to the other treatments ($P = 0.02$). As reported previously, the 90 °C processed SO had the greatest concentration of lipid peroxidation products including PV, AnV, DDE, and HNE (Table 2); because ISP is considered a specific measure of damage due to lipid peroxidation, this result was expected. While we have no livestock data from which to compare our data to, in human research, urinary ISP concentrations have been shown to be elevated under smoking-induced oxidative stress (Obata et al., 2000). In the current experiment, the combination of increased TBARS and ISP in the urine; and TBARS in the liver indicates that lipid damage occurred in response to the consumption of lipid peroxidation products.

Proteins and amino acids are another major target of pro-oxidants of which carbonyls are a by-product of oxidatively damaged proteins (Beal, 2002). In the current study, serum from pigs fed the 45 °C SO and 90 °C SO exhibited elevated PC ($P = 0.01$) compared to pigs fed the fresh SO (Table 3). This is in agreement with Lu et al. (2014) who also reported an increase in plasma PC in pigs fed peroxidized SO after 55 d. In the current experiment, no differences in liver PC values were detected ($P = 0.81$) which is in contrast to Lu et al. (2014) who observed an increase in liver PC in pigs fed peroxidized SO. This was unexpected because pigs were fed for a similar duration and with a peroxidized SO with a similar PV (180 mEq/kg oil compared to 145.3 mEq/kg oil in the current experiment), so we would have expected a similar outcome because lipid peroxidation has been shown to stimulate protein oxidation (Fellenberg and Speisky, 2006).

DNA is especially sensitive to oxidative damage of which, guanine is the most readily oxidized nucleic acid (Wu et al., 2004; Mateos and Bravo, 2007).

Therefore, we chose to measure 8-OH-2dG in the urine and liver. During DNA repair, products such as 8-OH-2dG are excreted in the urine, and because 8-OH-2dG is water soluble, is concentrated and stable to measure in urine (Wu et al., 2004). Feeding pigs the thermally processed SO treatments did not affect urinary excretion of 8-OH-2dG ($P = 0.15$). In contrast, liver 8-OH-2dG was affected by dietary treatments, where it was observed that liver concentration of 8-OH-2dG was greatest in pigs fed the 90 °C SO ($P = 0.01$) in comparison with other treatment groups. This may be explained by the decreased CAT activity in pigs fed the 90 °C processed SO (Table 3) which shifts free radical binding from the antioxidant defense system to DNA, possibly causing oxidative damage. We cannot explain why we observed treatment differences in liver 8-OH-2dG but not urine 8-OH-2dG, and to our knowledge, we have no literature from which to compare our data to.

Peroxidized Soybean Oil and Antioxidant Machinery

Antioxidant enzyme activities including SOD, GPx, and CAT were measured to determine antioxidant status in the liver and are presented in Table 3. The biochemical reactions of SOD, GPx, and CAT are well known (Kalyanaraman, 2013). In the current experiment, liver SOD activity was not affected by dietary treatment ($P = 0.22$). These results are similar to data in broilers where oxidative status was measured in response to heat stress where no differences were reported in liver SOD activity between heat stressed and thermal neutral broilers (Lin et al., 2006). Simplistically speaking, the function of GPx and CAT is to metabolize H_2O_2 to water, but if this cannot be done effectively, H_2O_2 is rapidly converted to the OH free radical, which is reactive with lipids, proteins, and DNA (Shu et al., 1979). Serum from pigs fed the 90 °C SO had reduced GPx activity ($P = 0.01$) in comparison to pigs fed fresh SO and the 45 °C processed SO, with the GPx activity of pigs fed the 180 °C SO being intermediate. This is similar to results reported by others who also observed a decrease in GPx activity in plasma of pigs fed peroxidized oils (Yuan et al., 2007; Boler et al., 2012).

Liver GPx was not affected by dietary treatment ($P = 0.83$) which is supported by Boler et al. (2012) who observed no differences in liver GPx between pigs fed peroxidized corn oil in comparison to pigs fed fresh corn oil. While liver SOD and GPx were not affected by dietary treatment in

the current experiment, liver CAT was increased in pigs fed the 180 °C SO diet compared to the other SO treatments ($P = 0.01$). The reduction in activity of GPx in serum and CAT in liver could be explained by the lack of pro-oxidant substrates for these enzymes. Because the activity of antioxidant enzymes were reduced, this could be driving the increase in serum PC and the increases in 8-OH-2dG and TBARS in the liver in addition to the increased liver weight in pigs fed the 90 °C processed SO.

Lastly, antioxidant capacity was analyzed by assessing FRAP activity in serum and urine. [Gabler et al. \(2005\)](#) demonstrated that feeding antioxidant compounds improves blood FRAP activity. In the current study, serum and urinary excretion of FRAP were not affected by dietary treatment ($P > 0.12$). This is in contrast to [Lin et al. \(2006\)](#) who observed a decrease in plasma FRAP in broilers that were heat stressed to induce disruption of oxidative balance.

Correlations between Lipid Peroxidation Products and Oxidative Stress Markers

Because consumption of lipid peroxidation products in SO was clearly shown to affect markers of oxidative stress, it was determined that

conducting a correlation analysis between lipid peroxidation products and markers of oxidative stress was worthwhile. Even though a correlation analysis does not represent a cause and effect relationship, the lack of comprehensive measures of lipid peroxidation products and oxidative stress measures in the literature and in the current experiment led us to conduct this analysis to provide guidance on research involving lipid peroxidation status and the effects on animal production and oxidative status in the future. However, because we considered the U:S, FFA, and TBARS differences among the SO treatments to be insignificant ([Table 2](#)) we elected not to report or discuss these relationships. Correlations between SO lipid composition and peroxidation products with measures of oxidative status are reported in [Table 4](#). There were no significant correlations noted between the SO quality ([Table 2](#)) with serum TBARS, serum FRAP, urine FRAP, liver PC, liver SOD, and liver GPx ([Table 3](#)) so they are not reported in [Table 4](#). The observation that urinary and liver TBARS exhibited very few correlations to the lipid composition and peroxidation measures analyzed in the SO is in contrast to [Shurson et al. \(2015\)](#) who reported that TBARS is one of the most frequently measured compounds for both lipid

Table 4. Pearson correlation coefficients among soybean oil composition and lipid peroxidation products with measures of oxidative status¹

Criterion ²	Soybean oil composition and peroxidation measures ²											
	PV	AnV	OFA	TPC	PTAG	OSI	HEX	ACR	DDE	HNE	Ratio	TOC
Serum PC	0.57 (0.01)	0.29 (0.04)	0.57 (0.01)	0.45 (0.01)	0.37 (0.01)	-0.46 (0.01)	0.25 (0.08)	-	0.31 (0.03)	0.30 (0.04)	-	-0.50 (0.01)
Serum GPx	-0.27 (0.06)	-0.47 (0.01)	-0.28 (0.05)	-0.44 (0.01)	-0.43 (0.01)	0.43 (0.01)	-0.47 (0.01)	-0.31 (0.03)	-0.47 (0.01)	-0.47 (0.01)	-0.47 (0.01)	0.44 (0.01)
Urinary TBARS	0.25 (0.09)	-	0.24 (0.10)	-	-	-	-	-	-	-	-	-
Urinary ISP	0.36 (0.02)	0.38 (0.01)	0.36 (0.02)	0.43 (0.01)	0.44 (0.01)	-	0.35 (0.02)	-	0.39 (0.01)	0.38 (0.01)	0.33 (0.03)	-0.39 (0.01)
Urinary 8-OH-2dG	0.24 (0.10)	-	0.24 (0.10)	-	-	-	-	-	-	-	-	-
Liver TBARS	-	-	0.24 (0.10)	-	-	-0.31 (0.03)	-	-	-	-	-	-
Liver 8-OH-2dG	0.41 (0.01)	0.47 (0.01)	0.42 (0.01)	0.52 (0.01)	0.50 (0.01)	-0.41 (0.01)	0.45 (0.01)	-	0.48 (0.01)	0.47 (0.01)	0.42 (0.01)	-0.51 (0.01)
Liver CAT	-0.39 (0.01)	-	-0.38 (0.01)	-	-	-	-	0.49 (0.01)	-	-	-	-

¹Top 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 $P \leq 0.10$. There were no correlations observed between SO treatment and serum TBARS, serum FRAP, urinary FRAP (ferric reducing ability of serum), liver PC, liver SOD, and liver GPx so they were removed from the table.

²PV = peroxide value; AnV = p-anisidine value; TBARS = thiobarbituric acid reactive substances; OFA = oxidized fatty acids; TPC = total polar compounds; PTAG = polymerized triacylglycerides; OSI = oxygen stability index; HEX = hexanal; DDE = 2,4-decadienal; HNE = 4-hydroxynonenal; Ratio = ratio of aldehydes as described by Wang et al., 2016; TOC = total tocopherols; PC = protein carbonyls; GPx = glutathione peroxidase activity; ISP = F₂-isoprostanes; 8-OH-2dG = 8-hydroxy-2'-deoxyguanosine; CAT = catalase activity.

quality assessment and is a commonly reported indicator of an animals' response to oxidative stress. However, given that TBARS is a non-descript measure of lipid peroxidation damage (Yin et al., 2011), this was not completely unexpected. Urinary 8-OH-2dG and liver CAT also exhibited few correlations to lipid peroxidation measures, which for urinary 8-OH-2dG was surprising given that this product is concentrated in the urine (Wu et al., 2004). The remaining oxidative stress markers (e.g., serum PC, serum GPx, urinary ISP, and liver 8-OH-2dG) had the greatest relationships to lipid composition and peroxidation measures. Of these lipid composition and peroxidation measures, PV, TBARS, and oxidized fatty acids (OFA) had the most relationships to peroxidation status, 7, 7, and 8 correlations, respectively, followed by all other lipid peroxidation measures, 4 correlations each. Acrolein and the aldehyde ratio exhibited the fewest correlations, 2 and 3 correlations, respectively, Table 4. As to which tissue is most representative of measuring the overall oxidative balance, is not clear from the experiment herein. Complicating matters is that some oxidative stress metabolites could not be measured in a specific tissue in the current experiment. For example, because protein excretion is extremely low in urine, PC could not be analyzed and the kit we utilized was not validated for measuring 8-OH-2dG in serum. We also elected to conduct correlations between measures of oxidative stress and pig performance and digestibility data reported by Lindblom et al. (2018). As shown

in Table 5, there were no consistent correlations except for liver 8-OHdG which had the greatest number of correlations to animal performance and digestibility components measured. We attribute this mainly due to the small effect that peroxidation status, particularly the 45 °C and 180 °C treatments, had on measures of either pig performance and digestibility or oxidative stress.

Neutrophil Extracellular Traps

Oxygen-derived free radicals (e.g., hypochlorous acid, hydroxyl radical, and nitric oxide) are potent oxidant bactericidal agents in both natural and acquired immunity (Knight, 2000). Immune cells are sensitive to oxidative stress because the high percentage of PUFA in their plasma membranes, where it has been shown that feeding peroxidized lipids reduces oxidative burst capacity in canines fed peroxidized poultry fat (Turek et al., 2003). In the current study, we were also interested if pigs fed peroxidized SO may also have exhibited altered immune capacity. Neutrophils, the most abundant group of leukocytes in the peripheral blood, engulf bacteria and kill them intracellularly when their antimicrobial granules fuse with the phagosome. In addition, neutrophils can immobilize and kill microorganisms by releasing granule proteins and chromatin that form extracellular fibers that bind pathogens, called neutrophil extracellular traps (Brinkmann et al., 2004), where in general a reduced NET value reflects a reduction in immune

Table 5. Pearson correlation coefficients among pig performance and digestibility parameters and measures of oxidative stress in pigs fed soybean oil with different degrees of peroxidation¹

Criterion ²	Urinary		Serum		Liver			
	TBARS	8-OHdG	PC	8-OHdG	PC	GPx	CAT	SOD
ADG	-	-	-0.27 (0.06)	-0.24 (0.09)	-0.24 (0.08)	0.27 (0.06)	-	-
GF	0.33 (0.02)	0.28 (0.06)	-	-	-	-	-0.37 (0.01)	-
DEGE	-	-	-0.25 (0.08)	-0.34 (0.01)	-	-	-	-0.30 (0.03)
ND	-0.25 (0.09)	-0.39 (0.01)	-	-	-	-	-	-
NR	-	-	-	-0.30 (0.04)	-0.24 (0.09)	-	-	-
LM	-	-	-	-0.28 (0.05)	-	-	-	-

¹Top 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* ≤ 0.10. There were no correlations of pig performance and digestibility parameters with urinary ISP, serum TBARS, serum GPx, or liver TBARS. There were no correlations of ADFI or ME as a percent of DE with any oxidative stress parameter.

²TBARS = thiobarbituric acid reactive substances; 8-OHdG = 8-hydroxy-2'-deoxyguanosine; PC = protein carbonyls; GPx = glutathione peroxidase activity; ISP = F₂-isoprostanes; CAT = catalase activity; SOD = superoxide dismutase activity; DEGE = energy digestibility; ND = nitrogen digestibility; NR = nitrogen retention as a percent of nitrogen digested.

Table 6. Neutrophil extracellular trap (NET) assay¹

	Processed soybean oil ²				Statistics	
	22.5	45	90	180	SEM	P value
Simulation index ³	22.5	45	90	180	SEM	P value
PMA/ionomycin	14.6 ^a	13.0 ^{ab}	10.5 ^b	14.4 ^a	1.55	0.05
Opsonized zymosan	3.4	3.6	3.2	4.6	0.48	0.15
LPS	1.27 ^b	1.49 ^a	1.12 ^b	1.13 ^b	0.074	0.01

¹Blood collected while pigs were on feed.

²Processing temperatures, °C: 22.5, fresh oil; 45, SO heated for 288 h at 45 °C; 90, SO heated for 72 h at 90 °C; 180, SO heated for 6 h at 180 °C. All processed oil treatments were heated with constant compressed air flow rate at 15 liters/minute. Superscripts reflect peroxidized soybean oil treatment differences (^{abc} $P \leq 0.05$).

³PMA = phorbol myristate acetate; LPS = lipopolysaccharide. The stimulation index is determined by dividing the average fluorescence of stimulated cells by the average fluorescence of the nonstimulated cells for each animal and each stimulus.

defense capacity. In the current study, pigs fed SO processed at 90 °C had reduced ($P = 0.05$) NET formation after activation compared to pigs fed the fresh SO. In addition, pigs fed the 90 °C and 180 °C processed SO had reduced ($P = 0.01$) NET formation after LPS stimulation (Table 6). These data suggest that the lipid peroxidation products in SO can compromise innate immune defenses, and is supported by the slight increase in oxidative stress noted in pigs fed the peroxidized SO, namely the 90 °C processed SO, Table 3; and the depression in growth noted as well (Lindblom et al., 2018). The mechanism by which dietary peroxidized lipids alters neutrophil function is unknown, but may be related to changes in membrane fluidity as a result of high dietary lipid peroxidation, which has been shown to impact macrophage function (Calder et al., 1990).

Lipid Peroxidation and Brain Catecholamines

The brain is composed of 35% to 65% lipid on a DM basis, with myelin composition being approximately 80% lipid (O'Brien and Sampson, 1965) with differential degrees of unsaturation depending upon the phospholipid measured (Martinez and Mougan, 1998). Because of its high lipid content and high oxygen consumption, the brain is highly susceptible to oxidative damage (Naudi et al., 2012), with many neurodegenerative diseases related to varying degrees of oxidative stress in the brain (Adibhatla and Hatcher, 2010). Because biogenic amines are implicated in a wide range of body functions and behavior (Neuroscience, 2004), we elected to determine if pigs fed the SO processed at 90 °C, pigs that exhibited reduced ADG and G:F (Lindblom et al., 2018), would have had altered brain catecholamine concentrations and that this is a contributing factor to how dietary peroxidized lipids antagonize health and performance of growing pigs. Catecholamines

and associated metabolites in the hippocampus, striatum, and prefrontal cortex are reported in Table 7. The concentration of epinephrine and dopamine were reduced ($P = 0.10$) in the right hippocampus of pigs fed the SO thermally processed at 90 °C compared to control pigs, with no differences noted for other catecholamines in the right hippocampus; and no differences noted for any catecholamines in either the right striatum or right prefrontal cortex due to feeding the SO processed at 90 °C. Consequently, it can be concluded that catecholamines and associated metabolites in the hippocampus, striatum, and prefrontal cortex were not influenced by feeding 10% oxidized SO (90 °C) for 49 d to growing pigs. It is possible that provision of oxidized oils earlier in life might have a greater influence on the developing brain, as these pigs were approximately 63 d of age (25 kg) at the initiation of dietary treatments.

In summary, thermally processing SO at 90 °C for 72 h was a successful model to induce oxidative stress in growing pigs, as measured by several common measures of oxidative stress. In contrast, thermally processing SO at 180 °C for 6 h or thermally processing at 45 °C for 288 h having small effects on measures of oxidative stress compared to fresh SO. These data suggest that markers of oxidative stress in the liver are not well correlated with lipid peroxidation products and suggest that further research should focus on markers of oxidative stress in blood and urine. This experiment was unable to determine a single-specific marker that should be focused on in future research. The data do, however, suggest that several lipid composition and lipid peroxidation products need to be measured, specifically PV and total tocopherols, and suggests that specific aldehydes may also need to be considered. Moreover, serum, urinary, and liver TBARS were poorly correlated with lipid composition and peroxidation measures analyzed in SO; however, other measures of lipid, protein,

Table 7. Catecholamine concentrations in the hippocampus, striatum, and prefrontal cortex of grower pigs fed SO with differing peroxidation levels

Catecholamine ² , ng/mg	Processed soybean oil ¹		Statistics	
	22.5	90	SEM	P value
Hippocampus³				
Norepinephrine	58.53	48.35	8.30	0.39
Epinephrine	13.91	10.40	1.48	0.10
DOPAC	0.26	0.18	0.04	0.21
Dopamine	0.21	0.13	0.03	0.10
HIAA	0.09	0.11	0.01	0.29
Striatum⁴				
Norepinephrine	6.20	4.07	1.32	0.26
Epinephrine	14.45	14.83	0.78	0.73
DOPAC	2.31	2.45	0.27	0.70
Dopamine	7.24	8.84	0.81	0.17
HIAA	0.33	0.35	0.03	0.61
HVA	5.32	6.24	0.79	0.41
Prefrontal cortex⁵				
Norepinephrine	4.65	4.22	2.04	0.88
Epinephrine	13.92	13.03	0.71	0.38
DOPAC	0.18	0.13	0.02	0.11
Dopamine	0.18	0.14	0.03	0.30
HIAA	0.27	0.22	0.03	0.22

¹Processing temperatures, °C: 22.5, fresh oil; 90, SO heated for 72 h at 90 °C with constant compressed air flow rate at 15 Liters/minute.

²DOPAC = 3,4-dihydroxyphenylacetic acid; HVA = homovanillic acid; HIAA = 5-hydroxyindoleacetic acid.

³Homovanillic acid and 5-hydroxytryptamine were quantified in fewer than three subjects for one or both dietary groups and thus, these catecholamines were considered to be below the detectable limit and not presented.

⁴SER was quantified in fewer than three subjects for one or both dietary groups and thus, this catecholamine was considered to be below the detectable limit and not presented.

⁵HVA and SER were quantified in fewer than three subjects for one or both dietary groups and thus, these catecholamines were considered to be below the detectable limit and not presented.

and DNA damage (as measured by urinary ISP, serum PC, and liver 8-OH-2dG, respectively) have the greatest relationship to the lipid composition and peroxidation measures analyzed in SO. Collectively, these data provide clear evidence that feeding peroxidized SO sources modulates markers of oxidative stress in growing pigs. Feeding peroxidized SO was also shown to compromise innate immune defenses as shown by the slight decrease in neutrophil extracellular trap formation in pigs fed the 90 °C processed SO, but did not appear to have any significant impact on brain catecholamine concentrations.

Conflict of interest statement. None declared.

LITERATURE CITED

- Adibhatla, R. M., and J. F. Hatcher. 2010. Lipid oxidation and peroxidation in CNS health and disease: from molecular mechanisms to therapeutic opportunities. *Antioxid. Redox Signal.* 12:126–179.
- Anjum, M. I., I. H. Mirza, A. G. Khan, and A. Azim. 2004. Effect of fresh versus oxidized soybean oil on growth performance, organs weights and meat quality of broiler chicks. *Pakistan Vet. J.* 24:173–178.
- Beal, F. 2002. Serial review: oxidatively modified proteins in aging and disease. *Free Radic. Biol. Med.* 32:797–803.
- Benzie, I. F., and J. J. Strain. 1996. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Anal. Biochem.* 239:70–76.
- Boler, D. D., D. M. Fernández-Dueñas, L. W. Kutzler, J. Zhao, R. J. Harrell, D. R. Campion, F. K. McKeith, J. Killefer, and A. C. Dilger. 2012. Effects of oxidized corn oil and a synthetic antioxidant blend on performance, oxidative status of tissues, and fresh meat quality in finishing barrows. *J. Anim. Sci.* 90:5159–5169.
- Brinkmann, V., U. Reichard, C. Goosmann, B. Fauler, Y. Uhlemann, D. S. Weiss, Y. Weinrauch, and A. Zychlinsky. 2004. Neutrophil extracellular trap kill bacteria. *Science* 303:1532–1535.
- Calder, P. C., J. A. Bond, D. J. Harvey, S. Gordon, and E. A. Newsholme. 1990. Uptake and incorporation of saturated and unsaturated fatty acids into macrophage lipids and their effect upon macrophage adhesion and phagocytosis. *Biochem J.* 1990, 269:807–814.
- Cardoso, A. R., P. A. H. B. Kakimoto, and A. J. Kowaltowski. 2013. Diet-sensitive sources of reactive oxygen species in liver mitochondria: role of very long chain acyl-CoA dehydrogenases. *PLoS One* 8:1–13.
- Dalle-Donne, I., R. Rossi, D. Giustarini, A. Milzani, and R. Colombo. 2003. Protein carbonyl groups as biomarkers of oxidative stress. *Clin. Chim. Acta* 329:23–38.

- Dionex. 2009. Determination of catecholamines in human plasma by liquid chromatography with electrochemical detection. Application Note 228. Sunnyvale (CA): Dionex Corp.
- Eder, K. 1999. The effects of a dietary oxidized oil on lipid metabolism in rats. *Lipids* 34:717–725.
- Fellenberg, M. A., and H. Speisky. 2006. Antioxidants: their effects on broiler oxidative stress and its meat oxidative stability. *Worlds. Poult. Sci. J.* 62:53–70.
- Gabler, N. K., E. Ostrowska, S. J. Sterling, R. B. Jones, B. G. Tatham, D. R. Eagling, M. Jois, and F. R. Dunshea. 2005. Consumption of raw brown onions variably modulate plasma lipid profile and lipoprotein oxidation in pigs fed a high-fat diet. *J. Sci. Food Agric.* 85:154–160.
- Gonzalez-Muñoz, M. J., S. Bastida, and F. J. Sanchez-Muniz. 1998. Short-term in vivo digestibility of triglyceride polymers, dimers, and monomers of thermoxidized palm olein used in deep-frying. *J. Agric. Food Chem.* 46:5188–5193.
- Halliwell, B., and S. Chirico. 1993. Lipid peroxidation: its mechanism, measurement, and significance. *Am. J. Clin. Nutr.* 57:715–725.
- Heit, B., G. Jones, D. Knight, J. M. Antony, M. J. Gill, C. Brown, C. Power, and P. Kubes. 2006. HIV and other lentiviral infections cause defects in neutrophil chemotaxis, recruitment, and cell structure: immunorestorative effects of granulocyte-macrophage colony-stimulating factor. *J. Immunol.* 177:6405–6414.
- Holman, R. 1954. Autoxidation of fats and related substances. *Prog. Chem. Fats Other Lipids* 2:51–98.
- Kalyanaraman, B. 2013. Teaching the basics of redox biology to medical and graduate students: oxidants, antioxidants and disease mechanisms. *Redox Biol.* 1:244–257.
- Kanazawa, K., and H. Ashida. 1998. Dietary hydroperoxides of linoleic acid decompose to aldehydes in stomach before being absorbed into the body. *Biochim. Biophys. Acta - Lipids Lipid Metab.* 1393:349–361.
- Knight, J. A. 2000. Review: free radicals, antioxidants, and the immune system. *Annals Clin. Lab. Sci.* 30:145–158.
- Lin, H., E. Decuypere, and J. Buyse. 2006. Acute heat stress induces oxidative stress in broiler chickens. *Comp. Biochem. Physiol.* 144:11–17.
- Lindblom, S. C., N. K. Gabler, and B. J. Kerr. 2018. Influence of thermally oxidized soybean oil on growth performance, digestibility, and gut integrity in growing pigs. *J. Anim. Sci.* In press.
- Liu, F., J. J. Cottrell, J. B. Furness, L. R. Rivera, F. W. Kelly, U. Wijesiriwardana, R. V. Pustovit, L. J. Fothergill, D. M. Bravo, P. Celi, B. J. Leury, N. K. Gabler, and F. R. Dunshea. 2016. Selenium and Vitamin E together improve intestinal epithelial barrier function and alleviate oxidative stress in heat stressed pigs. *Exp. Physiol.* 7:801–810.
- Liu, P., B. J. Kerr, C. Chen, T. E. Weber, L. J. Johnston, and G. C. Shurson. 2014b. Methods to create thermally oxidized lipids and comparison of analytical procedures to characterize peroxidation. *J. Anim. Sci.* 92:2950–2959.
- Liu, P., B. J. Kerr, T. E. Weber, C. Chen, L. J. Johnston, and G. C. Shurson. 2014c. Influence of thermally oxidized vegetable oils and animal fats on intestinal barrier function and immune variables in young pigs. *J. Anim. Sci.* 92:2971–2979.
- Liu, P., C. Chen, B. J. Kerr, T. E. Weber, L. J. Johnston, and G. C. Shurson. 2014a. Influence of thermally oxidized vegetable oils and animal fats on growth performance, liver gene expression, and liver and serum cholesterol and triglycerides in young pigs. *J. Anim. Sci.* 92:2960–2970.
- Loving, C. L., M. E. Kehrli Jr., S. L. Brockmeier, D. O. Bayles, D. D. Michael, S. N. Schlink, and K. M. Lager. 2013. Porcine granulocyte-colony stimulating factor (G-CSF) delivered via replication-defective adenovirus induces a sustained increase in circulating peripheral blood neutrophils. *Biologicals* 41:368–376.
- Lu, T., A. F. Harper, J. Zhao, M. J. Estienne, and R. A. Dalloul. 2014. Supplementing antioxidants to pigs fed diets high in oxidants: I. Effects on growth performance, liver function, and oxidative status. *J. Anim. Sci.* 92:5455–5463.
- Martinez, M., and I. Mougán. 1998. Fatty acid composition of human brain phospholipids during normal development. *J. Neurochem.* 71:2528–2533.
- Mateos, R., and L. Bravo. 2007. Chromatographic and electrophoretic methods for the analysis of biomarkers of oxidative damage to macromolecules (DNA, lipids, and proteins). *J. Sep. Sci.* 30:175–191.
- Monaghan, P., N. B. Metcalfe, and R. Torres. 2009. Oxidative stress as a mediator of life history trade-offs: Mechanisms, measurements and interpretation. *Ecol. Lett.* 12:75–92.
- Montuschi, P., P. J. Barnes, and L. J. Roberts. 2004. Isoprostanes: markers and mediators of oxidative stress. *FASEB J.* 18:1791–1800.
- Naudi, A., J. Jove, V. Ayala, O. Ramirez, R. Cabre, J. Prat, M. Portero-Otin, I. Ferrer, and R. Pamplona. 2012. Region specific vulnerability to lipid peroxidation in the human central nervous system. In: Catala, A., editor. *London (UK): Lipid peroxidation*. InTech. p. 437–456. doi:10.5772/46022
- Neuroscience. 2004. Neurotransmitters and their receptors, Ch. 6, pp 129–164, In: Purves, D., G. Augustine, D. Fitzpatrick, W. Hall, A. LaMantila, J. McNamara, and M. Williams, editors. *Neuroscience*, 3rd ed. Sunderland (MA): Sinauer Associates, Inc.
- O'Brien, J. S., and E. L. Sampson. 1965. Lipid composition of the normal human brain: gray matter, white matter, and myelin. *J. Lipid Res.* 6:537–544.
- Obata, T., K. Tomaru, T. Nagakura, Y. Izumi, and T. Kawamoto. 2000. Smoking and oxidant stress: assay of isoprostane in human urine by gas chromatography-mass spectrometry. *J. Chromatogr. B Biomed. Sci. Appl.* 746:11–15.
- Ringseis, R., N. Piwek, and K. Eder. 2007. Oxidized fat induces oxidative stress but has no effect on NF- κ B-mediated proinflammatory gene transcription in porcine intestinal epithelial cells. *Inflamm Res* 56:118–125.
- Rosero, D. S., J. Odle, A. J. Moeser, R. D. Boyd, and E. van Heugten. 2015. Peroxidised dietary lipids impair intestinal function and morphology of the small intestine villi of nursery pigs in a dose-dependent manner. *Br. J. Nutr.* 114:1985–1992.
- Roth, J. A., and M. L. Kaeberle. 1981. Evaluation of bovine polymorphonuclear leukocyte function. *Vet. Immunol. Immunopathol.* 2:157–174.
- Shu, H., R. E. Talcott, S. A. Rice, and E. T. Wei. 1979. Lipid peroxidation and paraquat toxicity. *Biochem. Pharmacol.* 28:327–331.
- Shurson, G. C., B. J. Kerr, and A. R. Hanson. 2015. Evaluating the quality of feed fats and oils and their effects on pig growth performance. *J. Anim. Sci. Biotechnol.* 1–6:10.
- Tavárez, M. A., D. D. Boler, K. N. Bess, J. Zhao, F. Yan, A. C. Dilger, F. K. McKeith, and J. Killefer. 2011. Effect

- of antioxidant inclusion and oil quality on broiler performance, meat quality, and lipid oxidation. *Poult. Sci.* 90:922–930.
- Turek, J. J., B. W. Watkins, I. A. Schoenlein, D. G. D. Allen, M. G. Hayek, and C. G. Aldrich. 2003. Oxidized lipid depresses canine growth, immune function, and bone formation. *J. Nutr. Biochem.* 14:24–31.
- Wang, S. Y., W. Bottje, P. Maynard, J. Dibner, and W. Shermer. 1997. Effect of Santoquin and oxidized fat on liver and intestinal glutathione in broilers. *Poult Sci* 76:961–967.
- Wu, L. L., C. C. Chiou, P. Y. Chang, and J. T. Wu. 2004. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetes. *Clin. Chim. Acta.* 339:1–9.
- Yin, H., L. Xu, and N. A. Porter. 2011. Free radical lipid peroxidation: mechanisms and analysis. *Chem. Rev.* 111:5944–5972.
- Yuan, S., D. Chen, K. Zhang, and B. Yu. 2007. Effects of oxidative stress on growth performance, nutrient digestibilities and activities of antioxidative enzymes of weanling pigs. *Asian-Aust. J. Anim. Sci.* 20:1600–1605.